



# Results of Intracytoplasmic sperm injection (ICSI) performed with sperm retrieved by microscopic testicular sperm extraction (m-TESE) in azoospermic patients

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## ABSTRACT

**Objective:** The absence of any sperm in the ejaculate is called azoospermia and it is detected in 1% of males and 10-15% of those with infertility complaints. Azoospermia may be due to obstructive (OA) and non-obstructive (NOA) causes. Today, healthy pregnancies can be achieved in azoospermic patients by intracytoplasmic sperm injection (ICSI) performed using sperm retrieved from microscopic testicular sperm extraction (m-TESE). In this study, we examined the sperm retrieval rates with m-TESE in azoospermic patients, the results of ICSI in OA and NOA patients with sperm and the underlying testicular pathologies in patients without sperm.

**Material and methods:** Patients who underwent m-TESE at IVF unit of our hospital between January 2005 and April 2017 were retrospectively reviewed. A total of 342 azoospermic patients (117 OA and 225 NOA cases) with regular follow-up were included in the study. In these cases, sperm retrieval and clinical pregnancy rates after ICSI were compared.

**Results:** Mean duration of infertility was 28.2±7.8 months in the OA group, 34.2±5.4 years in patients, 30.3±2.9 years in spouses. In the NOA group, the mean duration of infertility was 30.3±6.5 months, the mean age of the patients was 35.3±3.4, and the mean age of the spouses was 30.6±3.3 years. In 68.9% of the cases, the therapeutic indication was male factor alone, while 31.1% had female factor infertility. In the OA group, 68.9% of the cases had only male factor infertility, while 31.1% of them had also female factor infertility. In the NOA group, 65.4% of the cases had only male factor infertility, and female factor was found in 34.6% of the cases. OA patients had a mean serum FSH level of 11.7±3.7 mIU/mL and mean testicular volume of 12.5±2.6 mL, NOA patients had a mean serum FSH level of 13.7±5.4 mIU/mL and mean testicular volume of 9.8±3.4 mL. In the m-TESE procedure, motile sperm was found in all of the OA patients and in 52.4% (118/225) of the NOA patients. Clinical pregnancy rate in the OA group was 29.9% (35/117) and live birth rate was 25.6% (30/117). In the NOA group, the clinical pregnancy rate was 27.1% (32/118) and the live birth rate was 23.7% (27/118). Histopathologic evaluation was made in 107 cases in the NOA group with no testicular sperm, revealing that 59 cases with germ-cell aplasia (sertoli-cell only syndrome), 42 cases with maturation arrest, and 6 cases with hypospermatogenesis. Postoperative hematoma developed in 3 of m-TESE cases and subsided with conservative treatment.

**Conclusion:** If motile sperm is retrieved with m-TESE application in azoospermic patients, pregnancy resulting in one live birth in about 4 couples who undergo ICSI application can be achieved. In the presence of motile sperm, live birth rates are similar between OA and NOA case with very low complication rates.

**Keywords:** Azoospermia; histopathology; infertility; m-TESE; pregnancy.

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## Introduction

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 infertil-

ity.<sup>[1,2]</sup> 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.<sup>[3]</sup>

It has been shown that increasing the number of biopsy specimens obtained with conventional TESE procedures also increases the chances of picking up motile spermatozoa.<sup>[4]</sup> However excess number of tissue samples retrieved may increase the risk of intratesticular hematoma, infection, and fibrosis, and lead to decrease in serum testosterone levels.<sup>[5,6]</sup> m-TESE was firstly defined by Schlegel in the year 1998.<sup>[5-7]</sup> Nowadays, thanks to higher sperm retrieval, and lower complication rates, m-TESE has been used much more frequently.

In our study, the patients who were referred to our clinic because of azoospermic infertility (obstructive, and non-obstructive) and undergone m-TESE procedure were retrospectively evaluated. Sperm retrieval, clinical pregnancy, and live birth rates, and also histopathological diagnosis in azoospermic patients were analyzed.

## Material and methods

A total of 342 patients who referred to our center with the indication of infertility, and subjected to clinical, and biochemical evaluation and scheduled for m-TESE procedure with the diagnosis of obstructive (n=117), and non-obstructive (n=225) azoospermia were retrospectively evaluated. The patients in the OA group had previously undergone TESE procedures once (n=24) or twice (n=13), and spermatozoa had been retrieved. In the NOA group the patients had undergone m-TESE procedures once (n=46), and twice (n=21), and spermatozoa were retrieved. In the NOA group previous m-TESE procedures performed in 19 patients could not reveal the presence of any spermatozoa.

In the OA group mean duration of infertility was  $28.2 \pm 7.8$  months, mean age of the patients, and spouses were  $34.2 \pm 5.4$ , and  $30.3 \pm 2.9$  years, respectively. In the NOA group mean duration of infertility was  $30.3 \pm 6.5$  months, and mean age of the patients, and spouses were  $35.3 \pm 3.4$ , and  $30.6 \pm 3.3$  years, respectively.

