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Does Male Factor Infertility Affect Intracytoplasmic Sperm Injection Pregnancy Results?

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Abstract

Objective: Male infertility has conflicting results in assisted reproductive technology (ART). In this study we aimed to investigate whether male factor infertility affect intracytoplasmic sperm injection (ICSI) outcomes and pregnancy results in ICSI cycles.

Materials and Methods: A total of 1118 ICSI cycles from January 2007 through November 2014 were analyzed retrospectively:596 patients that were treated for male factor infertility, and 522 patients for tubal or unexplained infertility were included in the study. It was investigated whether sperm count has any effect on fertilization and implantation rates. Also the results of embryo quality and pregnancy were compared with the groups.

Results: In both groups there was no difference between the numbers of collected oocytes and mature oocytes. Although there was a higher fertilization rate in the control group, no significant difference was spotted between fertilization failure and embryo developmental arrest in groups ($P=0.07$ vs $P=0.4$, respectively). Between groups, there were no statistically significant differences according to the clinical pregnancy rate per transfer and live birth rates ($P=0.3$ vs $P=0.5$, respectively). The risk of preterm birth in the infertile male group was significantly higher ($P<0.007$). When the patients whom sperm was obtained with surgery were compared with oligozoospermic patients with ejaculated sperm, no significant difference between the implantation rates and clinical pregnancy rates was found ($P=0.1$ vs $P=0.3$, respectively).

Conclusion: The patients who underwent ICSI due to male factor and tubal-unexplained infertility showed no difference according to fertilization, implantation and clinical pregnancy rates. The good quality of sperm retrieved from surgery indicated positive effects on the clinical results.

Keywords: Clinical pregnancy rate, Male factor infertility, Surgical sperm retrieval.

Introduction

In developing countries, one couple out of four has an infertility problem (1). It has been reported that 9% of couples have infertility problems, while 8% of males of reproductive age are being treated for infertility (2). Male infertility equates to 50% of all infertility (3).

With the start of the usage of intracytoplasmic sperm injection (ICSI) in 1992 (4), a new era began in assisted reproductive technology (ART) in which male infertility cases had a chance of being treated (5). Following pregnancies obtained with the use of testicular sperm (6), testicular sperm extraction (TESE), percutaneous epididymal sperm aspiration (PESA) and testicular sperm aspiration (TESA) in the case of severe male infertility, fertilization rates have been reported at around 70-80% while clinical pregnancy rates have been reported at up to 45% (7).

Epigenetic regulation of embryogenesis in sperm involves different roles in gamete fusion and cleavage, which affect male fertility and fertilization (8). In order for fertilization to occur, 90%-95% of the histones in a sperm cell must change sides with protamines (9). Arginine-rich protamines are small proteins synthesized in the later stages of spermatogenesis and are located in the nucleus. Protamine rate variations are associated with many different phenotypes, such as decreased sperm count and function as well as low embryo quality in IVF (in vitro fertilization) (10,11).

In pregnancies obtained by ART, preterm birth, low or very low birth weight, congenital malformations and cerebral palsy are encountered more often. Even though it has been suggested that these results are related to advanced female age and multiple pregnancies, they have also been found to be similar to singleton pregnancy results (12,13). In addition, research has been carried out on micromanipulation techniques, extended culture media and the potential negative effects of the drugs used in ICSI (14)

In this study, it was investigated whether sperm count has any effect on fertilization and implantation rates. Also the

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results of embryo quality and pregnancy were compared with the groups.

Materials and Methods

The medical records of 1876 treatment cycles from January 2007 through November 2014 at the Etlik Zubeyde Hanım Women's Health Teaching and Research Hospital's Center of Assisted Reproduction were reviewed using a computer-based database. The study included patients who had male factor, tubal or unexplained infertility.

