

# The Outcome of Intracytoplasmic Injection of Testicular Spermatozoa and Epididymal Spermatozoa Obtained from Azoospermic Men

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Received date: July 19, 2016, Accepted date: August 03, 2016, Published date: August 10, 2016

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## Abstract

This study was carried out to compare the reproductive capability of testicular sperm injection (ICSI) technique. From November 2006 to December 2007, 198 intracytoplasmic sperm injection cycles were studied. One hundred thirty of 198 cycles were carried out using epididymal sperm and 68 were carried out using testicular sperm. The results showed that no significant differences were observed in the rates of fertilization and embryo transfer between obstructive and nonobstructive etiology and between epididymal and testicular spermatozoa. The abortion rate of motile epididymal or testicular spermatozoa was lower than that of immotile spermatozoa.

**Keywords:** Azoospermia; ICSI; Epididymis; Fertility; Male; Testis

## Materials and Methods

## Introduction

A common etiology of azoospermia in infertile men was surgically unreconstructable obstruction of the male genital tract [1]. Historically, a patient was considered infertile with obstructive azoospermia caused by congenital absence of the vas deferens (CAVD) or those had failed in reconstructive surgery. However, recent studies show that it is worthwhile to offer new surgical reconstruction to patients in whom previous seminal tract reconstructive surgery failed [2]. After the first successful pregnancies and the success reported using intracytoplasmic sperm injection (ICSI) in patients with severe oligoasthenoteratozoospermia the treatment of azoospermic infertile patients changed [3,4]. Actually, this novel therapy relating to retrieval of spermatozoa by surgery combined with aided reproduction has provided hopes to patients considered previously as an untreatable infertile. Spermatozoa retrieved by microsurgical aspiration of epididymal sperm (MESA) have been used for more than a decade for in vitro fertilization (IVF) [5]. Silber et al. first documented conception with sperm aspirated from the head of the epididymis and IVF in cases of bilateral CAVD [6]. The fertilization and pregnancy rates with MESA and standard IVF are low at not higher than 20% and 11%, respectively [5,7]. Yet, the micromanipulation for aided fertilization has improved the rate of fertilization significantly [8,9]. Moreover, ICSI may be considered an effective fertility treatment for patients with nonobstructive azoospermia. In nonobstructive origin azoospermia patient, the sperm cells can be taken only from testicles by testicular sperm aspiration (TESA) [10-14]. The fertilization and pregnancy rates of ICSI have been assessed in several studies [15-24]. Because of the contradictions in published reports, which may be partially due to sperm unrelated factors, the impact of the source of spermatozoa (testicular versus epididymal) and the etiology of azoospermia (obstructive versus nonobstructive) on sperm reproductive capacity is not well established. The ICSI result in obstructive azoospermia group was compared with nonobstructive azoospermia group, epididymal azoospermia group was compared with testicular azoospermia, and motile was compared with immotile spermatozoa.

## Data collection

Our retrospective study between November 2006 and December 2007 included 60 consecutive azoospermic patients in 62 ICSI cycles. In 198 cycles, 68 cases were manipulated in nonobstructive azoospermic patients and 130 cases were carried out in obstructive azoospermic patients. 52 cycles (40 patients) were performed using percutaneous epididymal sperm aspiration (PESA), 130 cycles (110 patients) were handled using TESA and 16 cycles (16 patients) were manipulated using PESA plus TESA. Sperm was taken from the testis until 1998 in obstructive azoospermia patients. After that, spermatozoa were retrieved from the epididymis in obstructive azoospermia patients and spermatozoa were retrieved from the testicles in nonobstructive azoospermia patients. Nonobstructive azoospermia was diagnosed by testis biopsy showing spermatogenesis failure. In obstructive azoospermia patients with surgically irreparable obstruction, CAVD or previous vasectomy reversal failure and in those who did not elect vasectomy reversal, ICSI was performed. Female partners were younger than 39 years and baseline serum follicle-stimulating hormone (FSH) was lower than 10 mIU/ml in all cases. Complete history, physical examination and hormone evaluation were performed. Patients with nonobstructive azoospermia and abnormal karyotype examination of peripheral leukocytes were excluded from study. Patients were informed about the ICSI technique using epididymal and testicular spermatozoa, and possible complications of the surgical procedures, such as infection, hematoma and impaired blood flow. All patients were counseled and signed a consent form approved by our internal ethics committee.

