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Research Article

The Predictive Value of Testicular Fine Needle Aspiration for Sperm Retrieval from the Contralateral Testis – A Prospective Randomized Study

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Abstract

Testicular sperm extraction (TESE) and testicular sperm detection by fine needle aspiration (TEFNA) are both acceptable methods for sperm retrieval for non-obstructive azoospermia (NOA) men. The aim of the study was to determine the predictability of TEFNA to sperm detection by either TEFNA or TESE of the second testicle and to compare fertilization rate (FR) of testicular spermatozoa retrieved by each method. Sixty one men diagnosed with non-obstructive azoospermia (NOA) participated in this prospective study. All patients had a sperm recovery trial by TEFNA on a single randomly selected testicle (10-20 punctures with 23-gauge butterfly needle) and either TEFNA or TESE on the contralateral testicle at the same surgical session. The procedure was considered successful if at least 1 spermatozoon per 5μL was retrieved for use in the coming cycle of IVF-ICSI.

We found that TEFNA could successfully predict all successful TESE cases (100% PPV and 88% NPV), whereas unsuccessful TEFNA was followed by successful TESE in 12.5% of cases. The mean number of spermatozoa collected by TEFNA vs. TESE was 1749±3175 (range 0-10,000) vs. 14129 +18005 (range 24-40800), respectively (p=0.033). TEFNA could successfully predict all successful TEFNA of the second testis (100% PPV and 95% NPV). The FR of MII oocytes was similar for sperm retrieved by either TEFNA or TESE. We conclude that in NOA patients successful TEFNA is fully predictive of both successful TESE and TEFNA on the contralateral testis. However, unsuccessful TEFNA may not predict the outcome of TESE in 12.5% of cases, most probably due to the numerical superiority of TESE. Spermatozoa collected by both methods share similar fertilization potential.

Keywords: Azoospermia; ART; Biopsy; Testis; TEFNA; TESE

Introduction

Advances of the last two decadesin the treatment of azoospermic men enabled recovery of testicular spermatozoa and fertilization of the oocytes with ICSI. Since the introduction of the retrieval of testicular sperm by tissue extraction [1,2], ICSI with testicular sperm has become a routine procedure initially for patients with obstructive azoospermia (OA) [1,2], and later for non-obstructive azoospermia (NOA) [3-5]. Several methods have developed for testicular sperm recovery, each with its own pros and cons [6]. Earlier, testicular percutaneous fine needle aspiration (TEFNA) was considered solely for OA patients, whereas testicular sperm extraction (TESE) by open multiple biopsies was reserved for men with NOA due to gonadal failure. In these hyper gonadotrophic azoospermic men the sperm retrieval rate by TESE was around 50%. Later, the use of TEFNA for NOA was introduced by [7] and was shown to be efficient, safe, less invasive and well-tolerated [8]. Since then, the reported recovery rate of testicular sperm with TEFNA which ran as low as 11% [9] and high as 58.5% and 64.7% [7,10], was subjected to much controversy. With a lack of randomized controlled trials to compare the two methods, non-randomized comparative trials provided data which supported the use of TESE for patients with defective spermatogenesis [9,11,12]. For example, Friedler et al. [9] used TEFNA and TESE consecutively in a single session in 37 NOA patients.The reported superiority of TESE over TEFNA (43% vs. 11% retrieval rate) was questioned by others partly because of the fewer puncture sitesper testicle (6 entries) in this study vs. 15-20 entries per testicle in the original Lewin's report [8].

As spermatogenesis is believed to be patchy in the testis of NOA patients, the better retrieval results reported with open biopsy may be explained by the greater potential for more suitable tissue to be obtained by this technique [13].

We sought to (i) study the predictability of TEFNA to sperm detection by either TEFNA or TESE of the second testicle and (ii) compare the sperm retrieval rate by TESE vs. TEFNA in the same individual in a single session and fertilization rate (FR) of testicular spermatozoa retrieved by each method.

Materials and Methods

Study design

This was a prospective study conducted between May 2008 and December 2010. Follow up period for spouses of NOA patients for outcomes of IVF-ICSI cycles was till April 2012. The study has been performed according to the Declaration of Helsinki. Informed, written consent has been obtained from each participant.