The patients who had total progressive motile sperm count (TPMSC) <5 million/mL were accepted as having oligozoospermia, while those with an absence of sperm in the ejaculate were accepted as having azoospermia, which in turn defined what was meant by male factor (the study group). For all patients, spermiogram samples were taken after observing a standard 2–5 days of abstinence. Semen analysis was interpreted according to the World Health Organization (WHO) criteria (15). Patients in the study group were evaluated with a detailed examination and revisal by an urologist. All couples with non-obstructive azoospermia (NOA) were offered genetic screening.

Patients who were treated for tubal or unexplained infertility were accepted into the control group. Tubal infertility was diagnosed with laparoscopy. Unexplained infertility was diagnosed in women with an ovulatory menstrual cycle and patent fallopian tubes, with a spermiogram analysis in a normal range. Fresh cycle and patients with single factor-based infertility were also included in the study.

The demographic characteristics and laboratory results of patients were obtained from our hospital's IVF clinic database.

Ovarian stimulation was performed with gonadotropin-releasing hormone (GnRH) agonist or antagonist protocols. In accordance with the protocol that had been chosen for the treatment, 2-3 days of spontaneous and induced menstruation were started. Pure recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropin was used in the treatment. Gonadotropin doses between 100-450 IU/d was set individually for each patient according to body mass index (BMI) and antral follicle count (AFC). The cycle was monitored with serial serum E2 levels and transvaginal ultrasound measurements. When the average diameter of at least three follicles ≥17 mm were determined, recombinant human chorionic gonadotropin (rhCG) was applied. HCG was given according to the BMI. Between 34 and 36 hours after hCG, oocyte pick-up (OPU) was performed, accompanied by transvaginal ultrasound. After OPU, hyaluronidase containing solution and cumulus oophorus was removed from the oocyte.

The remaining cells were mechanically removed with the help of offensive pipettes denudation. On the scheduled surgical sperm extraction day, the spermatozoa from ejaculate was examined before the operation on the azoospermic patients. If sufficient spermatozoa for ICSI were identified in the ejaculate, surgery was cancelled. For the other patients, a microdissection approach was employed for sperm retrieval (16). All patients underwent ICSI. After the preparation of the spermatozoa, microinjection was performed (17).

Seeing 2 distinct pronuclei and 2 clear polar bodies were accepted as fertilization. Embryonic cleavage was performed every day (18). The scoring of cell number, size and degree of fragmentation were noted. The blastocyst-stage embryo scoring was done according to visible inner cell mass and continuous trophectoderm with sufficient cells. Embryos were classified from grades 1 to 5 (best to worst). Top quality embryos were defined as having four or eight even-sized blastomeres at day 2 or 2, respectively, without multinucleation and containing 20% of cytoplasmic fragments. Good quality embryos were defined by three to five and six to 10 even-sized blastomeres at day 2 or 2, respectively, without multinucleation and containing <20% fragmentation. The good quality embryos were transferred into the uterine cavity on the third or fifth day after the microinjection procedure (19).

The cycle cancellation rate was defined as the number of cycles with an absence of sperm in surgical sperm retrieval, absence of oocyte in OPU, failed fertilization or embryonic developmental arrest divided by the total number of cycles studied. The fertilization rate (FR) was defined only by the proportion of mature oocytes with one polar body resulting in pronucleids. The implantation rate (IR) was defined as the total number of transferred embryos divided by the number of gestational sacs documented by transvaginal ultrasound at five to six weeks of gestation. Clinical pregnancy (CP) was accepted as fetal cardiac activity in the transvaginal ultrasound.

Pregnancy test serum was carried out along with β-hCG test on the ninth day after ET. Vaginal progesterone (Crinone 8% gel, Serono, Istanbul) twice a day or by vaginal progesterone along with 100 mg intramuscular progesterone was given from the embryo transfer to the pregnancy test. Stimulation and laboratory results of patients, pregnancy and perinatal outcomes were assessed.