## Ovarian stimulation and oocyte retrieval

Infertility evaluation in female partner consisted of assessment and hysterosalpingography. Average age of the female partners was  $32.3 \pm 5.5$  years. No statistically significant differences in average female age in the obstructive and nonobstructive azoospermia groups ( $33.5 \pm 4.2$  and  $34.2 \pm 3.8$  years, respectively) were observed. A unique protocol of

controlled ovarian stimulation including gonadotropin-releasing hormone agonist (leuprolide acetate) for at least 14 days was performed in female patients. When serum estradiol concentrations were less than 40 pg/ml and there are no ovarian cystic structures on ultrasound image, FSH was used for ovarian stimulation in a step-down protocol to achieved at least 2 follicles with average diameter of 18 mm. 36 hours after the administration of 10,000 IU human chorionic gonadotropin, oocyte was taken under transvaginal ultrasonography. Human tubular fluid medium added with 7.5% synthetic serum was used for oocytes preserving for approximately 3 to 5 hours. After that, the cumulus cell was removed from the oocytes and placed in hyaluronidase at a concentration of 80 IU/ml for about 30 to 60 seconds. Then, oocytes were taken to fresh medium. Corona cells were removed by gently pipetting in and out.

### ICSI procedure

ICSI was carried out with oocytes in metaphase II according to the method described by Palermo et al. ICSI was done with a heated microscope stage. After being injected, the oocytes were rinsed and placed in suitable growing medium. These oocytes were then incubated in a culture dish. About 16 to 18 hours after the injection procedure, the oocytes were checked for fertilization. Healthy oocytes were kept otherwise were removed from the dish. The fertilized oocytes were assessed after 24 hours cleavage. Embryo was transferred about 72 hours after injection.

### Sperm retrieval

On the day of ovum retrieval percutaneous PESA or TESA was performed. All procedures were done in our outpatient procedure room using local anesthesia. Povidone-iodine was used for the area after shaving of the anterior scrotum. Lidocaine 1% was administered for anesthesia in the pubic tubercle area when no sperm was obtained. The testicle was immobilized for PESA by holding the inferior two thirds of the testicle and pulling the epididymis. The epididymis carefully stabilized between the thumb and index finger by surgeon to expose the superior pole of the patient's testicle. The suction of sperm was performed by a 23 gauge butterfly needle inserted into the epididymis and pulling back the plunger of the 20 cc syringe. When

the plunger reached 20 cc, the butterfly tubing was clamped at a point closed to the hub of the syringe. This made the 20 cc syringe replacement easier with a 1 cc tuberculin syringe without releasing negative pressure. Massaged the epididymis to knead fluid into the tubing. When the fluid return stopped, the needle was removed. Sperm was collected in similar fashion for TESA. A 19 gauge butterfly needle was used. The surgeon immobilized the testicle by grasping the epididymis and cord between the fingers, while pulling the scrotal skin taut. The depth of the needle excursion was controlled to protect the epididymis from injury. Inserted the needle into the inferior pole of the testis, and rapidly advanced and pulled back several times toward the superior pole. The seminiferous tubules were aspirated by withdrawing the needle slightly and redirected several times to disrupt the testicular architecture. Repeated the procedure until an opaque to yellow fluid and tissue was flowed into the butterfly tubing. Washed the aspirate within the needle or proximal tubing with a small volume of culture medium into a Falcon tube maintained at 37°C. The testicular tissue was minced into small pieces with sterilized scissors and forced through a 25 gauge needle. The homogenized tissue was washed twice with culture medium and the pellets were suspended and recovered. Postoperative pressure was kept constantly on the aspiration site in the operating room for 5 minutes. After that, a fluff compression dressing and scrotal supporter were applied and left in place for 24 hours. Student's t, the chi-square and Fisher's exact tests was used for statistical significance evaluation. Statistically significant differences were considered at  $p \leq 0.05$ .

### Results

No significant differences in the mean age of patients with obstructive and nonobstructive azoospermia were observed ( $32.2 \pm 3.7$  and  $30.6 \pm 5.1$  years, respectively,  $p \geq 0.56$ ). Of the 198 cycles 68 (34.3%) and 130 (65.6%) were performed in nonobstructive and obstructive azoospermia cases, including 71% and 28.4 % of obstructive azoospermia cases involving vasectomy and nonvasectomy conditions (epididymis obstruction, CAVD and herniorrhaphy), respectively. ICSI using motile spermatozoa was done in 71.7% of the cycles, while immotile spermatozoa were retrieved and injected in 17.2%.

