ICSI outcome of patients with severe oligospermia vs non-obstructive azoospermia

B. Demir[1], I.I. Arikan[2], G. Bozdag[3], I. Esinler[3], L. Karakoc Sokmensuer[4], S. Gunalp[3]

[1]Department of Gyneacology and Obstetrics, Ergani State Hospital, Diyarbakir
[2]Departments of Gynecology and Obstetrics, Zonguldak Karaelmas University, Faculty of Medicine, Zonguldak
[3]Department of Obstetrics and Gynecology, Hacettepe University, Faculty of Medicine, Ankara
[4]Department of Histology and Embryology, Hacettepe University, Faculty of Medicine, Ankara (Turkey)

Summary

Objective: To compare the results of intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) cycles in men with severe oligoospermia and non-obstructive azoospermia. *Materials and Methods:* This study included 91 ICSI cycles performed due to male factor infertility. Patients are divided into two groups according to source of spermatozoa. Group 1 consisted of 38 cycles in which sperm was obtained from testicles (cases with non-obstructive azoospermia). In Group 2, 53 consecutive cycles were included in which ejaculated sperm was available for ICSI in spite of severe oligospermia (< 100,000/ml). Fertilization, embryo quality and clinical pregnancy rates were compared between the groups. *Results:* Although, the female age and mean number of oocytes retrieved were similar among the two groups, fertilization rate was significantly lower in the non-obstructive azoospermia (34.6%) group compared to group in which patients underwent ICSI with ejaculate spermatozoa (55.3%) ($p < 0.05$). However, there were no differences regarding mean number of available grade 1 embryos on day 3 and pregnancy rate between the two groups. *Conclusion:* Testicular sperm from non-obstructive azoospermia patients had significantly lower fertilization rates than the ejaculated spermatozoa from severe oligospermia patients in ICSI cycles. However, it did not bring about improved pregnancy rate.

Key words: Non-obstructive azoospermia; Intracytoplasmic sperm injection; Oligozoospermia.

Introduction

After it was first reported by Palermo *et al*. in 2002 intracytoplasmic sperm injection (ICSI) has become the preferred technique for fertilization in assisted reroductive technologies (ART) not only in severe male factor infertility as azoospermia or oligoasthenoteratozoospermia but also in unexplained and tubal factor infertility [1-4].

Characteristics of male patients as obstructive (OA) or nonobstructive azoospermia (NOA); semen parameters indicating number, motility and morphology and the sperm source as fresh ejaculate or surgically removed are the conflicting factors affecting treatment outcomes in ICSI cycles, and the debate has continued these topics for two decades. Some reports demonstrated improved fertilization and pregnancy rates with ejaculated or testicular sperm in obstructive azoospermia according to testicular sperm from nonobstructive azoospermia [5-14] while others showed comparable results [15-19].

In the current study, we aimed to understand whether the source of sperm differs in means of fertilization rate, embryo quality and pregnancy outcome in ICSI cycles. Therefore, ICSI cycles in men with severe oligozoospermia and non-obstructive azoospermia were compared in which sperm was obtained with either ejaculation or testicular biopsy, respectively.

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Materials and Methods

We retrospectively analyzed the records of patients who underwent ICSI at the 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.

Patients are divided into two groups according to spermatozoa source for ICSI: 1) sperm was successfully obtained from testicles (cases with non-obstructive azoospermia and normal peripheral karyotype) (group 1; n = 38), and 2) ejaculated (severe oligozoospermia: < 100,000/ml) (group 2; n = 53).

Preoperative evaluation included a complete history and physical examination. In both groups, neither preoperative hormonal treatment nor a diagnostic biopsy was planned. In group 1, testicular sperm extraction (TESE) was performed under local anesthesia by widely opening the testes in an equatorial plane, one day prior the day of oocyte retrieval. Microdissection was carried out with examination of the seminiferous tubules using an operating microscope (Carl Zeiss, OPMI Pico Surgical Microscope) at x 20 magnification. Enlarged seminiferous tubules were selected, removed and evaluated by an embryologist. Each sample was mechanically cut and dispersed in 1 ml of G-IVF (Vitrolife, Kungsbacka, Sweden) supplemented with 10% HSA (Vitrolife, Kungsbacka, Sweden) in a petri dish (Falcon Plastics, Becton-Dickinson). Each specimen was evaluated under phase-contrast microscope at x 200 magnification. If intact spermatozoa were noted, the procedure was terminated. If no sperm were seen, microdissection of additional areas of testicular parenchyma was carried out and additional samples were taken. After dissection, tunica albuginea was closed with 5-0 polypropylene. The tissue was collected in a sterile conic tube (Falcon Plastics, Becton-Dickinson). Following washing