Statistical Analyses

For the statistical analyses SPSS version 21 were used. The distribution of the variables was checked by using the Kolmogorov-Smirnov and student's *t* test. Comparisons between the groups were tested using the Mann-Whitney U test. The chi-square test and Fisher exact test were used to analyze nominal variables. Continuous variables were expressed as mean + SD. $P < 0.05$ was considered as statistically significant.

Results

According to the criteria, a total of 1118 ICSI cycles were analyzed retrospectively:596 patients in study group and 522 patients in control group were enrolled for the study. While there was no statistically significant difference between BMI and third day baseline hormone results, women's age, male's age and infertility duration were

found to be statistically and significantly lower in study group ($P<0.001$, $P=0.02$, $P<0.001$, respectively). The demographic characteristics of patients and laboratory results are shown in Table 1.

No significant difference was found between the numbers of collected oocytes and mature oocytes. Although there was a higher FR in the control group, no significant difference was spotted between total fertilization failure and embryo developmental arrest in both groups. The laboratory results are given in Table 2. Between the two groups, there were no statistically significant differences between the clinical pregnancy rate per transfer and live birth rates (respectively, $P=0.3$ vs. $P=0.5$). Pregnancy outcomes of the groups are shown in Table 2.

When the patients from whom sperm was obtained with surgery were compared with oligozoospermic patients with ejaculated sperm, it was found that there were no significant difference between the implantation rates and clinical pregnancy rates ($P=0.1$ vs. $P=0.3$, respectively). The data relating to these patients and the results of stimulation are presented in Table 3.

Discussion

This study primarily examined the outcomes of patients who underwent ICSI due to male infertility. According to our results there was no difference between clinical pregnancy results and live birth rates but the risk of preterm birth in the infertile male group was significantly higher ($P<0.001$).

In fertilization, the centromere comes from the male gamete cells. In the first cycle of the zygote meiosis, the paternal origin of the centromere is copied (20). Centrosome morphology and function affect the results of early embryogenesis and is able to predict the results of ART (21). In a study that examined 116 ICSI cycles on 17 patients with cryptospermia, there were no significant difference between the FR. In this study, when surgically obtained sperm and ejaculate sperm were compared, higher IR, higher pregnancy rates and higher baby take-home rates were shown (22). It can be argued that sperm was damaged during its passage through the male genital system, which negatively affected the success of ART for the ejaculate sperm (23). Greco et al have suggested that better quality embryos may be obtained along with the spermatozoa obtained by TESE (24). In the current study, more cycle cancellations were performed on the study group since sperm could not be found during surgical sperm retrieval. As interesting no statistically significant difference between total fertilization failure and embryo developmental arrest. As a result of getting good quality sperm from surgery, FR and IR and live birth rate were similar between the groups. Zheng and colleagues compared obtained oocytes from the same mother in the same cycle, IVF with severe male infertility (husbands) and donor sperm. Although severe male infertility resulted in a lower FR, the rate of good quality embryos was not different (25).

There are published reports that state that perinatal outcomes of ART pregnancies are worse than for spontaneous pregnancies. Preterm birth, low birth weight and premature rupture of membranes are more common, even in singleton ART pregnancies. It has also been stated that corresponding birth weights did not change as a result of the cause of infertility (26). Wang et al, who examined the results of 17726 newborns resulting from 15035 ART cycles, found that preterm birth risk was higher in the female factor infertility group than the male factor infertility group. They explained that this was due to the delay in fetal growth caused by uterus-induced uteroplacental defects, stating that normal fetal growth should happen in couples with only male infertility providing there was no uterine problem (27). However, in infertile male patients, sperm increases the likeliness of DNA damage. Term pregnancy chance is higher in normal embryonic genome formation with normal DNA-structured sperm (28). In our study, we found that the risk of preterm birth was higher in the group with male infertility. This could be caused by the sperm's DNA damage that exists in the infertile male group.

