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The Effect of the Sperm Source on the Outcome of Intracytoplasmic Sperm Injection-Embryo Transfer Cycles in Normal Responder Women

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Abstract

Introduction: Poor semen quality is the main cause of male factor. The aim of this study was to evaluate the effect of sperm source on the outcome of intracytoplasmic sperm injection-embryo transfer cycles (ICSI-ET) in normoresponder women.

Methods: A total of 884 normoresponder women, underwent first ICSI cycles were evaluated. Fertilization, cleavage, embryo morphology, clinical pregnancy, miscarriage and live birth rates were compared. Ejaculated semen samples were collected by masturbation. Sperm retrieval from the testis was performed percutaneously by microdissection testicular sperm extraction (m-TESE) under local anesthesia.

Results: Patients were divided four group according to source of sperm and sperm parameters. Testicular spermatozoa obtained from men with azoospermia (group 1, n=43), severe oligoasthenoteratozoospermia (sOAT), (group 2, n=93), oligoasthenoteratozoospermia (OAT) (group 3, n=209) and normal semen analysis (group 4, n=539). The most reduced fertilization rate was observed with testicular spermatozoa and the fertilization rate increased as semen quality increased from Group 2 to 4. Our results indicated that semen quality can affect the fertilization process. We observed that the cleavage and high-quality embryo rates were highest in group I (88.4% and 93%, respectively) compared to other groups. Clinical outcomes of ICSI did not show statistically significant differences in the rates of clinical pregnancy, miscarriage and live birth rate.

Conclusion: Neither sperm parameters nor the source of spermatozoa affects live birth rate in normoresponder women when motile/morphologically normal spermatozoa is present.

Keywords: Azoospermia, Oligo-astheno-teratozoospermia, Semen quality, Intracytoplasmic sperm injection.

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Introduction

Infertility, defined as unable to conceive within the one-year period, affects approximately 15% of sexually active couples (1). Male factors account for half of all causes of infertility (2,3). Reduced semen quality is the main cause of male factor (2–4) and oligoasthenoteratozoospermia (OAT) is the most common diagnosis of abnormal semen quality (2). Several studies have shown that both testicular spermatozoa and poor semen quality are associated with a higher rate of sperm chromosome aneuploidy, improper meiotic recombination (5,6), sperm DNA fragmentation and mitochondrial dysfunction in patients with azoospermia (OA), OAT or severe OAT (7–10). Although, a recent study indicated that impaired testicular function is reversible with different treatment modalities (11), intracytoplasmic sperm injection (ICSI) using the spermatozoa of these men may result in poor cycle outcome.

ICSI has commonly been used for couples with male factor infertility and reliable pregnancy rates have been achieved by using ejaculated spermatozoa, as well as testicular spermatozoa. ICSI provides mechanical assistance through the injection of a morphologically normal and motile spermatozoa 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 adversely affect ICSI outcomes and result in impaired rates of successful embryo transfer, implantation, pregnancy and live birth (12–14).

In the current study, we report an analysis of ICSI cycles in normoresponder women to investigate the effect of sperm source on the outcome of ICSI when ejaculated or testicular spermatozoa are used for microinjection.

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Material and Methods

Patients and Study Design

Overall 884 women who underwent assisted reproduction in our Institute between January 2005 and June 2013 were retrospectively enrolled. The study was approved by the Institutional Review Board. Only normal responders to controlled ovarian hyperstimulation were included. Women were considered as normal responders provided that the number of follicles were ≥4 following controlled ovarian stimulation. Those with basal FSH ≥ 10 IU/ml, history of pelvic surgery and previous diagnosis with unexplained infertility were excluded from the study. All women had normal uterine cavity confirmed with hysterosalphingography and/or saline infusion sonography.

Semen Evaluation and Sperm Preparation

The sperm quality of the male partners of all couples attending for ICSI cycles were extracted from the hospital database. All semen samples were analyzed according to the methods and standards outlined by World Health Organization (WHO) laboratory manual for the examination and processing of human semen (fifth edition, WHO, 2010). All of the couples were divided in four groups according to the sperm quality of male partners: Testicular spermatozoa obtained from men with azoospermia (Group 1; n=43), severe OAT (Group 2; n=93 sperm concentration, <5x106/ml), OAT (Group 3; n=209, sperm concentration between ≥5x106/ml, ≤15x106/ml) and normal semen analysis (Group 4; n=539, normal semen parameters).