with the gradient method (Isolate sperm separation medium, Irvine Scientific, Santa Ana, CA, USA), the prepared sample was incubated at 37°C with 7% CO_2. In the morning of scheduled oocyte retrieval day, the sample was transferred into a petri dish (Falcon Plastics, Becton-Dickinson) and covered with oil (Vitrolife, Kungsbacka, Sweden) for identification and collection of spermatozoa.

All patients underwent controlled ovarian hyperstimulation (COH) 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) and antral follicle count at baseline transvaginal ultrasonography (TVS). Ovarian response was monitored with frequent serum estradiol (E2) measurements and TVS, as described previously. The criterion for hCG (Pregnyl; Organon, Istanbul, Turkey) administration was the presence of two 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 with oligozoospermia were collected by masturbation after two to seven days of sexual abstinence on the day of egg retrieval.

The most morphologically normal motile spermatozoa were selected for ICSI. Where all sperm had morphological defects, sperm with fully developed tails and grossly normal heads were injected. The presence of fertilization was evaluated by examining oocytes 12-17 h after injection for the presence of distinct two pronuclei and two polar bodies.

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 [20]. The embryos with even blastomeres and no fragmentation were graded as grade 1, the embryos with even blastomeres and < 20% fragmentation as grade 2a, the embryos with uneven blastomeres and no fragmentation as grade 2b, the embryos with uneven blastomeres and < 20% fragmentation as grade 2ab. The embryos with 20-50% fragmentation and > 50% fragmentation were graded as the grade 3 and 4 embryos, respectively. Grades 1-3 were considered as transferable embryos.

Clinical pregnancy was defined as the presence of an intrauterine gestational sac with fetal heart beat at TVS.

Statistical analyses were performed using Statistics Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL). The normal distribution of the variables was tested with Kolmogorov-Smirnov. Parametric and numeric variables were compared with Independent samples T-test. The χ^2 test was used to analyze nominal variables in the form of frequency tables: p values of < .05 or less were considered statistically significant. Values were expressed as mean ± SD, unless stated otherwise.

Results

Female age, body mass index (BMI), duration of infertility and basal antral follicle count were comparable among the two groups (Table 1). The COH performance of the two groups also revealed similar mean number of metaphase-II oocytes retrieved (Table 2). However, fertilization rate was significantly lower in the non-obstructive azoospermia (34.6%) group when compared to the group in which patients underwent ICSI with ejaculate spermatozoa (55.3%) ($p < 0.05$, Table 2).

In group 1, 51 of 53 cycles reached embryo transfer (ET); however, only 18 of 38 cycles succeeded in reaching ET. There was no difference according to ovarian response to stimulation, embryo quality and clinical pregnancy/ET between the ejaculated and non-obstructive azoospermia groups (Table 2). However, it is noteworthy to mention that pregnancy rate seems to be higher when ejaculated sperm is available, but no statistical significance was reached probably due to the small study group.

Table 1. — *General characteristics of patients who underwent ICSI with ejaculated and testicular spermatozoa*.

	Group 1 (non-obstructive azoospermia) (n = 38)	Group 2 (severe oligospermia) (n = 53)	*p* value
Female age (years)	32.5 ± 4.6	31.3 ± 4.7	NS
BMI (kg/m²)	24.3 ± 2.1	24,6 ± 4.7	NS
Infertility duration (months)	107.03 ± 58.8	107.9 ± 62.9	NS
AFC	9.1 ± 2.7	9.3 ± 5.1	NS
E2 level on the day of hCG administration (pg/ml)	2150 ± 910,9	2029,7 ± 1333	NS

BMI: body mass index, AFC: antral follicle count, hCG: human chorionic gonadotropin, E2: estradiol, NS: not significant. Note: Values are expressed as mean ± SD.