Study population

The study population included 61 men with NOA who underwent

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	Study population (n=61)	Next TESE (n=32)	Next TEFNA (n=29)	P Value*
Age (mean ± SD)(years)	33.9 ± 8.1	34.1± 8	33.8 ± 2.8	0.9
FSH (IU/L)	15.9 ± 13.4	17.6 ± 12.7	13.7 ± 14.1	0.06
LH (IU/L)	11.1 ± 13.2	14.3 ± 16.1	7.2 ± 6.9	0.04
Testosterone (IU/L)	16.5 ± 8.7	15.6 ± 6.6	17.5 ± 10.7	0.5
Small size testicles (< 15 cc)	26.1%	29.2%	22.7%	0.6
Pathology				
Hypospermatogenesis	41.6%	29%	55.1%	0.13
Germ cell aplasia	40%	54.9%	24.2%	
Spermatogenic maturation arrest	6.7%	6.5%	6.9%	
Not defined	11.7%	9.7%	13.8%	

*t test, Chi test for testicle size and pathology

Table 1: Characteristics of the study population.

their first testicular sperm retrieval procedure at the IVF unit of Hadassah Hebrew University Mt. Scopus Medical Center in Jerusalem. The diagnosis of azoospermia was made on the basis of at least two semen analyses after high velocity centrifugation (1800g X 5 min). The men underwent a clinical evaluation including a physical examination of the genitalia, hormonal assessment (FSH, LH and testosterone), testicular ultrasound and karyotype analysis. The patients were diagnosed as NOA based on histopathology of germ cell aplasia (Sertoli cell-only syndrome), sperm maturation arrest, and tubular sclerosis/atrophy [14]. In the case of a mixed histological pathology, the most prominent pattern was used for classification. Hypospermatogenesis indicated complete but reduced spermatogenic activity and was considered a separate subpopulation. Azoospermic patients with histology of normal spermatogenesis were classified as OA and were excluded from the study. The histopathology profile of the study group is shown on Table 1.

The intervention

The surgical procedure was undertaken under general anesthesia with a view to cryopreservation of the detected sperm. The patients consented to the procedure prior to the operation. Initially the patients had a sperm recovery trial by TEFNA on a randomly selected single testicle and immediately thereafter, 29 patients were randomly allocated for a TEFNA (TEFNA group) and 32 allocated for TESE (TEFNA-TESE group) on the contralateral testis. The procedure was considered successful if enough spermatozoa for one cycle of IVF-ICSI were collected and cryopreserved. The concentration of 1 spermatozoon per 5μL was defined as the lowest limit necessary for one cycle of IVF-ICSI.

Methodology of TEFNA

The surgical technique of TEFNA was previously described in detail [8] and carried out with several minor modifications. In brief, the scrotal skin was shaved, cleansed with 0.5% chlorhexidine/alcohol and draped. While holding and lightly pressing the testis between the thumb and index fingers, multiple punctures (mean 15, range 10-20) of the testis were performed using 23-gauge butterfly needles attached to an aspiration device with negative pressure of 600 mmHg. The butterfly needle was passed directly through the scrotal skin, into the testis, moved up and down at various sites, sampling various locations and directing the needle to the rete testis. Before retrieving the needle from the testis, a small artery forceps was used to clamp the butterfly's microtubing. Following each aspiration, the needle and microtubing were transferred to the IVF laboratory, and there flushed with HEPES medium (Irvine Scientific; IR-90126, U.S.A) with 4% synthetic serum supplement (SSS) (Irvine Scientific; IR-99193, U.S.A) into one well of a 4-well plate (Nunc, Copenhagen, Denmark). A new butterfly needle was used for each puncture and the punctures continued as long as a yellowish non-bloody fluid continued to flow into the tube. Occasional scant testicular tissue that was aspirated along was resected and flashed into a well of a 4-well plate (Nunc, Copenhagen, Denmark).The testicular aspirates were examined under inverted microscope (Nikon, Diaphot) at X 200 magnification. The collected samples were then transferred separately into 15 ml conical tubes (Falcon; Becton Dickinson Labware, NJ, USA) and left to stand for 15 min.The supernatant was transferred to new conical tubes and a small fraction ofthe pellet was sent to histopathology examination fixed in 4% formaldehyde. The rest of the pellet was grounded and added to the supernatant.After centrifugation at 3400 rpm for 5 min the final sperm suspension was achieved. Finally, microdroplets of 1-5μL supernatant were placed on a dish (Falcon 35 3801; Becton Dickinson Labware, NJ, USA) under mineral oil and microscopically searched for spermatozoa at X200 and X400 magnification. Sperm positive samples were cryopreserved in –70°C overnight and then transferred into liquid nitrogen.

