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The influence of sperm parameters on the outcome of intracytoplasmic sperm injection-embryo transfer cycle in poor responder women under 35 years of age

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Summary

To evaluate the influence of sperm parameters on the outcome of intracytoplasmic sperm injection (ICSI) cycles in poor responder women under the age of 35 years in a retrospective analysis in a fertility center. *Materials and Methods:* A total of 432 poor responder women who underwent ICSI cycles were evaluated. The interventions included ICSI and microdissection testicular sperm extraction (m-TESE). Main outcome measures included fertilization, cleavage, clinical pregnancy, and delivery rates. *Results:* Patients were divided into four groups according to the sperm parameters and the source of sperm; testicular spermatozoa obtained from men with azoospermia (group 1; n=26), severe oligoasthenoteratozoospermia (OAT) (group 2; n=35), OAT (group 3; n=104), and normal semen analysis (group 4; n=267). Average age of the women, antral follicle count, FSH level, male age, number of previous ICSI cycles, duration of infertility, and the maximal endometrial thickness were similar among the groups. In group 1, the fertilization rate was lower than those in all other groups. Cleavage, clinical pregnancy, and delivery rates were similar among the groups. *Conclusions:* Neither sperm parameters nor the source of spermatozoa affects delivery rate through ICSI in poor responder women < 35-years-old.

Key words: ICSI, microdissection TESE, male factor, OAT.

Introduction

Infertility affects approximately 15% of sexually active couples and male factors account for about half of the cases [1]. Idiopathic oligoasthenoteratozoospermia (OAT) is the most common medical diagnosis of abnormal semen quality [2]. Several studies reported increased rate of centrosome dysfunction, sperm DNA fragmentation [3, 4], deficiency of oocyte-activating factors [5], higher sperm chromosomal abnormalities, and sperm related aneuploidies in the deriving embryos [6-11] in patients with OAT, severe OAT, obstructive azoospermia (OA), and non-obstructive azoospermia (NOA). Thus, ICSI with spermatozoa of these men may affect outcome.

Intracytoplasmic sperm injection (ICSI) has been commonly used for couples with male infertility since its first successful introduction in 1992 [12]. Reliable pregnancy rates were achieved by using spermatozoa from OAT patients as well as testicular spermatozoa [13].

ICSI provides a mechanical assistance of injection of a morphologically normal and motile spermatozoon into the oocyte. Although aneuploid spermatozoa may retain the ability to fertilize an oocyte through ICSI, resultant embryo has an increased risk of chromosomal abnormalities which may negatively affect ICSI outcome.

A poor ovarian response, although the definition varies widely, to ovulation induction is a common problem which severely diminishes live birth rate [14]. Even though the number of oocytes affects the outcome, female age seems a better predictor on pregnancy rate.

In the current study, the authors aimed to evaluate the influence of semen quality on the outcome of ICSI in relatively young women (< 35-years-old) with poor ovarian response.

Materials and Methods

Patients and design

A retrospective analysis of 432 women with the diagnosis of poor ovarian response (POR) that underwent ICSI-ET cycles between May 2005 and June 2012 at the Assisted Reproduction Unit of Ota-Jinemed Hospital was carried out. The study was approved by the Institutional Review Board of Ota-Jinemed Hospital. Women who had < five oocytes as a response to controlled ovarian stimulation were classified as poor responders. All women had normal uterine cavity confirmed with hysterosalphingography and/or saline infusion sonography.

Semen evaluation and preparation

Semen analysis of male partners of all couples attending ICSI cycles were recruited from hospital database. All semen samples were analyzed according to the World Health Organization 2010

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Table 1. — *Baseline characteristics of groups.*

	Group 1 (n=26)	Group 2 (n=35)	Group 3 (n=104)	Group 4 (n=267)	*p* value
Female age (years)	30.9 ± 2.2	31.7 ± 1.4	32.3 ± 1.8	31.8 ± 2.1	NS
Antral follicle count	3.8 ± 1.2	3.7 ± 1.5	3.9 ± 1.4	3.7 ± 2.1	NS
FSH (mIU/ml)	10.7 ± 1.4	9.8 ± 2.3	10.7 ± 2.5	10.5 ± 3.2	NS
Male age (years)	37.3 ± 4.3	38.1 ± 4.4	37.9 ± 5.2	36.9 ± 5.8	NS
No. of previous IVF/ICSI cycles	1.5 ± 1.4	1.7 ± 1.3	1.6 ± 1.5	1.6 ± 1.9	NS
Duration of infertility (years)	2.9 ± 1.3	3.2 ± 2.8	3.1 ± 3.3	3.1 ± 4.2	NS
Maximal endometrial thickness (mm)	8.5 ± 1.39	8.4 ± 1.32	8.4 ± 1.41	8.6 ± 1.53	NS

Note: All values are expressed as mean ± SD. NS= not significant.

criteria. Couples were divided into four groups according to male partners' semen analysis: 1) testicular spermatozoa obtained from men with azoospermia (group 1; n=26), severe OAT (group 2; n=35), 3) OAT (group 3; n=104), and 4) normal semen analysis (group 4; n=267). Baseline characteristics of the patients are given in Table 1.