All semen samples were collected on the day of oocyte retrieval by masturbation at the laboratory after a 3 or 4 days abstinence from ejaculation. Semen samples was evaluated manually within one hour after the semen collection in the laboratory. The Liquefaction was made at 37oC for 20 min. After 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 under both 10x and 40x a magnification of objectives. All semen samples were prepared by centrifuga¬tion on a density gradient us¬ing MOPSE/MRC supplemented with 10% serum substitute supplement.

The counting of spermatozoa was done using Meckler's counting chamber. Semen samples were categorized onthe basis of sperm count per milliliter of semen inaccordance with WHO normal and pathological ranges including normospermia (normal sperm count), oligospermia, and azoospermia.

Sperm retrieval from the testis was performed by micro-surgical testicular sperm extraction (m-TESE), under local anesthesia on the day of oocytes retrieval. The specimen was washed into apetri dish with a small volume of modified human tubal fluid (M-HTF; Irvine Scientific, CA, USA), and the presence of sperm was confirmed using an inverted microscope at 400× magnification. ICSI was performed immediately. Azoospermia was confirmed via analysis of two different semen analyses according to WHO criteria. Only the male partners with a count of motile testicular spermatozoa for ICSI of each oocytes were included the study.

Ovarian Stimulation and Oocyte Retrieval

The GnRH agonist or antagonist pro¬tocol with recombinant FSH were used during the ovarian stimulation. After one or two follicles with 18 mm in diameter was detected with transvaginal monitorization, hCG (10,000 IU) was administered. After 36 hours following HCG injection, oocyte pick-up was performed with a needle by transvaginal route. Sperm separation was performed with light microscope under 200× to 400× magnification. The cumulus cells were removed from oocytes with 0.1% hyaluronidase, after two hours folloving incubation. ICSI was performed as described elsewhere. Fertilization was evaluated as appearance of two pronuclei and two polar bodies after 16 to 18 hours following insemination. Cleavage was checked 48-72 hours after ICSI.

Embryos were evaluated with the scoring system established by Staessen et al.(15). even and homogeneous blastomeres without fragmentation was accepted as a Grade 1; even and homogeneous blastomeres with <20% fragmentation was accepted as a Grade 2; uneven and non-homogeneous blastomeres with 20-50% fragmentation was accepted as a Grade 3; uneven and non-homogeneous blastomeres with >50% fragmentation as a Grade 4. Those in Grades 1 or 2 were accepted as high-quality embryos.

After three days following the oocyte retrieval embryo transfer was performed. The luteal phase was supported by 50 mg of P in oil injections (IM) which was continued until the detection of fetal hearth beat. Clinical pregnancy was verified by the

presence of fetal cardiac activity with transvaginal ultrasonography performed at six to seven weeks of gestation. Miscarriage was defined as disappearance of fetal heartbeat.

Statistical Analysis

Data analysis was carried out using MedCalc Statistical Software Program version 13.1.0. Female age, antral follicle count, FSH level, male age, number of previous ICSI cycles, duration of infertility, and maximal endometrial thickness were assessed 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 884 normoresponder women were included in this study. Patients' characteristics of are summarized in Table 1. Female age, antral follicle count, FSH level, men's age, number of previous ICSI cycles, length of infertility, and maximal endometrial thickness were similar among the four groups. The number of oocytes retrieved during the oocytes pick-up did not differ significantly between the groups (Table 2).

Motile, normal-looking spermatozoa were found easily in male partners' ejaculates. But, in 7 patients with azoospermia, in whom spermatozoa was detected by testicular biopsies, a few sperm cells with a slowdown type of motility were observed. These 7 patients were included in the study. Following m-TESE, scrotal hematoma was noted and disappeared within a few days in two cases.