Methodology of TESE

The surgical procedure of TESE and the laboratory handling of the specimen were described before in length [15]. In brief, an incision of approximately 1 cm was made through the skinand underlying layers. The protruding testicular mass from three distant regions of the testis was resected. The testicular tissue was placed inHEPES medium (Irvine Scientific; IR-90126, U.S.A) with 4% synthetic serum supplement (SSS) (first media) (Irvine Scientific; IR-99193, U.S.A), and handed to the adjacent laboratory. During surgery, a single randomly taken biopsy of the testis was sent for histological examinationfixed in 4% formaldehyde. In the laboratory,the specimens were transferred into new HEPES medium with 4% SSS (later media) and the first media were decanted into new tubes. The first media and the specimens in the later media were processed separately as previously described [15]. The sperm-containing suspensions were frozen for later use, or saved for histopathology confirmation if no spermatozoa were found.

Statistical analysis

Mean, standard deviation and percentages are presented where appropriate. T test was used for mean comparison of age, BMI, FSH, LH, testosterone, fertilization rate, implantation rate and cleavage rate. Chi test was used for categorical variables such as testicles size, pathology and for pregnancy rate. Wilcoxon rank sum test was used to comparemean ranks between the study groups for number of cycles and number of eggs aspirated. Paired t test was done for comparing the mean total sperm count for men underwent both TEFNA and TESE. All P values are two-sided, and P values <0.05 were considered to be statistically significant. Statistical analyses were performed with SAS 9.1 (SAS Institute, Cary, NC).

Results

The Baseline characteristics of the study groupare shown in Table 1. The mean age of the male patients was 33.9±8.1 years (range 23-63) with mean FSH ± SD levels of 15.9±13.4. Of the study group 40.4% of men were diagnosed with testicular pathology of hypo spermatogenesis and 42.3% had germ cell aplasia.

Of the 61 patients with NOA, half (n= 29) underwent bilateral TEFNA and 32 underwent both TEFNA and TESE. The groups were comparable regarding age, yet FSH level was lower and rate of hypo spermatogenesis higher in the TEFNA-only group (Table 1). In 20% of the TEFNA-only group pathological analysis could not take place due to the condition of the specimen. The overall sperm retrieval rate for TEFNA was 65.5 % for any spermatozoon and 34.4% for enough spermatozoa for one IVF-ICSI cycle.

In the TEFNA-only group (n=29) 10 men had successful retrieval from the first testis and 11 from the second. Analysis of the TEFNA-only group showed that failed retrieval from one testicle, correctly predicted subsequent failure at the second testicle in 95% of the cases (NPV). Successful retrieval of first testis by TEFNA predicted a positive outcome in the second testis in all cases 100% (PPV) of the patients. Kappa coefficient showed high agreement between the two testicles (0.93).

32 men underwent TEFNA and subsequent TESE for sperm retrieval of whom 8 had successful TEFNA and 11 had successful TESE. Classification analysis of the TEFNA-TESE group where results from TESE procedure served as gold standard, revealed that successful TEFNA could predict a successful TESE in all cases, with a positive predictive value of 100% (8/8). However, unsuccessful TEFNA (defined as a failure to detect enough spermatozoa for one IVF-ICSI cycle) predicted unsuccessful TESE in 87.5% (21/24) (negative predictive value).Of note, 12.5% (3/11 false negative)of patients with failed TEFNA had subsequent successful TESE. Therefore, the sensitivity of TEFNA in predicting TESE outcome was 73% (8/11) and the specificity - 100%.

A significant difference was noted between the mean number of spermatozoa collected by TEFNA vs. TESE in the TEFNA-TESE group with successful sperm retrieval, 1749±3175 (range 0-10,000) vs. 14129 +18005 (range 24-40800), respectively (p=0.033).

Thirty spouses of NOA men with successful testicular sperm collection underwent 65IVF-ICSI cycles. Comparable baseline characteristics of the women and results of controlled ovarian hyperstimulation in both groups are shown in Table 2. IVF-ICSIoutcomesof sperm originated by TEFNA or TESE are shown in Table 3. The total FR of MII oocytes by testicular sperm was 43%. TEFNA and TESE yielded testicular sperm with similar FR 43% vs. 44%, respectively (p= 0.91). The FR of sperm which was detected only by TESE after unsuccessful TEFNA was 22%(p=0.44). Cleavage and implantation rates of embryos derived from fertilization with testicular sperm retrieved by both methods were comparable (Table 3).

