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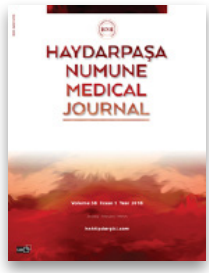

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ORIGINAL ARTICLE

Comparison of the ICSI Results From Sperm Obtained by m-TESE at Three Different Periods in Patients with Azoospermia: Retrospective Study

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Abstract

Introduction: The absence of any spermatozoa in the ejaculate is called azoospermia, and it is seen in 1% of males, resulting in 10%–15% of infertility problems. Azoospermia may be due to obstructive (OA) and non-obstructive (NOA) causes. Microscopic testicular sperm extraction (m-TESE) is often applied today to obtain sperm from patients with azoospermia, and healthy pregnancies can be obtained by performing intracytoplasmic sperm injection (ICSI) with the testicular sperm obtained. In this study, we aimed to compare the ICSI cycle results from fresh sperm obtained by m-TESE on the day of oocyte collection or the day before, and frozen m-TESE sperm.

Methods: Between January 2008 and April 2017, patients who underwent m-TESE in the In Vitro Fertilization Unit of our hospital were retrospectively analyzed. A total of 342 patients with azoospermia (117 OA and 225 NOA cases) with regular follow-up were included in the study. The first group underwent m-TESE on the same day, the second group underwent m-TESE 24 hours before, and in the third group, the sperm was frozen after m-TESE.

Results: A total of 235 patients, 117 patients with OA (%100) and 118 NOA patients (52.4%), had motile spermatozoa. In 150 patients (85 OA, 65 NOA), the sperm were used the same day, and in 51 patients (24 OA, 27 NOA), one day later. Sperm in 34 patients (8 OA, 26 NOA) was frozen for later use. When ICSI results were evaluated in these groups, the number and rates of fertilization in the OA group were 56 (65.8%), 16 (66.6%), and 5 (62.5%); clinical pregnancy numbers and rates were 30 (35.2%), 9 (37.5%), and 3 (37.5%); and the live birth numbers and rates were 26 (30.5%), 7 (29.1%), and 2 (25.0%), respectively. In the NOA group, fertilization numbers and rates were 46 (70.7%), 19 (70.3%), and 18 (69.2%); clinical pregnancy numbers and rates were 24 (36.9%), 10 (37.0%), and 10 (38.4%); and the live birth numbers and rates were 20 (30.7%), 8 (29.6%), and 8 (30.7%), respectively.

Discussion and Conclusion: In the IVF-ICSI applications, there was no difference in terms of fertilization, pregnancy, and live birth rates between the use of frozen sperm or m-TESE on the oocyte collection day or 1 day before.

Keywords: Azoospermia; infertility; m-TESE; pregnancy; spermatozoa.

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Azoospermia means the absence of any spermatozoa in the ejaculate, and it affects 1% of male population and 10%–15% of patients who are admitted with the indication of infertility [1, 2]. Non-obstructive azoospermia (NOA) is defined as the absence of spermatozoa in the ejaculate because of the presence of very few mature testicular spermatozoa or failure of their production. However, in obstructive azoospermia (OA), testicular spermatozoa are produced, but ejaculatory ducts are occluded. In NOA cases, spermatozoa are retrieved using the conventional testicular sperm extraction (TESE) or microscopic sperm extraction (m-TESE) methods. The harvested spermatozoa may be used in the intracytoplasmic sperm injection (ICSI) method to obtain healthy pregnancies. ICSI was first applied by Palermo in the year 1992, and the first pregnancy was realized [3].

It has been shown that increasing the number of biopsies taken in conventional TESE operations increases the picking up of motile spermatozoa [4]. However, taking too many tissue samples can increase the risk of intratesticular hematoma, infection, and fibrosis, and it may lead to a decrease in serum testosterone levels [5, 6]. m-TESE was first defined by Schlegel in 1998 [7]. M-TESE is frequently used in patients with azoospermia because of the high rates of sperm retrieval and low complication rates. In the ICSI process, previously obtained and frozen sperm can be used. Or, m-TESE may be performed a day before the oocyte collection day or on the oocyte collection day [8]. It has been reported that the best fertilization rates are obtained with the sperm found in m-TESE before the HCG administration [9].

The aim of this retrospective study was to compare the ICSI results with sperm obtained from the m-TESE procedure and were obtained from frozen sperm, oocyte collection day, or 1 day before the oocyte collection day.