Comparisons between groups' laboratory and clinical outcomes are summarized in Table 2. The fertilization rate was significantly lower with testicular spermatozoa when compared to the ejaculated spermatozoa (65.1% vs. 74.5% for sOAT, 75.1% for OAT, and 80.0% for normal sperm parameters) (p=0.001). The cleavage and high-quality embryo rate were highest in group 1 (88.4% and 93% respectively) and there was significant difference between the groups (P<0.05). The proportion of implantation, rates of clinical pregnancy, miscarriage and delivery rate showed no statistical differences among any of the four groups (P>0.05). No major malformation was not noted of the 332 children who were born.

Discussion

In the present study, a reduced fertilization rate was observed with testicular spermatozoa from azoospermia group in ICSI cycles. But, clinical outcomes such as implantation, miscarriage and delivery rate were similar between groups.

ICSI has been commonly used for couples with male infertility since its first successful introduction in 1992 (16). However, ICSI is one of the most unphysiological methods of assisted reproductive technologies (ART), since spermatozoa are selected somewhat arbitrarily by an embryologist. Although, aneuploid spermatozoa may ability to fertilize an oocyte through ICSI, resultant embryo has an increased risk of chromosomal abnormalities which may negatively affect ICSI outcome. It can cause a decreased rates of successful embryo transfer, implantation, pregnancy and live birth.

In the current study, we have compared ICSI outcomes of men with different sperm parameters

Table 1. Characteristics of normoresponder women.

	Group I (n=43)	Group II (n=93)	Group III (n=209)	Group IV (n=539)	P value
Age (years)	31.3±3.6	30.7±3.6	29.9±3.4	30.4±3.5	0,06
Antral follicles count	10.1±2.8	9.4±2.4	9.9±3.2	9.7±2.7	0,38
FSH	6.3±1.5	5.9±1.8	6.1±1.5	6.4±1.9	0,08
Male age	33,4±3.4	32,9±3.5	32,6±3.9	33,5±4.1	0,09
Infertility period (year)	4.6±2.7	5.1±2.6	4.9±2.9	5.4±3.1	0,08
No. of previous IVF/ICSI cycles	1.9±1.3	1.7±1.1	1.6±1.1	1.7±1.1	0,35
Endometrial thickness (mm)	10.4±3.4	9.7±1.8	9.9±2.3	10.2±2.4	0,20

All values are expressed as mean ± SD.
Significant difference (p <0.05) between the groups by the ANOVA (rank transformation method).

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

	Testicular spermatozoa	Ejaculated spermatozoa			
	Group I (Azospermia) (n=43)	Group II (sOAT) (n=93)	Group III (OAT) (n=209)	Group IV (Normal) (n=539)	P value
No. of oocytes retrieved	12.5±2.87	11.9±3.4	12.6±4.3	13.1±4.5	0,06
No. of MII oocytes	9.8±2.4	8.9±3.2	9.8±3.7	10.1±4.3	0,06
Fertilization rate per oocytes (%)	65.1%	74.5%	75.1%	80.0%	<0.001
Cleavage rate (%)	88.4%	83.0%	81.9%	85.8%	<0.001
High quality embryo rate per embryo on day 3 (%)	40 (93,0%)	70 (75,3%)	179 (85.6%)	485 (90,0%)	<0.001
No. of embryos transferred	2.1±1.1	2.3±0.9	2.2±0.8	2.2±0.8	0,75
Implantation rate	20/90 22,2%	41/214 19,2%	108/460 23,4%	296/1186 24,9%	0,22
Clinical pregnancy rate (%)	(34.9%)15	(36.6%)34	(40.7%)85	(43.8%)236	0,41
Miscarriage rate (%)	2/15(13.3)	4/34(11.8)	9/85(10.6)	23/236(9.7)	0,94
Delivery rate (%)	13/43(30.2)	30/93(32.3)	76/209(36.7)	213/539(39.5)	0,37

Values are expressed as the mean±SD.
Significant difference (p<0.05) between the groups by the ANOVA (rank transformation method).

in normoresponder women. The outcome of ICSI as settled by fertilization, cleavage, high quality embryo, clinical pregnancy, miscarriage and delivery rate was compared between groups. Since female age is an independent predictor of success with ART (17,18), the groups was matched for women age. Moreover, normoresponder women were selected to assess the effect of semen parameters on the adequate number of oocytes. Also this inclusion criterion has helped to overcome heterogeneity in terms of infertility factors. All of the oocytes were injected using only the motile and normal-looking spermatozoa in the ICSI procedure.