	Next TESE (n=11)	Next TEFNA (n=19)	P Value
Age (mean ± SD)(years)	27.8±6.2	29.8±7.5	0.5*
BMI	24.1±5.4	23.4±2.6	0.7*
Day 3 FSH (IU/L)	6.7±3	6.2±2.6	0.75*
Number of cycles	1.4±0.5	1.8±0.8	0.12**
Total Number of eggs aspirated	25.3±24.3	28.9±19.9	0.57**

* t test
** Wilcoxon

Table 2: Characteristics of the spouses of NOA men and treatment cycles.

	Next TESE^ (n=7)	Next TEFNA (n=18)	P Value*
Fertilization rate	0.44±0.27	0.43±0.2	0.91
Cleavage rate	0.28±0.2	0.3±0.19	0.77
Implantation rate**	0.15±0.14	0.18±0.2	0.72
Total implantation rate**	0.12±0.14	0.15±0.18	0.68
Cumulative Pregnancy rate**	0.43	0.44	0.94

^ sperm originated from TESE
* t test, Chi test for pregnancy rate.
** fresh and frozen-thawed embryos

Table 3: Outcome of IVF-ICSI cycles by method of testicular sperm retrieval.

Discussion

TEFNA has been suggested as a successful approach for collecting mature testicular spermatozoa in cases of NOA [8]. This method was advocated as easy to perform, requiringless advanced surgical skills, reaching deeper multiple seminiferous tubules, less invasive and well tolerated by patients. With thereported comparable record of sperm retrieval [8,10], we investigatedthe option of TEFNA as a tool for prediction of TESE outcome andthe efficacy of TEFNA vs. TESE in the same patient.

Overall, the sperm retrieval rate of TEFNA was 65.5 % for any spermatozoon and 34.4% for enough spermatozoa for one IVF-ICSI cycle (1spermtozoon/5μL). This rate was relatively low in comparison to our [15] and other's previous reports regarding TESE [13,16].

By analysis of the group of bilateral TEFNA we showed thata high correlation of sperm production between the testicles can be anticipated. Successful sperm retrieval with TEFNA of one testicle predicted favorable outcome of the second testis in 100%. Likewise, a failure to collect spermatozoa with TEFNA from one testis predicted unsuccessful outcome of the contralateral side by the same method in 95% of cases. However, our analysis with the TEFNA-TESE group showed that failed sperm retrieval by TEFNA could not fully predict the chance of sperm retrieval with TESE. A portion of 12.5% of patients with unsuccessful TEFNA was found to have detectable sperm for ICSI with TESE at the contralateral side. The inferiority of TEFNA in sperm collection in comparison to TESE may be explained on the basis of sperm quantity, as the mean number of spermatozoa retrieved by TEFNA was 8 times lower than the number collected by TESE from the contralateral testis of the same individual.The lower numbers of spermatozoa retrieved by TEFNA has been reported in the past [8], where in almost half (46%) of the successful proceduresless than 10 spermatozoa! were recovered. The superiority of TESE over TEFNA shown in this study corroborates with the report of Friedler and his colleagues (1997), where 75% of successful TESE were initially considered a failure by TEFNA. According to these authors unsuccessful TEFNA could not predict successful TESE in 36%. The discordant numbers between that report and ours may be explained by the lower number of punctures in the Friedler's study (6 per testicle) compared to 10-20 in ours.

Our study design has enabled us to compare quality of spermatozoa retrieved by both methods. Based on similar fertilization, cleavage,implantation and pregnancy rates, we may conclude that both TEFNA and TESE yielded sperm of comparable quality.

Our analysis has several weaknesses (i) the number of subjects is relatively small. (ii)the study was not blinded to the physicians and embryologists.

On the other hand this was (i)a randomized prospective study, (ii) one of few studies comparing TEFNA and TESE on the same individual at the same first surgical session of testicular sperm retrieval.

Based on our results we suggest employing the following treatment algorithm in patients with NOA. Testicular sperm retrieval may start with TEFNA of one testicle. If enough spermatozoa for an IVF cycleare found; the search for sperm may be continued with TEFNA on the other testis based on full correlation of sperm detection between the testes. In this way the patient enjoys a well-tolerated procedure and good-quality sperm. If TEFNA is unsuccessful,we suggest switching to TESE on the contralateral testis to enhance the chance to collect testicular sperm based on TESE numeral superiority.

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Disclosure

Authors do not have any financial interests (e.g. employment, significant share ownership, patent rights, consultancy, research funding, etc.) in any company or institution that might benefit from their publication.

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