

Testicular Sperm Aspiration (TESA) in 327 ICSI Cycles

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ABSTRACT: **Objective**—To evaluate the efficiency of testicular sperm recovery by testicular sperm aspiration (TESA) in an IVF program. **Design**—Retrospective Data Analysis. **Setting**—The Center of Reproductive Medicine, Department of Obstetrics and Gynecology, Arcispedale S. Maria Nuova, Reggio Emilia, Italy. **Patient(s)**—Couples undergoing TESA/ICSI for obstructive or nonobstructive azoospermia. **Intervention(s)**—TESA/ICSI. **Main outcome measure(s)**—Efficiency of testicular sperm recovery, fertilization rate, implantation rate and clinical pregnancy rate. **Result(s)**—Between March 1, 1997 and March 31, 2005, 327 cycles of TESA/ICSI were performed in couples in which the male had obstructive or nonobstructive azoospermia. The efficiency of testicular sperm recovery was 99.4% and 99.3%, fertilization rate 57.1% and 49.1%, implantation rate 5.7% and 6.2%, and the clinical pregnancy rate 12.9% and 15.4% in men with obstructive and nonobstructive infertility, respectively. **Conclusion(s)**—The efficiency of TESA is very high in both obstructive and nonobstructive azoospermia. Because TESA is less invasive than TESE, it should be considered a valuable alternative to TESE in IVF programs, especially in setting where resources are limited. *Int J Fertil* 51(4):177-182, 2006

KEY WORDS: obstructive /nonobstructive azoospermia, testicular sperm aspiration (TESA), testicular sperm extraction (TESE), intracytoplasmic sperm injection (ICSI), assisted reproduction technology (ART)

INTRODUCTION

THE DEMONSTRATION OF THE FERTILIZATION capacity of epididymal spermatozoa obtained by microsurgical epididymal sperm aspiration (MESA) and of testicular spermatozoa obtained by testicular sperm extraction (TESE), as well as the introduction of intracytoplasmic sperm injection (ICSI), have opened new

possibilities in the therapy of male infertility secondary to azoospermia [1-4].

The first patients to be successfully treated were those with obstructive azoospermia (OA) [1,3,4]. Subsequently, TESE and ICSI were also successfully used for treatment of male infertility due to nonobstructive azoospermia (NOA) [5,6]. In the mid-1990s, following the remarkable results obtained in patients with OA, success was reported using less

invasive methods of sperm recovery, such as the testicular aspiration by fine needle (TEFNA) or testicular sperm aspiration (TESA) [7-10].

To our knowledge, few papers reported findings about TESA, and it was difficult to collect data reflecting efficiency in terms of sperm retrieval and pregnancy rate [11-12]. As a result, the application of TESA did not expand in the last ten years, and today TESE still remains the most widely used procedure, supported by the data, reporting that TESE allows a higher sperm retrieval than TESA and permits the testicular sperm cryopreservation [13,14].

In contrast to the majority of IVF centers, our center never used TESE, and in this work we report the results of 327 TESA/ICSI cycles performed in couples where the male partner was affected by OA or NOA.

MATERIALS AND METHODS

We analyzed a dataset constructed from 329 TESA procedures and 327 TESA/ICSI cycles performed in couples where the male partner was affected by OA or NOA. The study was conducted from March the 1st 1997 to March the 31st 2005 in our clinic at Arcispedale S. Maria Nuova in Reggio Emilia, Italy.

According to the ART protocols in Reggio Emilia, cryopreservation and disposal of excess embryos are not allowed, and all transferable embryos were transferred in each cycle on day 2 or 3 [15].

Patients

We performed a preliminary diagnostic TESA (dTESA) in all azoospermic patients who were candidates for IVF. Azoospermic patients with no sperm retrieval at dTESA were excluded from the IVF program. All 156 patients of this study—75 with OA and 81 with NOA—had a positive sperm retrieval during the preliminary dTESA.

The mean age of male patients was 35.6 ± 5.2 years, without significant differences between the groups of OA and NOA. All 156 patients underwent a preliminary extensive work-up of at least three ejaculates, medical history and examination, hormonal assay (FSH and LH) and karyotype where indicated. Specifically, 57 patients with OA underwent a cystic fibrosis gene mutation analysis, and 39 NOA patients underwent a Y-chromosome microdeletion analysis. The female patients underwent routine preliminary examination. The mean female age was 33.7 ± 4.3 years, without significant differences between the groups of OA and NOA.

We performed 179 TESA procedures and 178 TESA/ICSI cycles in couples with OA and 150 TESA procedures and 149 TESA/ICSI cycles in couples with NOA. Totally, we performed 329 TESA procedures and 327 TESA/ICSI cycles.

The study was approved by our institutional review board.

