Original Articles

Reproductive Biology Section

ICSI cycle outcomes in oligozoospermia

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Summary

Aim: The aim of this study was to evaluate the sole effect of sperm concentration on fertilization, embryo quality and pregnancy rates in patients undergoing ICSI cycles. *Materials and Methods:* 560 ICSI cycles performed for male factor infertility were divided into four groups according to sperm concentration retrospectively. Group 1 consisted of 86 couples whose sperm concentration was less than 1 x 10^6, group 2 consisted of 169 couples whose sperm concentration ranged between 1 x 10^6 and 5 x 10^6, group 3 consisted of 95 couples whose sperm concentration ranged between 5 x 10^6 and 10 x 10^6 and group 4 consisted of 210 couples whose sperm concentration ranged between 10 x 10^6 and 20 x 10^6. *Results:* Fertilization rate was significantly lower in the first three groups compared to the last group ($p < 0.05$). The first three groups were comparable with each other. There were no differences according to ovarian response to stimulation, embryo quality and clinical pregnancy rates between the four groups. *Conclusion:* Lower sperm concentration has detrimental effects on the outcomes of ICSI cycles. This situation is more evident in men with severe and extremely severe oligozoospermia.

Key words: ICSI; Oligozoospermia; Fertilization rate.

Introduction

Great progress has been made in male subfertility by the introduction of ICSI by Palermo *et al*. in 1992 [1]. From then on, only sperm has been thought to be enough for a successful pregnancy independent of the sperm defect severity. In the following years investigations of the effect of sperm defects on the outcomes of ICSI were published. Morphology is the most evaluated parameter of semen analysis with conflicting results; some report adverse effects [2-6] and others comparable results [7-10]. However there is a paucity of data regarding the effect of sperm concentration on the outcomes of ICSI cycles [11, 12].

The aim of this study was to evaluate the sole effect of sperm concentration on fertilization, embryo quality and pregnancy rates in patients undergoing ICSI cycles.

Materials and Methods

We retrospectively reviewed the records of patients who underwent ICSI for male factor infertility with oligozoospermia as the sole cause at Hacettepe University, Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Fertility and Reproductive Endocrinology between July 2001 and January 2010. Before the beginning of data collection, institutional review board approval was obtained.

The patients were divided into four groups according to sperm concentration which was analyzed according to Kruger's strict criteria [13]. Group 1 consisted of 86 couples whose sperm concentration was less than 1 x 10^6, group 2 consisted of 169 couples whose sperm concentration ranged between 1 x 10^6 and 5 x 10^6, group 3 consisted of 95 couples whose sperm concentration ranged between 5 x 10^6 and 10 x 10^6 and group 4 consisted of 210 couples whose sperm concentration ranged between 10 x 10^6 and 20 x 10^6. All patients underwent controlled ovarian hyper-stimulation using luteal-long leuprolide acetate (LA; Lucrin; Abbott, Cedex, Istanbul, Turkey) and recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) using the step-down protocol. The starting dose of gonadotropin was determined based on the woman's age, body mass index (BMI), antral follicle count at baseline transvaginal sonography (TVS), and day 3 FSH and E2 levels. Ovarian response was monitored with frequent serum E2 measurements and TVS. The criterion for hCG (Profasi; Serono, Istanbul, Turkey) administration was the presence of three or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound-guided puncture of follicles 36 hours after hCG administration.

Semen samples of the male patients were collected by masturbation after two to seven days of sexual abstinence on the day of egg retrieval.

ICSI was performed for all metaphase II oocytes, as described by Van Steirteghem *et al*. [14]. Spermatozoa were selected for injection based on motility. Nonmotile sperm were used when motile sperm were not collected. Fertilization was controlled 16 to 18 hours after ICSI for the presence of distinct two pronuclei and two polar bodies [15].

Embryos were graded on day 3 according to a 1-4 scoring system (with 1 being the best), which was based on fragmentation, cell symmetry and blastomere number. The embryos with even blastomeres and no fragmentation were graded as grade 1, embryos with even blastomeres and < 20% fragmentation as grade 2a, embryos with uneven blastomeres and no fragmentation

Revised manuscript accepted for publication September 19, 2011

as grade 2b, and embryos with uneven blastomeres and < 20% fragmentation as grade 2ab. Embryos with 20-50% fragmentation and > 50% fragmentation were graded as grade 3 and 4 embryos, respectively [16]. Grades 1-3 were considered as transferable embryos. All the procedures of embryo transfer were performed with a soft catheter under TVS. The luteal phase was supported by daily vaginal progesterone suppositories (Crinone, Serono, Istanbul, Turky) starting one day after oocyte pick-up.

Clinical pregnancy was determined by ultrasound demonstration of a gestational sac at TVS.

Statistical analyses were performed using Statistics Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL). The normal distribution of variables was tested with Kolmogorov-Smirnov. Parametric and numeric variables were compared with the Independent samples t-test. The χ^2 test was used to analyze nominal variables in the form of frequency tables; *p* values of 0.05 or less were considered statistically significant. Values are expressed as mean ± SD, unless stated otherwise.

Results

A total of 560 ICSI cycles were included in the analysis. The baseline characteristics of patients and contents of the groups are given in Table 1. The demographic characteristics and responses of the females to controlled ovaran hyperstimulation were comparable between groups (Tables 1 and 2).

Fertilization rate was significantly lower in the first three groups compard to the last group ($p < 0.05$, Table 2). The first three groups were comparable with each other.

There was no difference according to ovarian response to stimulation, embryo quality and clinical pregnancy rates between the four groups (Tables 1 and 2).

