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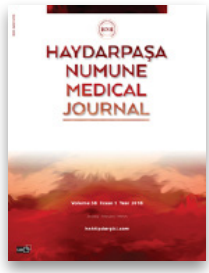

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ORIGINAL ARTICLE

Comparison of ICSI Results from Sperms Obtained by m-TESE at Three Different Periods in Azoospermic Patients: Retrospective Study

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Abstract

Introduction: The absence of any sperm in the ejaculate is called azoospermia and it is seen in 1% of males and 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 azoospermic patients, 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 sperms obtained by m-TESE on the day of oocyte collection or the day before and frozen m-Tese sperms.

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

Results: A total of 235 patients, 117 patients with OA (%100) and 118 of NOA patients(%52,4) had motile spermatozoa. In 150 patients (85 OA, 65 NOA), sperm were used the same day, in 51 patients (24 OA, 27 NOA) one day later. Sperms in 34 patients (8 OA, 26 NOA) were frozen for later use. When ICSI results were evaluated in these groups, the number and rates of fertilization in 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%), 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%), live birth numbers and rates were 20 (30.7%), 8 (29.6%) and 8 (30.7%).

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

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

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Azoospermia means absence of any spermatozoa in the ejaculate, and it is found in 1% of male population, and 10-15% of patients who were 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 cases with NOA, spermatozoa are retrieved using 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 firstly 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 motile spermatozoa [4]. However, taking too many tissue samples can increase the risk of intratesticular hematoma, infection and fibrosis, and 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 azoospermic patients 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 1 day before oocyte collection day or on oocyte collection day [8]. It has been reported that the best fertilization rates are obtained with the sperm found in m-TESE before HCG administration [9].

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

Materials and Methods

A total of 342 male patients who were admitted to our center due to lack of children were evaluated retrospectively by deciding to have m-TESE by determining that obstructive and non-obstructive azoospermia was 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, the women were 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, detailed medical and reproductive history of each patient was obtained and physical examinations were performed. Testis sizes were measured by orchidometer and vascularity and echogenicity were evaluated by color doppler ultrasonography. Serum FSH, LH and testosterone and prolactin were measured. Semen analysis was performed 3 times totaly, 2 times for 15 days and third time on 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 were 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
Motil sperm retrieval rate	117 (%100)	118 (%52.4)	p>0.05

OA: Obstructive Azoospermia; NOA: Non-Obstructive Azoospermia; FSH: Follicle Stimulated Hormone.

m-TESE procedure was started with scrotal midline incision under general anesthesia, After passing through scrotal layers, under optical magnification (8X) tunica albuginea was incised transversely for 2-3 cm dependent on the testicular volume, Then testicular parenchyma was examined under 20X magnification. Opaque-white dilated, and rotund seminiferous tubuli were identified, picked up with a microsurgical pincette, and dissected. If normal tubuli cannot 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 modified Eagle's MEM solution with HEPES, and delivered to the embryologists present in the operating suit. Seminal plasma, and contents of tubuli dissected, and disintegrated by the embryologists were taken out. Under microscope, at magnification of 200x, and 400x, spermatozoa in fragmented tubuli were sought. If spermatozoa were found in the material sent, then 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 the patients in Group 1, the sperm were used by the embryologist in the embryology laboratory for 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 were frozen for later use. The freezing was diluted 1:1 with 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 the sperm were kept ready at 37° C for use with ICSI.

The wives of azopermic patients had normal uterine cavity and those with a age above 40 were excluded from the study. Controlled ovarian stimulation was performed until at least 2 dominant follicles reached a diameter of 17 mm using the GnRH-antagonist and recombinant FSH (Gonal-F, Serono). Oocyte retrieval was performed with transvaginal ultrasound 35 hours after hCG administration. The collected eggs were cultured in G-IVF-Plus (Vitrolife) medium with 10% HSA at 37° C, 6% CO 2. 2 hours after incubation, the cumulus-corona complex, hyaluronidase (type VIII, Sigma) was pipetted.

Fertilization was performed 16-18 hours after ICSI and cleavage rates were controlled 48-72 hours after oocyte pick-up. Embryo transfer was performed on the 3rd day after oocyte pick-up. At least 1 embryo was transferred to all spouses. Pregnancy evaluation was made by measuring hCG levels in blood 12 days after embryo transfer and b-hCG level was considered as positive above 30 mIU/mL. A decrease in b-hCG level after a positive test was accepted as biochemical pregnancy. At the 7th week ultrasound, it was accepted as gestational sac and fetal heart beat 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 IBM Statistical Package for Social Sciences (IBM SPSS Statistics; Armonk, NY, ABD) Statistics Software 22 program was used. P<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.