Testicular Sperm Recovery

TESA was performed by urologists (L.S. and F.M.) on the day of ovum retrieval using local anesthesia. We obtained no more than four aspirations per testis, starting with one random aspiration of right or left testis in case of OA, and with two aspirations per each testis in case of NOA. We did not cryopreserve spermatozoa retrieved at TESA, and in all our TESA/ICSI cycles only fresh testicular spermatozoa were used.

After administration of local anesthesia, laboratory personnel carried to the surgery room a variable number of previously prepared dishes containing 100 μ l of medium to which was added heparin to avoid red blood cells aggregations. For each aspiration, one 21-gauge butterfly needle was attached to a 30 ml plastic syringe and, after the connection, the needle was filled with this medium. The testis was positioned as close as possible to the scrotal skin and held in this position. The needle was then perpendicularly and deeply inserted into the testis at the point of its major diameter. At the same time the syringe was energetically aspirated until complete retraction of the piston, after which the needle was almost totally extracted and the pressure in the syringe almost totally reduced. Subsequently, the needle was inserted again in a different direction and the aspiration process repeated. Finally, the needle was gradually retracted until the point of complete extraction and, at the same time, the pressure of the syringe was gradually reduced to zero. After the extraction, the 21-gauge butterfly with its connection was immediately delivered to the laboratory for the examination.

In laboratory, the aspirated sample was flushed in a dish with 8 μ l droplets (30 for each dish) of heparinized culture medium using a sterile 1 ml plastic syringe and immediately examined under the inverted microscope using the Nikon micromanipulator (DIAPHOT 300, Nikon Corp., Tokyo, Japan) to check for the presence of spermatozoa. The same technique has been repeated for all droplets until a sufficient number of spermatozoa were obtained. All droplets were observed under the microscope with great accuracy; in case no spermatozoa were detected at first, we waited 20 minutes for a second

TABLE I
Sperm retrieval, fertilization and cleavage rates after ICSI with testicular sperm in OA and NOA.

	OA	NOA	Total
Male patients	75	81	156
TESA procedures	179	150	329
TESA procedures with sperm retrieval	178/179 (99.4)*	149/150 (99.3)*	327/329 (99.3)*
TESA procedures with motile sperm retrieval	176/179 (98.3)*	130/150 (86.7)*	306/329 (93.0)*
ICSI cycles	178	149	327
Successfully injected oocytes	1139 (6.4±3.5)**	1010 (6.8±3.9)**	2149 (6.6±3.6)**
Oocytes showing 2PN	651/1139 (57.1)*	496/1010 (49.1)*	1147/2149 (53.4)*
Embryos obtained and transferred	637 (3.6±2.4)**	486 (3.4±2.2)**	1123 (3.5±2.3)**

* values are presented as (%).
** values are (means ± SD).

microscope observation in order to allow the sperm sedimentation. Each spermatozoa found was transferred in polyvinylpyrrolidone (PVP) solution using an injection pipette. All micromanipulation procedures were carried out in 5 µl droplets of buffer medium under light oil on a 37°C warming stage. The in situ or progressive motility of each spermatozoa recovered was used as a sperm vitality index. Two hours after TESA procedure patients were discharged from the hospital.

Ovarian Stimulation and ICSI Procedures

All female partners were stimulated using a gonadotropin releasing hormone analogue suppression and recombinant FSH or human menopausal gonadotropins. Transvaginal oocyte retrieval was performed 36–37 hours after the administration of 10,000 IU of Human Chorionic Gonadotropin (hCG). Following removal of the oocyte's surrounding cumulus and corona cells, nuclear maturation assessment was performed using an inverted microscope, to ensure injection of metaphase II oocytes only. ICSI was then performed as reported by Palermo et al [2].

Assessment of Fertilization, Embryo Cleavage and Establishment of Pregnancy

Fertilization was assessed at 18–20 hours after injection by confirmation of the presence of 2 pronuclei (2PN) and 2 polar bodies. Embryo cleavage was observed 48 hours after injection. Transfer was performed on day 2 to 3. All patients received 50 mg of progesterone in oil daily IM for luteal support, starting the afternoon of ET and continuing until a negative serum hCG or a viable fetus was documented by transvaginal sonography. A rise in serum hCG on two consecutive occasions from 11–13 days after transfer indicated the presence of a pregnancy. A clinical pregnancy was defined as at least 1 fetus with a positive heartbeat revealed by transvaginal sonography 4 to 5 weeks after ET.

Statistical Analysis

Data were prospectively collected and regularly updated. Data were analyzed by chi-squared test as appropriate. Values of $P < 0.05$ were considered to be statistically significant.

TABLE II
Clinical pregnancies and deliveries after TESA/ICSI in OA and NOA.