Table 2. — *Cycle characteristics of patients who underwent ICSI with ejaculated and testicular spermatozoa*.

	Group 1 (non-obstructive azoospermia) (n = 38)	Group 2 (severe oligospermia) (n = 53)	*p* value
No. of oocyte-cumulus complexes	10.7 ± 4.8	10.2 ± 7.3	NS
No. of metaphase II oocytes	8 ± 4.1	8.7 ± 6.5	NS
No. of 2-pronucleated oocytes	2.8 ± 3.1	4.8 ± 3.9	**p < 0.05**
Fertilization rate (%)	34.6	55.3	**p < 0.05**
No. of grade I embryo on day 3	0.7 ± 1.0	1.1 ± 1.3	NS
No. of grade 2 embryo on day 3	0.3 ± 0.6	0.8 ± 1.4	NS
No. of transferred grade I embryos	0.8 ± 0.9	0.9 ± 0.9	NS
No. of transferred grade 2 embryos	1.8 ± 1.8	1.5 ± 1.3	NS
No. of embryos transferred	2.8 ± 1.2	2.9 ± 1.1	NS
Clinical pregnancy/embryo transfer (%)	22.2	39.2	NS

Note: Values are expressed as mean ± SD, unless stated otherwise. NS = not significant

Discussion

According to results of our study, testicular sperm from non-obstructive azoospermia patients had worse fertilization rates than the ejaculated spermatozoa from severe oligospermia patients. The outcomes according to developing embryo quality and clinical pregnancy rates were comparable between the two groups.

Verza *et al*. have evaluated the effect of severity of sperm abnormality to treatment outcomes in ejaculated sperm in ICSI cycles and compared the ICSI results of obstructive and non-obstructive azoospermia patients [12]. Non-obstructive azoospermia patients had the worst results acoording to fertilization, embryo quality and clinical pregnancy rates among the all other groups. Miscarriage rates were comparable between the groups. Furthermore, they reported that fertilization rate decreased with the increasing severity of sperm abnormalities in patients of the ejaculated sperm group. We could only detect a decrease in the fertilization rate which is concor-

dant with these results, but clinical pregnancy rate was comparable between the groups in our study.

Also Göker *et al*. reported that patients with non-obstructive azoospermia had lower fertilization and pregnancy rates according to patients with normal and abnormal semen parameters [13]. They did not mention the severity of sperm abnormality. Clinical research will be deficient especially if not differentiating obstructive azoospermia from non-obsructive azoospermia and not mentioning the severity of sperm abnormality in semen analysis.

Aboulghar *et al*. also observed decreased fertilization and pregnancy rates in patients with non-obstructive azoospermia [11].

In contrast to these reports Bukulmez *et al*. concluded that severity of sperm defect and origin of spermatozoa did not effect ICSI outcome even in patients with non-obstructive oligospermia [19]. Naru *et al*. reported comparable ICSI results with epididymal, testicular and ejaculated sperm but they did not group the patients according to the sperm abnormality nor to the obstruction pattern [18]. Furthermore, Ghazzawi *et al*. also reported no difference in the outcomes between patients with oligoasthenoteratozoospermia, obstructive and non-obstructive azoospermia [17]. Different from the report by Bukulmez *et al*. they observed decreased live birth rates in patients with non-obstructive oligospermia.

ICSI is the most advanced procedure of ART primarily used in patients with azoospermia. The poorer ICSI outcomes with testicular spermatozoa in non-obstructive azoospermic patients and severily defective ejaculated spermatozoa indicate that spermatogenesis is very important via this procedure [5-7, 12, 15-17, 21, 22]. This may be due to higher deficiencies related to physiologic, metabolic and genetic materials found in the spermatozoa of such patients [23, 24].

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Address reprint requests to:
B. DEMIR, M.D.
Peyas Mah. 282. Sokak
Diyarpark 1 Sitesi, E blok, No: 15
Kayapinar, Diyarbakir (Turkey)
e-mail: bulentdemirmd@hotmail.com