Semen samples were collected by masturbation at the laboratory after two to four days of abstinence. All semen analyses were carried out manually within one hour after the semen collection. The semen samples were left to liquefy at 37°C for 20 minutes. Following liquefaction, a drop of the well-mixed specimen was placed on a clean glass slide, covered with a coverslip, and left for a few minutes. The preparation was examined at ×400 magnification. Sperm parameters were divided into four groups as severe OAT (sperm concentration $\leq 1\times10^6$ /ml, motility < 25% and morphology < 1%), OAT (sperm concentration = $1\text{-}15\times10^6$ /ml, motility < 25% and morphology = 1-4%), normal (sperm concentration > 15×10^6 /ml, motility > 25%, morphology ≥ 4%), and testicular sperm obtained with m-TESE. The semen samples were prepared by centrifugation on a density gradient and washed with HEPES buffered medium containing human serum albumin.

Azoospermia was confirmed on at least two semen samples and microdissection testicular sperm extraction was performed as described previously [15]. Procedures in which fresh motile testicular spermatozoa used for ICSI were included in the study. Twitching was accepted as a minimum criterion for motility. Four patients had the diagnosis of OA and 22 had NOA.

Ovarian stimulation and oocyte retrieval

Women were treated with either down-regulation protocol starting GnRH-agonist in the previous luteal phase or GnRH-antagonist protocol. Recombinant FSH was started when down-regulation was achieved or on cycle day 3 until at least one follicle reached to 17 mm in diameter. GnRH antagonist was administered routinely on cycle day 6 regardless of follicular size. Oocyte retrieval was performed by transvaginal route 35 hours after hCG injection.

After removing the cumulus cells attached to the oocytes with hyaluronidase (type VIII) after two hours of incubation, ICSI was performed as described elsewhere [16]. Fertilization was assessed 16 to 18 hours and cleavage was checked 48-72 hours after ICSI. The embryos were transferred three days after oocyte retrieval. The luteal phase was supported by 50 mg of P in oil injections IM and continued until the detection of fetal heart beat. Clinical pregnancy was verified by the presence of fetal cardiac activity with transvaginal ultrasonography performed at six to seven weeks of gestation. The implantation rate was shown by the ratio of the number of implanted embryos to the number of embryos transferred into the uterus. Miscarriage was defined as disappearance of gestational sac.

Statistical analysis

MedCalc Statistical Software Program version 13.1.0 was carried out. Female age, antral follicle count, FSH level, male age, number of previous ICSI cycles, duration of infertility, and maximal endometrial thickness were evaluated by one-way analysis of variance (ANOVA) and the log-transform Scheffé's method. Fertilization, cleavage, implantation, clinical pregnancy, miscarriage, twin pregnancy, and delivery rates were evaluated by one-way analysis of variance (ANOVA). A p-value < 0.05 was accepted as significant.

Results

A total of 432 poor responder women were included in this study. Patients' characteristics are summarized in Table 1. Female age, antral follicle count, FSH level, male age, number of previous ICSI cycles, duration of infertility, and maximal endometrial thickness were similar among the four groups classified according to sperm parameters. Ovarian response to controlled ovarian stimulation in terms of number of oocytes retrieved did also not differ significantly among the groups (Table 2). Sperm count was significantly higher in group 4 than those in group 3 and 2 ($33.7 \pm 1.0 \times 10^6$ ml, $8.4 \pm 1.4 \times 10^6$ ml, and $2.1 \pm 1.3 \times 10^6$ ml, $p < 0.01$, respectively). It was also higher in group 3 than that in group 2 ($p < 0.05$). Scrotal hematoma was noted in one case following m-TESE and disappeared within a few days.

Fertilization rate was significantly lower with testicular spermatozoa as compared to the ejaculated spermatozoa (57.4% *vs.* 74.2% for sOAT, 76.4% for OAT, and 76.2% for normal sperm parameters) ($p = 0.007$). However, there was no significant difference in the cleavage, number of embryos transferred, implantation, clinical pregnancy, miscarriage, twin pregnancy, and delivery rates among the four groups (Table 2). Major malformation was not noted in the 142 children who were born.

Discussion

Despite being the only effective therapy for couples with male subfertility, ICSI is one of the most unphysiological methods of assisted reproductive technologies (ART), since spermatozoa is somewhat selected arbitrarily by an embryologist. Theoretically, testicular spermatozoa as well as spermatozoa of men with sOAT and OAT may carry higher

Table 2. — *Outcome of ICSI-ET cycles of poor responder women in four different groups as classified according to semen parameters.*