	OA	NOA	Total	
TESA/ICSI	178	149	327	
ET	177	143	320	
Embryos obtained and transferred	637	486	1123	
Clinical pregnancies	23	23	46	
(Pregnancies/cycle)	23/178 (12.9)*	23/149 (15.4)*	46/327 (14.1)*	NS
(Pregnancies/ET)	23/177 (13.0)*	23/143 (16.1)*	46/320 (14.4)*	
Deliveries	16	20	36	
(Deliveries/cycle)	16/178 (8.9)*	20/149 (13.4)*	36/327 (11.0)*	NS
(Deliveries/ET)	16/177 (9.0)*	20/143 (14.0)*	36/320 (11.2)*	
Babies	25**	28†	53††	
Implantation rate	36/637 (5.7)*	30/486 (6.2)*	66/1139 (5.8)*	NS

NS= not significant.

*values are presented as (%).

**9 single, 5 twin and 2 triplet deliveries.

†14 single, 4 twin and 2 triplet deliveries.

††23 single, 9 twin and 4 triplet deliveries.

RESULTS

Screening for cystic fibrosis has been positive in 28 OA patients (28/57, 49.1%); DF508 mutation was observed in 25 (25/28, 89.3%) patients. Molecular study of microdeletion of Y-chromosome resulted positive only in 2 (2/39, 5.1%) patients.

Table I shows our results about TESA and IVF procedures. We retrieved spermatozoa in 99.3% of total TESA procedures. Of these, 93.0% were motile and 7.0% non-motile. A total of 2149 oocytes were injected: 1139 in couples with OA and 1010 in couples with NOA. The percentage of oocytes showing 2PN was 57.1% in the OA group and 49.1% in the NOA group. A total of 637 embryos was transferred in 177 ETs in the OA group and 486 embryos were transferred in 143 ETs in the NOA group.

Table II shows data about clinical pregnancies, deliveries and babies born. We performed a total of 320 ET. The clinical pregnancy rate per TESA/ICSI was 12.9% and 15.4%; the pregnancy rate per ET was 13.0% and 16.1%; and the embryo implantation rate was 5.7% and 6.2% in OA and NOA, respectively.

We obtained 36 total deliveries that resulted in the births of 52 healthy babies; one baby subsequently died as a result of the HELLP syndrome. The only complication was one case of scrotal haematoma with a spontaneous resolution (1/329, 0.3%).

DISCUSSION

The use of testicular spermatozoa in the therapy of male infertility caused by azoospermia is an accepted clinical procedure worldwide. The question then is which is the preferred means by which to obtain the sperm? It would appear that TESE is the most widely-used technique for testicular sperm retrieval, whereas only few ART centers use TESA [16]. TESE is often preferred to TESA because of the following advantages: a higher percentage of sperm retrieval and the possibility of testicular sperm cryopreservation [9,14]. However, in our experience, TESA permitted spermatozoa retrieval in almost 100% of the cases of patients with azoospermia: specifically, 99.4% in OA and 99.3% in NOA men, respectively.

In OA patients, our results were similar to the results generally obtained using TESE, whereas in NOA patients our results were better [17]. Moreover, to our knowledge our results obtained in OA and NOA were by far better than those reported by other authors using TESA or TEFNA [10,12]. The probable explanations for our results are numerous. The first and most important explanation could be that we regularly performed a preliminarily dTESA in all azoospermic male candidates. In 52 patients we didn't find spermatozoa, and these patients were excluded from our IVF program.

The second possible explanation could be linked to the TESA technique used. For each testicular puncture, we obtained two aspirations in two different directions, increasing the chance of hitting a rare site of active spermatogenesis even in severe NOA pathology. The last explanation could be the laboratory procedures and microscope observations performed. We flushed the testicular aspirations in droplets of heparinized medium; we then analyzed the samples after the total cell sedimentation. In some cases search continued for 3-4 hours at the microscope until we detected the spermatozoa. These results demonstrate that the efficiency of TESA in terms of sperm retrieval is not inferior to that of TESE.

In our OA and NOA populations we observed a fertilization rate of 53.4%, an implantation rate of 5.8% and clinical pregnancy rate per ET of 14.4%. Vermaeue et al reported a similar fertilization rate but higher implantation and pregnancy rates [16]. Comparing OA and NOA couples, we did not find a statistically significant difference in fertilization, pregnancy, and implantation rates (Table 1). This contrasts with the findings in the Vermaeue study, where statistically significant lower fertilization and implantation rates were noted in NOA patients [16].

In conclusion, the results of this study support the contention that, for many reasons, TESA should be preferred to TESE. It is a minimally invasive technique, provides intact testicular tubules adequate for the histological assessment of spermatogenesis in all cases [21], can be performed in an office setting with local anesthesia, has a very low rate of complication, is an easy, sure and quick technique, and permits a high testicular sperm retrieval.

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