Table 1. — *Baseline characteristics*.

	Group 1 (SC 0 < Ml/ml (n = 86)	Group 2 (SC 1 M/ml - 5 M/ml) (n = 169)	Group 3 (SC 5 M/ml - 10 M/ml) (n = 95)	Group 4 (SC 10 M/ml - 20 M/ml) (n = 210)	*p* value
Female age (years)	30.5 ± 4.7	30.9 ± 5	31.6 ± 4.6	32.1 ± 5	NS
Bady mass index (kg/m²)	24.8 ± 3.8	24.7 ± 2.4	24.3 ± 3.2	25.2 ± 4.2	NS
Duration of infertility (mo)	78.6 ± 58	90.7 ± 57	92.4 ± 62	96.1 ± 60	NS
Antral follicle count	11.5 ± 4.4	12.1 ± 7.6	12.2 ± 6.3	11.4 ± 6.4	NS

SC: Sperm concentration.

Discussion

There is not enough accurate data about the effects of sperm defects on ICSI cycle outcomes. Morphology, motility and sperm concentrations are the basic characteristics of a routine semen analysis. Many researchers are trying to find out the effect of male factor on treatment outcome by using these parameters.

According to our study embryo quality and clinical pregnancy rates are comparable in oligozoospermic couples. Only fertilization rate was significantly decreased in couples who had sperm concentration less than 10 M/ml (Table 2).

Hashimoto *et al*. evaluated the effect of severity of oligozoospermia on ICSI cycle outcome in the way we did and they also observed a decreased fertilization rate similar to our study, but the difference was significant in couples who had sperm concentration less than 1 M/ml [11]. Fertilization rates were similar in the other three groups. In our study the fertilization rate was decreased in the first three groups who had sperm concentration less than 10 M/ml compared to the last group (Table 2).

Strassburger *et al*. investigated the effect of extremely low sperm counts on outcome of ICSI cycles in which sperm concentration ranged between cryptozoospermia and 1 M/ml [12]. The couples with crytozoospermia had the worst outcomes. Fertilization and clinical pregnancy rates decreased and abortion rate increased in the couples in whom no visible spermatozoa were found before or after centrifugation which was called cryptozoospermia. Spermatozoa could only be discovered after extended sperm preparation in these patients.

Hashimoto *et al*. and Strassburger *et al*. evaluated the effects of oligozoospermia on ICSI cycle outcome by first investigating severe oligozoospermia and second extreme oligozoospermia; neither study had a control group with normal sperm analysis [11, 12]. They both demonstrated adverse outcomes with decreasing sperm counts similar to our study, especially for fertilization rates.

Several authors have reported increased sperm chromosome abnormalities with decreasing sperm concentration [17-20]. Nagvenkar *et al*. reported that men with severe oligozoospermia (sperm concentration < 5 M/ml) have

Table 2. — *Ovarian response, embryo quality and treatment outcomes*.

	Group 1 (SC 0 < Ml/ml) (n = 86)	Group 2 (SC 1 M/ml - 5 M/ml) (n = 169)	Group 3 (SC 5 M/ml - 10 M/ml) (n = 95)	Group 4 (SC 10 M/ml - 20 M/ml) (n = 210)	*p* value
E2 level on hCG day (pg/ml)	2446.5 ± 1624.6	2484.1 ± 1546.3	2524.3 ± 1623.2	2485.5 ± 1530.7	NS
No. of metaphase II oocytes	9.9 ± 6.2	10.1 ± 6	10.6 ± 5.9	10.7 ± 6.3	NS
No. of 2-pronucleated oocytes	6.8 ± 4.6	7.02 ± 4.6	7.4 ± 4.8	8.06 ± 4.8	NS
Fertilization rate (%)	69	68	70	74*	$p < 0.05$
No. of day 3 grade 1 embryos	0.87 ± 1.4	0.67 ± 1.2	0.82 ± 1.3	0.80 ± 1.2	NS
No. of day 3 grade 2 embryos	4.8 ± 3.3	5.3 ± 3.8	5.7 ± 3.9	6.2 ± 4.05	NS
No. of transferred grade 1 embryos	0.51 ± 0.8	0.43 ± 6.7	0.43 ± 0.7	0.50 ± 0.8	NS
No. of transferred grade 2 embryos	2.2 ± 1.2	2.2 ± 1.3	2.3 ± 1.2	2.4 ± 1.2	NS
No. of embryos transferred	2.8 ± 1.1	2.6 ± 1.1	2.8 ± 1.1	2.9 ± 1.2	NS
Clinical pregnancy/embryos Transfer (%)	47.5	50.6	50	47.5	NS

SC; Sperm concentration

higher frequencies of sperm aneuploidy compared to oligozoospermic (sperm concentration between 5 M/ml and 20 M/ml) and normozoospermic men [17]. They demonstrated that pregnancy and ongoing pregnancy rates were significantly lower in couples with severe oligozoospermia, however the fertilization rate was similar to our study and the above studies. These results were related to increased implantation failure and miscarriage of chromosomally abnormally developed embryos.

In a study reported by Won Bak *et al*. 24.6% of men with severe oligozoospermia (sperm concentration < 5 M/ml) developed azoospermia in a time period greater than six months and they advised considering sperm freezing in such patients [21].

It is clear from the literature that lower sperm concentration has detrimental effects on the outcomes of ICSI cycles. This situation is more evident in the men with severe and extremely severe oligozoospermia. Clinicians should consider these effects when performing ICSI cycles on couples with oligozoospermia.

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