We observed a reduced fertilization rate with testicular spermatozoa as compared to ejaculated semen. This finding is in contrast to some of prior studies that reported the similar fertilization rate with testicular and ejaculated sperm used in the ICSI procedure (19,20), 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. Our study, in accordance with Lautradi et al. who found a decreased fertilization potential of testicular spermatozoa and concluded that sperm can affect embryogenesis beginning from a very early stage of embryo development (21). Similarly, Demir et al. showed that fertilization rate was significantly lower with testicular spermatozoa coming from patients with non-obstructive azoospermia as compare to ejaculated spermatozoa coming from patients with severe oligospermia in ICSI cycles (22). Also, the fertilization rates with ejaculated spermatozoa increased as semen quality increased from Group 2 to 4. This results indicating that semen parameters can affect the fertilization process. Although ICSI can help sperm penetrate the zona pellucida and reach the cytoplasm of the oocytes, the completion of oocyte activation and the formation of the male and female pronuclei are determined by the intrinsic nature of the sperm and oocytes (23). High rates of DNA fragmentation (7,8), acrosomal defects and epigenetic factors such as sperm specific phospholipase C zeta (24) in poor-quality sperm are all considered to be possible reasons for fertilization failure.

In the ICSI procedure, only a single spermatozoon is required for injection and, individual sperm features such as motility (25,26) and morphology (27,28) appear to be the important causes for the successful outcome. Burrelo et al. found that even normally looking spermatozoa from OAT patients had a high

rate of aneuploidy (14). Vegetti et al. detected that patients who had poor semen parameters associated with higher aneuploidy rate in chromosomes 13, 18, 21, X, and Y in their spermatozoa than controls (10). 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.

On the other hand, the cleavage rate and high-quality embryo rate is highest with testicular spermatozoa. This observation may be explained by that after completing fertilization, different quantities, qualities, morphologies, or sources of sperm have no obvious effects on pronuclei syngamy, embryonic genome formation, or the initiation of mitotic division. Moreover, the development of a human embryo in the early stages is controlled by maternally inherited mRNA. The embryonic genome is activated at approximately the 8-cell stage, and then the paternal influences on embryo development begin to be apparent (29). These factors might explain the discrepancy between the effects of ICSI on cleavage and the formation of high-quality embryos.

Aside from these laboratory results, data on ICSI clinical outcomes such as implantation, miscarriage and delivery rate did not show significant differences. This result indicates that although different sources and different qualities of sperm could influence the fertilization process, cleavage and the formation of high-quality embryos, the potential viability of ICSI embryos after transfer is nearly equal no matter what type of sperm is used. Therefore, it is thought that there is no significant effect on the clinical outcome of ICSI when different qualities and sources of sperm are used for microinjection (30,31). Furthermore, this result also demonstrates that the fertilization process and the formation of high-quality embryos might act as a mechanism for selecting the truly normal sperm.

In Our study, sperm selection was made under 400× magnification, at the periphery of the PVP microdroplet, to guarantee that the sperm selected had a relatively normal form and good motility. However, a normal-looking sperm could still contain serious nuclear malformations and chromosomal aneuploidies (32). There are published reports that when intracytoplasmic morphologically selected sperm injection (IMSI) was performed with sperm selected under a magnification level above 6 000× (33), the rate of sperm nucleus normality was significantly higher. More importantly, with these two methods, ICSI outcomes were significantly improved (33). Therefore, in order to improve the clinical outcome in patients affected by severe male factor infertility, more detailed sperm selection might be considered.

In conclusion, our results demonstrate that neither sperm parameters nor the source of spermatozoa affects live birth rate in normoresponder women when motile/morphologically normal spermatozoa is present.

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