Poor responder women	Group 1: azoospermia (n=26)	Group 2: severe OAT (n=35)	Group 3: OAT (n=104)	Group 4: normal (n=267)	*p* value
No. of oocytes retrieved	2.6 ± 1.3	2.8 ± 1.2	2.9 ± 1.1	2.8 ± 1.1	NS
No. of MII oocytes	2.0 ± 1.2	1.9 ± 1.4	2.2 ± 1.5	2.1 ± 1.2	NS
Fertilization rate per oocyte(%)	57.4	74.2	76.4	76.2	0.007
Cleavage rate (%)	87.2	81.8	82.8	78.5	NS
No. of embryos transferred	1.8 ± 0.3	1.7 ± 0.4	1.7 ± 0.6	1.7 ± 1.1	NS
Implantation rate (%)	10/47 (21.3)	12/61 (19.7)	38/170 (22.4)	97/459 (21.1)	NS
Clinical pregnancy rate, n(%)	9 (34.6)	10 (28.5)	33 (31.7)	80 (29.9)	NS
No.of miscarriages, (%)	1 (11.1)	1 (10)	4 (12.1)	9 (11.2)	NS
Delivery rate, n(%)	8 (30.7)	9 (25.7)	29 (27.8)	71 (26.5)	NS
Twin pregnancy rate(%)	1/9 (11.1)	2/10 (20)	5/33(15.2)	17/80 (21.3)	NS

Note: values are expressed as the mean±SD. NS= not significant.

aneuploidy and DNA fragmentation rates, thereby may be associated with poor outcome in an ICSI cycle [3-11, 17-20]. In the current study, the authors compared ICSI outcomes of men with different sperm parameters in poor responder women. Since female age is an independent predictor of success with ART [21], the authors included women aged less than 35 years. Moreover, poor responder women were selected to assess the effect of semen parameters on the limited number of oocytes. Also this inclusion criterion has helped to overcome heterogeneity in terms of infertility factors.

A reduced fertilization rate was observed with testicular spermatozoa compared to those achieved with other semen parameters in the present study. This finding is in contrast to some of the prior studies which reported the same fertilization rate with testicular sperm as ejaculated sperm [22], but in accordance with Loutradi *et al.* who found a decreased fertilization potential of testicular spermatozoa with ICSI [23]. Possible explanations of the reduced fertilization rate are high rates of DNA fragmentation, mitochondrial dysfunction, and chromosomal aneuploidy found in the sperm of men with azoospermia [8]. The present authors observed decreased fertilization rate even though they only used motile testicular spermatozoa. Aside from fertilization, other variables such as implantation rate, miscarriage rate, and delivery rate did not differ.

In the ICSI procedure, only a single spermatozoon is required for injection and, individual sperm features such as motility [24] and morphology [25] seem to be the important factors for the successful outcome. However, Burrelo *et al.* found that even normally shaped spermatozoa from OAT patients had an increased aneuploidy rate [18]. Ushijimal *et al.* determined a significantly higher frequency of disomy for chromosomes 13, 21, sex chromosomes, and diploidy in the OAT group than the control group [26]. Vegetti *et al.* detected that patients with abnormal semen parameters showed a significantly higher aneuploidy rate for chromosomes 13, 18, 21, X, and Y in their spermatozoa compared to controls [27]. The risk of chromosomal aneuploidy in spermatozoa seems to be inversely correlated to sperm concentration and total progressive motility. These studies indicate that spermatozoa of men with OAT could influence the fertilization process and the potential viability of ICSI embryos. However, in the present study, the outcome of ICSI cycles in men with diminished sperm parameters did not differ from that with normal semen analysis.

Nagy *et al.* demonstrated that only microinjection of an immotile (presumably dead) spermatozoon into the oocyte had a strongly negative influence on the result of ICSI procedure [24]. Thus, the only ultimate criterion for successful ICSI seemed the presence of at least one living spermatozoon per oocyte in the pellet of the treated semen sample.

The association between sperm morphology and ICSI outcome has also been analyzed. Previous studies found an inverse relationship between the percentage of atypical forms and the percentage of aneuploidies [28]. Furthermore, globozoospermia, flagellar abnormalities, large-headed and multiple-tailed spermatozoa, and elongated-head spermatozoa were linked to increased aneuploidies [29,30]. De Vos *et al.* evaluated the influence of morphology of spermatozoa on the fertilization and pregnancy outcome and found lower fertilization, pregnancy, and implantation rates with the injection of morphologically abnormal spermatozoa (irrespective of origin) compared to the injection of morphologically normal spermatozoa [25]. Since only cases with morphologically normal motile spermatozoa were included, the present authors are not able to comment on the effect of morphologically abnormal spermatozoa on pregnancy rate via ICSI.

Minor sperm nuclei abnormalities (such as vacuoles) which may be related to poor outcome cannot normally be identified during the ICSI procedure [31]. The present authors selected sperm under ×400 magnification, at the periphery of the PVP microdroplet. The rate of sperm nucleus normality was significantly higher when intracytoplasmic morphologically selected sperm injection (IMSI) was performed with sperm selected under a magnification level above ×6,000 [32]. The role of more detailed sperm selection needs to be studied to improve the ICSI outcome.

The present authors do recognize some weaknesses such as its retrospective nature and the limited number of poor re-

sponder patients having ICSI with testicular spermatozoa. Moreover, they included only cases having motile/morphologically normal spermatozoa in each group. They assume that inclusion of men with no motile spermatozoa available could have changed the results.

In conclusion, neither sperm parameters nor the source of spermatozoa affects delivery rate through ICSI in poor responder women < 35 years of age, when motile/morphologically normal spermatozoa is present.

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