Materials and Methods

A total of 342 male patients who were admitted to our center due to the inability to conceive children with their partners were evaluated retrospectively by deciding to have m-TESE. OA and NOA were determined from the result of clinical evaluation and laboratory tests. In the OA group, the mean duration of infertility was 28.2±7.8 months, the age of men was 34.2±5.4 years, and the age of women was 30.3±2.9 years. In the NOA group, the mean duration of infertility was 30.3±6.5 months, the age of men was 35.3±3.4 years, and the age of women was 30.6±3.3 years (Table 1).

When the diagnosis of azoospermia was made, a detailed medical and reproductive history of each patient was obtained, and physical examinations were performed. Testes sizes were measured by an orchidometer, and vascularity and echogenicity were evaluated by color doppler ultrasonography. The serum follicle stimulated hormone (FSH), LH , and testosterone and prolactin were measured. The semen analysis was performed three times in total, two times for 15 days and the 3rd time during the m-TESE procedure. The m-TESE procedure was performed on the day of oocyte collection, one day before the day of oocyte collection or before the induction of ovulation. The sperm in the 3rd group patients was stored frozen.

Table 1. Comparative findings of patients

	OA patients (n=117)	NOA patients (n=225)	p
Male age (year)	34.2±5.4	35.3±3.4	p>0.05
Female age (year)	30.3±2.9	30.6±3.3	p>0.05
duration of infertility (month)	28.2±7.8	30.3±6.5	p>0.05
testicular volumes (ml)	12.5±2.6	9.8±3.4	p>0.05
FSH value (mIU/ml)	11.7±3.7	13.7±5.4	p>0.05
Motile sperm retrieval rate	117 (%100)	118 (%52.4)	p>0.05

OA: obstructive azoospermia; NOA: non-obstructive azoospermia; FSH: follicle stimulated hormone.

The m-TESE procedure was started with a scrotal midline incision under general anesthesia. After passing through the scrotal layers, under optical magnification (8X), the tunica albuginea was incised transversely for 2–3 cm dependent on the testicular volume. Then, the testicular parenchyma was examined under the 20X magnification. Opaque-white dilated and rotund seminiferous tubuli were identified, picked up with a microsurgical pincette, and dissected. If normal tubuli could not be detected, then the search was continued further. In cases with similar tubuli, random samples were extracted. Extracted tissue samples were placed in Petri dishes containing a modified Eagle's MEM solution with HEPES, and they were delivered to the embryologists present in the operating suit. The seminal plasma and contents of tubuli dissected and disintegrated by the embryologists were taken out. Under the microscope, at magnifications of 200x and 400x, spermatozoa in fragmented tubuli were sought. If spermatozoa were found in the material sent, then the micro-TESE procedure was terminated, but if the sperm could not be found under the microscope, the other testis was opened with the same method and the material was continued to be sent.

In patients in Group 1, the sperm was used by the embryologist in the embryology laboratory on the same day.

In patients in Group 2, the spermatozoa were incubated in the G-IVF-Plus medium (Vitrolife) until the following day

and used the next day.

From the patients in Group 3, the obtained sperm was frozen for later use. The freezing was diluted 1:1 with a sperm freezing medium (FertiPro N.V.) to 1.8 ml of cryovials (Nunc). The samples were then slowly cooled at room temperature for 30 minutes and stored at −196°C.

For thawing, frozen tissue samples were kept in a 37°C water bath for 3–5 minutes. Samples were then washed with G MOPS Plus Medim (Vitrolife) and kept ready at 37°C for use with ICSI.

The spouses of patients with azoospermia had a normal uterine cavity, and women older than 40 were excluded from the study. A controlled ovarian stimulation was performed until at least two dominant follicles reached a diameter of 17 mm using the GnRH-antagonist and recombinant FSH (Gonal-F, Serono). The oocyte retrieval was performed with transvaginal ultrasound 35 hours after the hCG administration. The collected eggs were cultured in the G-IVF-Plus (Vitrolife) medium with 10% HSA at 37°C, 6% CO_2. Two hours after the incubation, the cumulus–corona complex, hyaluronidase (type VIII, Sigma) was pipetted.

The fertilization was performed 16–18 hours after ICSI, and cleavage rates were controlled 48–72 hours after the oocyte pick-up. The embryo transfer was performed on the 3rd day after the oocyte pick-up. At least 1 embryo was transferred to all spouses. Pregnancy evaluation was made by measuring the hCG levels in blood 12 days after embryo transfer, and the b-hCG level was considered as positive if above 30 mIU/mL. A decrease in the b-hCG level after a positive test was accepted as biochemical pregnancy. At the 7th week ultrasound, a gestational sac and fetal heart beat were accepted as clinical pregnancy. As a result of interviews with the hospital or couples, the number of live births was determined.

Statistical Analysis

For statistical analysis, the IBM Statistical Package for Social Sciences (IBM SPSS Statistics; Armonk, NY, ABD) Statistics Software 22 program was used. A P-value <0.05 was considered statistically significant.