While making the diagnosis of azoospermia, detailed medical, and reproductive case histories of the patients were obtained. Their physical examinations were performed, and their FSH

levels were measured. Patients who received hormonal treatment, and diagnosis of hypogonadotropic hypogonadism were excluded from the study.

Genetic counselling was provided for the patients before m-TESE. In cases with AZF deletions, especially in cases with AZFa, and AZFb deletions, decrease in sperm retrieval rates was told to them, and these patients did not undergo routine genetic tests. Patients diagnosed as obstructive azoospermia were classified according to relevant etiologies. The patients previously experienced inguinal surgery (n=45), infection (n=27), vasectomy (n=15), while in 30 patients any etiology could not be detected.

m-TESE procedures were performed on the day of oocyte collection in women or the day before. Before m-TESE procedure, seminal samples of the patients were collected for the last time, and their azoospermic state was confirmed. The ejaculate volumes of the patients were not recorded, and patients whose ejaculates contained spermatozoa were excluded from the study. Patients in whom m-TESE procedures revealed the presence of only motile/mature spermatozoa were included in the study. Patients having immature or immotile spermatozoa were not included in the study.

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 can 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 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. However if no spermatozoon was found, then the contralateral testis was dissected, and the extracted material was also given to the embryologists.

Histopathology of testicular material was classified as hypospermatogenesis (spermatogenesis was detected at every stage,

but quantitative inadequacy was observed); maturation arrest (sperm maturation did not progress beyond spermatogonium, spermatocyte or spermatid levels) or germ-cell aplasia (sertoli cell-only syndrome, absence of any germ cell in seminiferous tubuli).

This retrospective study was conducted in compliance with ethical principles defined in Helsinki Declaration. All study participants preoperatively read, and signed enlightened consent forms.

### 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 accepted as the level of statistical significance.

### Results

In 68.9% of the patients in the OA group the indication for m-TESE was only male factor infertility, while in 31.1% of them also female factor infertility was detected. In 65.4% of the patients in the NOA group only male factor infertility was detected, while in 34.6% of them also female factor infertility was revealed. In 52.4% of the patients with the diagnosis of NOA, m-TESE procedure detected motile spermatozoa, while motile spermatozoa were retrieved from all ( $n=117$ ) OA patients ( $p < 0.05$ ). Mean serum FSH values, and testicular volumes of OA, and NOA patients were  $11.7 \pm 3.7$  mIU/mL vs.  $13.7 \pm 5.4$  mIU/mL, and  $12.5 \pm 2.6$  mL vs.  $9.8 \pm 3.4$  mL, respectively. FSH levels did not differ statistically significantly between groups ( $p > 0.05$ ). In NOA patients testicular volumes were relatively lower, but still without any statistically significant intergroup difference ( $p > 0.05$ ).

Clinical pregnancy, and live birth rates in OA, and NOA patients who underwent ICSI procedure were 29.9% (35/117) vs. 27.1% (32/118), and 25.6% (30/117) vs. 23.7% (27/118), respectively ( $p > 0.05$ ) (Table 1).

Histopathological examination results of testicular tissue samples obtained from 107 patients in the NOA group who underwent m-TESE without any sperm retrieval revealed the presence of germ-cell aplasia in 59, maturation arrest in 42, and hypospermatogenesis in 6 cases (Table 2).

Postoperative hematoma developed in 3 patients who underwent m-TESE which regressed during follow-up period. Mean procedural time was  $55.2 \pm 25.5$  min, and any anesthetic complication was not seen.

**Table 1. Comparative data of the patients**

	OA patients (n=117)	NOA patients (n=225)	p
Male partner, age (years)	34.2±5.4	35.3±3.4	>0.05
Female partner (years)	30.3±2.9	30.6±3.3	>0.05
Duration of infertility (months)	28.2±7.8	30.3±6.5	>0.05
Testicular volume (mL)	12.5±2.6	9.8±3.4	>0.05
FSH values (mIU/mL)	11.7±3.7	13.7±5.4	>0.05
Motile sperm retrieval rate	117 (100%)	118 (52.4%)	<0.05
Clinical pregnancy rate after ICSI	35 (29.9%)	32 (27.1%)	>0.05
Live birth rate after ICSI	30 (25.6%)	27 (23.7%)	>0.05

OA: obstructive azoospermia; NOA: non-obstructive azoospermia; FSH: Follicle stimulating hormone; ICSI: intracytoplasmic sperm injection

**Table 2. Histopathological results of the patients without any retrieved spermatozoa in the NOA group**

Germ-cell aplasia	n=59 (55.1%)
Maturation arrest	n=42 (39.2%)
Hypospermatogenesis	n=6 (5.6%)

NOA: non-obstructive azoospermia

### Discussion

In our study, motile spermatozoa could be obtained using m-TESE procedure in nearly half of the cases with NO, and live births could be delivered in 23.7% of these cases. In all cases with OA, motile spermatozoa could be retrieved with m-TESE, and live births could be realized in 25.6% of these cases.