Motil 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), sperm were used the same day, 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 ICSI results were evaluated in these groups, fertilization numbers and rates in 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%), 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%), live birth numbers and rates were 20 (30.7%), 8 (29.6%) and 8 (30.7%), and no statistical difference was found between the groups (Table 2). There was no major or minor malformation in the children.

Discussion

In this study, the ICSI results were compared which sperm obtained by m-TESE 1 day before of oocyte collection or on the day of oocyte collection and with frozen m-TESE sperm. A total of 235 ICSI cycles with motile sperm were evaluated. Fertilization, implantation, clinical pregnancy rates, 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 of 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 be avoided to prevent unnecessary ovula-

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 azospermia; ICSI: Intracytoplasmic sperm injection.

tion induction of women, possible complications and cost prevention. Using frozen frozen sperm or m-TESE 1 day before oocyte removal may reduce the stress of pairs. Performing m-TESE prior to oocyte retrieval may contribute to more time spent looking for spermatozoa in terms of physicians and laboratory staff.

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

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

In our study, only patients with motile sperm were included in NOA and OA patients and similar results were obtained. Park et al.[14] In his study, 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 are obtained, it is seen that live birth rates are much lower than motile sperm use.

Another feared situation in IVF-ICSI operations is the malformation risk in the child. 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, minor or major malformation was not detected in infants born.

Conclusion

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

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References

1. Willott GM: Frequency of azoospermia. Forensic Sci Int 1982; 20:9-13.
2. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. J Urol 1989; 142:62-7.
3. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of a single spermatozoon into an oocyte. Lancet 1992; 340:17-8.
4. Amer M, Haggar SE, Moustafa T, Abd El-Naser T, Zohdy W. Testicular sperm extraction: impact of testicular histology on outcome, number of biopsies to be performed and optimal time for repetition. Hum Reprod 1999; 14:3030-45.
5. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. Hum Reprod 1997; 12:1688-92.
6. Takada S, Tsujimura A, Ueda T. Matsuoka Y, Takao T, Miyagawa Y, Koga M, Takeyama M, Okamoto Y, Matsumiya K, Fujioka H, Nonomura N, Okuyama A. Androgen decline in patients with nonobstructive azoospermia after microdissection testicular sperm extraction. Urology 2008; 72:114-8.
7. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod 1999; 14:131-5.
8. Levran D, Ginath S, Fahri J, Nahum H, Glezerman M, Weissman A. Timing of testicular sperm retrieval procedures and in vitro fertilizationintracytoplasmic sperm injection outcome. Fertil Steril 2001; 76:380–3.
9. Urman B, Alatas C, Aksoy S, Nuhoglu A, Sertac A, Balaban B. Performingtesticular or epididymal sperm retrieval prior to the injection of hCG. J AsistReprod Genet 1998; 15:125-8.
10. Vehement G, Verna eve V, van Landry L, Tournaye H, Devroey P, vanSteirteghem A. Should diagnostic testicular sperm retrieval followed by cryopreservation for later ICSI be the procedure of choice for all patients withnon-obstructive azoospermia? Hum Reprod 2004; 19:2822–30.
11. Kanto S, Sugawara J, Masuda H, Sasano H, Arai Y, Kyono K. Fresh motile testicular sperm retrieved from nonobstructive azoospermic patients has the same potential to achieve fertilization and pregnancy via ICSI as sperm retrieved from obstructive azoospermic patients. Fertil Steril 2008; 90:2010.e5–7.
12. Haberman H, Seo R, Cieslak J, Niederberger C, Prins GS, Ross L. In vitro fertilization outcomes after intracytoplasmic sperm injection with fresh or frozenthawed testicular spermatozoa. 2000; 73:955-60.
13. Palermo GD, Schlegel PN, Hariprashad JJ, Ergun B, Mielnik A, Zaninovic N,et al. Fertilization and pregnancy outcome with intracytoplasmic sperm injectionfor azoospermic men. Hum Reprod 1999; 14:741-8.
14. Park YS, Lee SH, Song SJ, Jun JH, Kong MK, Seo JT. Influence of motility onthe outcome of in vitro fertilization/intracytoplasmic sperm injection with fresh vs. frozen testicular sperm from men with obstructive azoospermia. Fertil Steril 2003; 80:526–30.
15. Tournaye H. Update on surgical sperm recovery-the European view. HumFertil (Camb) 2010; 13:242-6.
16. Fedder J, Loft A, Parner ET, Rasmussen S, Pinborg A. Neonatal outcome andcongenital malformations in children born after ICSI with testicular or epididymalsperm: a controlled national cohort study.HumReprod 2013; 28:230-40.