Results

There were no significant differences in the main characteristics of all three groups, such as female/male age, infertility etiology, male FSH values, female initial FSH values, and ovulation induction protocols.

Motile spermatozoa were obtained from a total of 235 patients (118%) and 117 (52.4%) of 117 OA patients (Table 1).

In 150 patients (85 OA, 65 NOA), the sperm was used on the same day, and in 51 patients (24 OA, 27 NOA), one day later. In 34 patients (8 OA, 26 NOA), the spermatozoa were frozen for later use.

When the ICSI results were evaluated in these groups, fertilization numbers and rates in the OA group were 56 (65.8%), 16 (66.6%), and 5 (62.5%); clinical pregnancy numbers and rates were 30 (35.2%), (37.5%), and 3 (37.5%); and live birth numbers and rates were 26 (30.5%), 7 (29.1%), and 2 (25.0%). In the NOA group, fertilization numbers and rates were 46 (70.7%), 19 (70.3%), and 18 (69.2%); clinical pregnancy numbers and rates were 24 (36.9%), 10 (37.0%), and 10 (38.4%); and live birth numbers and rates were 20 (30.7%), 8 (29.6%), and 8 (30.7%), respectively; and no statistical difference was found between the groups (Table 2).

There were no major or minor malformations in the children.

Discussion

In this study, the ICSI results compared the sperm obtained by m-TESE 1 day before the oocyte collection, on the day of oocyte collection, and frozen m-TESE sperm. A total of 235 ICSI cycles with motile sperm were evaluated. The fertilization, implantation, clinical pregnancy rates, and low and live birth rates were similar between the three groups. In the light of this information, the m-TESE procedure was performed 1 day before the egg collection, or the use of frozen sperm was done before; M-TESE can provide many practical and medical advantages over the same-day egg collection. Approximately, 20%–30% of patients with azoospermia cannot be found in all efforts [10]. For this reason, if m-TESE is done before and sperm cannot be found, it can

Table 2. ICSI results by groups

	1. Group (n=150)		2. Group (n=51)		3. Group (n=34)		
	OA patients (n=85)	NOA patients (n=65)	OA patients (n=24)	NOA patients (n=27)	OA patients (n=8)	NOA patients (n=26)	p
Fertilization rate (%)	56 (65.8)	46 (70.7)	16 (66.6)	19 (70.3)	5 (62.5)	18 (69.2)	p>0.05
Clinical pregnancy rate (%)	30 (35.2)	24 (36.9)	9 (37.5)	10 (37.0)	3 (37.5)	10 (38.4)	p>0.05
Live birth rate (%)	26 (30.5)	20 (30.7)	7 (29.1)	8 (29.6)	2 (25.0)	8 (30.7)	p>0.05

OA: obstructive azoospermia; NOA: non-obstructive azoospermia; ICSI: intracytoplasmic sperm injection.

be avoided to prevent unnecessary ovulation induction of women, possible complications, and unnecessary cost. Using frozen sperm or m-TESE a day before the oocyte removal may reduce the stress for couples. Performing m-TESE prior to oocyte retrieval may provide more time for physicians and laboratory staff spends looking for spermatozoa.

When we found live sperm in OA and NOA patients, it was determined that there was no difference between the fertilization, pregnancy, and live birth rates in all three groups. Fertilization rates (52.9%, 55.6%) and pregnancy rates (33.2%, 30%) in Kanto et al. [11] were found to be similar to our study in ICSI patients with NOA and OA.

In the other two studies, it was reported that there was no difference between fertilization, pregnancy, and live birth rates in the ICSI cycles with fresh and frozen sperm obtained from NOA and OA patients [12, 13].

In our study, only patients with motile sperm were included as NOA and OA patients, and similar results were obtained. In the study by Park et al., [14] higher fertilization and pregnancy rates were reported in ICSI cycles in which motile sperm was used compared to non-motile sperm ICSI cycles.

In some recent studies, successful results have been reported in ICSI cycles using round spermatids [15]. Although successful results have been obtained, it is seen that live birth rates are much lower than in the motile sperm use.

Another situation feared in IVF-ICSI operations is the risk of child malformations. In studies conducted on this subject, the rates of malformations in ICSI cycles with epididymal, testicular, or frozen sperm were found to be similar with ICSI cycles with ejaculate sperm and with similar rates compared to normal pregnancies [11, 16]. In our study, no minor or major malformation was detected in born infants.

Conclusion

The use of the sperm obtained during the m-TESE procedure on the same day or a day later and its freezing and use in later periods does not affect the success of the ICSI procedure in terms of fertilization, clinical pregnancy, and low and live birth rates.

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