Mäkinen et al, in their study examining the results of 1079 newborns after IVF / ICSI, showed that BMI, fetal sex and parity had an effect on fetal weight, whereas the embryo culture period had an effect as an independent factor, while the cause of infertility and maternal age showed

Table 1. Patient characteristics and hormone levels of groups

	Study Group (Male Factor)	Control Group (Tubal Factor/Unexplained Infertility)	*P* Value
No. of patients	596	522	
Female age, years	29.2±4.8	31.15 ± 4.71	<0.001
Male age, years	33.1 ± 5.7	33.9 ± 4.8	0.02
Duration of infertility (month)	68.9±51.7	77.5 ± 48.9	<0.001
BMI (kg/m^2)	6.5±25.3	8.3±24.5	0.07
Day 3 FSH (IU/mL)	7.1±2.7	7.3±3.6	0.4
Day 3 LH (IU/mL)	5.1±3.1	5.4± 3.6	0.7
Day 3 E2 (pg/mL)	45.9 ± 27.8	49.2 ±59.6	0.8

Abbreviations: OPU, oocyte pick-up; AFC, Antral follicle count; BMI= body mass index; FSH= follicle stimulating hormone; LH= luteinizing hormone; E2= estradiol. Data are presented as % or mean ± standard deviation.

Table 2. Laboratory Results and Pregnancy Outcomes of Male Factor (Study Group) and Tubal Factor or Unexplained Infertility (Control Group) Groups.

	Study Group	Control Group	*P* Value
No. of patients	596	522	
AFC	13.7± 6.5	12.7±6.5	0.01
Mature oocyte count	8.5± 6.1	9.1 ±5.6	0.01
FR (%)	50.8	51.2	0.4
Cycle cancellation rate following OPU (%)			
Total fertilization failure	6.1	5.9	0.07
Embryo developmental arrest	6.2	5.1	0.4
Surgical sperm negative	8.6	-	-
Implantation rate/ET (%)	35.1	37	0.6
Clinical pregnancy rate/ET (%)	26.2	30.8	0.3
Live births/ET (%)	24.5	21.4	0.3
Mean gestational weeks	36.94±3.76	37.45±2.62	<0.001
Mean birth weight (g)	2810.6±758.6	2937.9±717.1	0.3

Abbreviations: OPU, oocyte pick-up; ET, embryo transfer; FR, fertilization rate; AFC, Antral follicle count.
Data are presented as % or mean ± standard deviation.

Table 3. Laboratory Results and Pregnancy Outcomes of Male Factor Infertility Group According to Sperm Method Used

	Surgical Extraction	Ejaculated	*P* Value
No. of patients	92	369	
AFC	14.1± 6.7	13.7±6.4	0.4
No. of mature oocyte	6.5± 6.1	9.7 ±5.3	0.02
Fertilization rate (%)	43.5	51.8	0.07
Cycle cancellation rate following OPU (%)			
Total fertilization failure	7.8	3.2	0.07
Embryo developmental arrest	1.8	5.9	0.4
Implantation rate/ET (%)	34.2	38	0.1
Clinical pregnancy rate/ET (%)	30.5	35	0.3
Live births/ET (%)	20.1	25.4	0.1
Mean gestational weeks	38.1±1.4	38±3.6	0.01
Mean birth weight (g)	3140±481	2925.±728.5	0.01

Abbreviations: OPU, oocyte pick-up; ET, embryo transfer; FR, fertilization rate; AFC, Antral follicle count.
Data are presented as % or mean ± standard deviation.

no effect (29). In addition, the effect of embryo culture media on fetal weight as an independent factor was stated. While their mechanism is not clearly understood, embryos undergo epigenetic modification during the preimplantation period;in the meantime, any kind of developing failure might have an effect on fetal growth (30).

In summary, the results of the patients who underwent ICSI due to male factor and tubal-unexplained infertility showed no difference according to fertilization, implantation and clinical pregnancy rates. The good quality of sperm retrieved from surgery indicated positive effects on the clinical results.

Ethical Issues

The ethics committe approval of the study was obtained from our hospitals' editorial board as the number 190/2 in February 2015.

Conflict of Interests

The authors declare no conflict of interests.

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