	Obstructive Azoospermia (1)	Non-obstructive Azoospermia (2)	P1-2
Mean age $\pm$ SD	32.2 $\pm$ 3.7	30.6 $\pm$ 5.1	0.56
% Normal fertilization (2 PN)	60.5	54	0.4
% Abnormal fertilization (1,3 PN)	16.6	16.4	0.5
% Embryo transfer	73.6	74.2	0.5
% Pregnancy/cycle	30	22	0.04
% Pregnancy/transfer	32.5	23.8	0.03
% Pregnancy/patient	39.8	28.3	0.03
% Abortion	28	40	0.01
PN: Pronucleus			

**Table 1:** Fertility outcome after ICSI in azoospermic patients

Spermatogenic cells, namely round and elongated spermatids, were used in 9.1% and 2.01% of cases, respectively. The abnormal fertilization (1 plus 3 pronuclei), normal fertilization (2 pronuclei), and embryo transfer rates for obstructive and nonobstructive azoospermia were 16.6%, 60.5%, and 73.6%, and 16.4%, 54%, and 74.2%, respectively (p>0.05; Table 1).

For obstructive azoospermia, the pregnancy rate per cycle, per transfer and per patient, and the abortion rate were 30%, 32.5%, 39.8% and 2%, respectively, and for nonobstructive azoospermia, these rates were 22%, 23.8%, 28.3% and 40%, respectively (p<0.05). For PESA, the normal, abnormal fertilization, and embryo transfer rates were 58.7%, 21.4% and 73.6%, respectively. For PESA plus TESA, these rates were 62.3%, 10.4% and 78%, respectively. For TESA, these rates were, 57.3%, 14.5% and 73.4%, respectively (p>0.05, Table 2).

No significant differences in mean patient age in the 3 groups were observed (31.4, 32.7 and 30.8 years, p>0.05). For PESA, the pregnancy

rate per cycle, per transfer and per patient, and the abortion rate were 34.6%, 40%, 54.5% and 11.1%, respectively. For PESA plus TESA these rates were, 37.5%, 37.5%, 37.5% and 33.3%, respectively. For TESA these rates were 26.1%, 28.3%, 31% and 41%, respectively (PESA versus PESA plus TESA p>0.05, and PESA and PESA plus TESA versus TESA p>0.05). The pregnancy and miscarriage rates as well as the type of injected spermatozoa were shown on Table 3. No significant differences were demonstrated in the pregnancy rate per cycle and per patient for motile (41.04% and 45.7%) and immotile (29.4% and 36.8 %, respectively) sperm (p>0.05). The lowest pregnancy rate per cycle and per patient was in patients in whom round and elongated spermatids were retrieved (15% and 13.63 %, respectively). The abortion rate was higher in those in whom immotile versus motile spermatozoa were retrieved (70% versus 25.5%, p<0.05). The round and elongated spermatids gave 100 % rate of miscarriage.

Variables	PESA(1)	PESA-TESA(2)	TESA(3)	p
Mean age	31.4	32.7	30.8	P1-2; P2-3; P 3-1 ≥ 0.05
%Normal fertilization (2 PN)	58.7	62.3	57.3	P1-2; P2-3; P 3-1 ≥ 0.05
%Abnormal fertilization (1,3 PN)	21.4	10.4	14.5	P1-2; P2-3; P 3-1 ≥ 0.05
%Embryo transfer	73.6	78	73.4	P1-2; P2-3; P 3-1 ≥ 0.05
%Pregnancy/cycle	34.6	37.5*	26.1	P1-2; P2-3 ≤ 0.05
%Pregnancy/transfer	40	37.5*	28.3	P1-2; P2-3 ≤ 0.05
% Pregnancy/patient	54.5	37.5*	31	P1-2; P2-3 ≤ 0.05
% Abortion	11.1	33*	41	P1-2; P2-3 ≤ 0.05