Regarding sperm retrieval rates in cases with NOA, Ramasamy et al.<sup>[8]</sup> reported a 60% sperm retrieval rate with m-TESE in their study encompassing 792 patients. In another study, spermatozoa were obtained with m-TESE in 36% of the patients with nonobstructive azoospermia.<sup>[9]</sup> Vloeberghs et al.<sup>[10]</sup> found sperm retrieval, and pregnancy rates related to TESE procedure as 40.5, and 37%, respectively. They determined that 13.4% of all patients who had undergone TESE had delivered healthy babies. In a meta-analysis performed by Bernie et al.<sup>[11]</sup> superiority of m-TESE over conventional TESE as for sperm retrieval rates (52 vs. 35%) was demonstrated. Also in our study, motile sper-

matozoa were retrieved in 52.4% (118/225) of the patients. This sperm retrieval rates are in accordance with those previously reported in the literature.

Sperm retrieval rates in cases with NOA have differed in various studies between 25, and 50 percent.<sup>[11-13]</sup> The reason for this discrepancy may be that some OA patients were evaluated as NOA, and in some studies immotile, and immature spermatozoa were used for ICSI. In addition conventional TESE or m-TESE methods might be used in different studies.

Higher sperm retrieval rates are achieved with m-TESE, besides greater number of spermatozoa can be obtained exposing testicular tissue to lesser degree of trauma. In a study performed in our country, higher probability of sperm retrieval was demonstrated in a testicular tissue with large seminiferous tubuli.<sup>[14]</sup> Amer et al.<sup>[15]</sup> compared m-TESE, and conventional TESE, and revealed that acute, and chronic complications as hematoma, infection, fibrosis, lower testosterone levels are less frequently seen in the m-TESE group without development of permanent devascularization in both groups. In our study, in only 3 patients hematoma developed during early postoperative period which regressed with conservative treatment.

Seo et al.<sup>[16]</sup> tried to determine predictors for the presence of spermatozoa before micro-TESE in NOA patients, and compared testicular size, pathology, and FSH values. Probable sperm retrieval rates based on histopathological diagnoses were compared within themselves. They reported that presumptive sperm retrieval rates had not correlated with testicular size, and FSH values, while these rates had correlated with histopathological findings.

Tun et al.<sup>[17]</sup> investigated the factors effective on successful sperm retrieval rates with TESE in patients with NOA. To this end, preoperative serum FSH, inhibin levels, and testicular volumes of the patients were measured. Spermatozoa were detected in 59.6% of the patients, while none of the parameters demonstrated a significant correlation with sperm retrieval rates. In our study lower testicular volumes were found in patients with NOA, but this difference did not reach a level of statistical significance. Besides FSH levels were not effective on sperm retrieval rates in m-TESE. Most frequently germ-cell aplasia was detected in NOA patients without any retrieved spermatozoa.

m-TESE may be performed at the same day or one day before oocyte aspiration, and spermatozoa may be used following 24 hours of incubation. In previous studies any difference between

fertilization, and clinical pregnancy rates was not reported when m-TESE, and ICSI performed with testicular spermatozoa used at the same day or 24 hours after incubation.<sup>[18,19]</sup> Therefore in our study outcomes of ICSI performed with testicular spermatozoa retrieved one day apart were evaluated in the same group.

In previous studies, pregnancy outcomes of NOA, and OA patients using testicular spermatozoa were investigated, some studies detected higher rates of pregnancy in OA compared with NOA patients, while others found comparable pregnancy rates between both groups.<sup>[19,20]</sup> Differences are thought to stem probably from testicular spermatozoa used.

In cases where immature testicular spermatozoa are used pregnancy rates regress considerably. Tanakaa et al.<sup>[21]</sup> reported that when compared with ICSIs performed using normal spermatozoa very low rates of healthy pregnancies were obtained in patients who had undergone round spermatid injections. Besides Park et al.<sup>[22]</sup> reported higher pregnancy rates in cases where motile testicular spermatozoa were used relative to those who used non-motile spermatozoa. Similarly, in our study we detected that the etiology of azoospermia did not affect live birth rates in cases with azoospermia where only motile spermatozoa were used.

In conclusion, if motile spermatozoa are retrieved with m-TESE in azoospermic patients ICSI can achieve successful pregnancies with live births in 1 in nearly 4 couples. Live birth rates achieved with IVF-ICSI procedures using spermatozoa retrieved during m-TESE surgery do not differ between cases with OA, and NOA. m-TESE can be achieved with very low complication rates in azoospermic patients.

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**Ethics Committee Approval:** Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

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