**Table 2:** Fertility outcome in patients with epididymal and testicular spermatozoa

	Round – Elongated Spermatids	Motile Spermatozoa	Immotile Spermatozoa
No. obstructive azoospermia (%)	3 (2.3)	107 (82.3)	20 (15.4)
No. nonobstructive azoospermia (%)	19 (27.9)	35 (51.47)	14 (20.58)
No. cycles	22	142	34
No. pregnancies/cycle (%)	3 (13.63)	55 (41.04)	10 (29.4)
No. pts.	20	127	19
No. pregnancies/pt.	3 (15)	58 (45.7)	7 (36.8)
No. abortions (%)	3 (100)	14 (25.5)	7 (70)

**Table 3:** Outcome according to azoospermia etiology and the results of spermatozoa retrieval

## Discussion

New technique of testicular sperm aspiration combined with ICSI have been developed since the introduction of MESA for obstructive azoospermia caused by bilateral CAVD [6]. Consequently, many azoospermic patients with various causes can be offered IVF. As a result of the high fertilization and pregnancy rates that may be achieved by ICSI, this technology is now routinely used to improve the

results of IVF after epididymal sperm aspiration. For couples with infertility secondary to nonobstructive azoospermia no corrective treatment is available except hypogonadotropic hypogonadism. However, some of these couples were able to have genetic offspring using the small number of spermatozoa in the testicles with ICSI. It seems that these azoospermic patients have small foci of spermatogenesis in the testes. It was estimated that there must be at least 4 to 6 mature spermatids per tubule for any spermatozoa to reach

the ejaculate [25,26]. Conventionally, azoospermic patients with serum FSH greater than 2 to 3-fold normal, who have severe testicular failure were not amenable to any conventional therapy. However, testicular sperm retrieval resulted in a lower pregnancy rate as well as a higher abortion rate than epididymal sperm retrieval. Our study demonstrated that the normal fertilization rate (2 pronuclei) in nonobstructive azoospermic patients was 54%, similar to the normal fertilization rate achieved in obstructive azoospermic patients (60.5%). Also, fertilization rates were similar for epididymal and testicular spermatozoa. The reason why there were no differences in the fertilization rate in patients with obstructive (epididymal spermatozoa) and nonobstructive azoospermia (testicular spermatozoa) was not known. However, the pregnancy rate per cycle and per patient in patients with nonobstructive was significantly lower than those with obstructive azoospermia. We also noted that the pregnancy rate was higher for epididymal versus testicular spermatozoa. Furthermore, testicular spermatozoa were related to a higher abortion rate than epididymal spermatozoa. Actually, there is severe impairment of spermatogenesis and even testicular failure nonobstructive azoospermic patients [27]. Nonobstructive azoospermic patients may have a defect in genes or a genetically determined barrier inhibited reproduction. Therefore, it is reasonable that despite successfully extracting live spermatozoa in, the pregnancy rate of nonobstructive azoospermic patients was significantly lower than that of obstructive azoospermic patients. The fertilization rate of ICSI was similar in patients with obstructive (epididymal) and nonobstructive (testicular) spermatozoa in this retrospective analysis. In our study, testicular spermatozoa resulted in a higher abortion rate than epididymal spermatozoa. In contrast to the findings of Silber et al. [28], although similar fertilization and cleavage can be obtained in epididymal and testicular spermatozoa, there was still different in pregnancy rates. However, the pregnancy rates can be determined by other factors other than spermatozoa, such as maternal age and ovarian reserve thus impairing the outcome [28]. As described, the higher miscarriage rate associated with testicular sperm may be associated with azoospermia etiology. These cells probably carry genetic disorders preventing pregnancy establishment, mainly in nonobstructive azoospermia cases. Genetically defective sperm can lead to fetal loss and genetic disease in the offspring [29,30]. We also found out that motile spermatozoa had lower abortion rate than immotile spermatozoa. Sperm acquire the capacity for vigorous forward motility during transit through the epididymis, which has an important role in sperm maturation [31]. During this process, the function of the sperm centrosome may be affected by some alterations resulting in impaired motility and a low percent of vital sperm.

## Conclusions

Our study shows that the fertilization rate of ICSI is similar in patients with obstructive (epididymal) and nonobstructive (testicular) spermatozoa. Testicular spermatozoa are associated with a higher abortion rate than epididymal spermatozoa. Also, the abortion rate of motile epididymal or testicular spermatozoa was lower than that of immotile spermatozoa.

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