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

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Research Article

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 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 decades in 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 hypergonadotropic azoospermic men the sperm retrieval rate by TESE was around 50%. Later, the use of TEFNA for NOA was introduced by [7] and 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 sites per 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
their first testicular sperm retrieval procedure at the IVF unit of Hadassah Hebrew University Mt. Scopus Medical Center in Jerusalem. The diagnosis of azospermia 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. Azospermic 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 of TESE and the laboratory handling of the specimen were described before in detail [15]. In brief, the protruding testicular mass from three distant regions of the testis was resected. The testicular tissue was placed in HEPES 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 specimen were described before in length [15]. In brief, an incision of approximately 1 cm was made through the skin and underlying layers. The protruding testicular mass from three distant regions of the testis was resected. The testicular tissue was placed in HEPES 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 examination fixed in 4% formaldehyde. In the laboratory, the specimens were transferred into new HEPES medium with 4% SSS (later media) and the first media was resected. The testicular tissue was placed in HEPES 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 examination fixed in 4% formaldehyde. In the laboratory, the specimens were transferred into new HEPES medium with 4% SSS (later media) and the first media was 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.

Methodology of TESE

The surgical procedure of TESE and the laboratory handling of the specimen were described before in detail [15]. In brief, the protruding testicular mass from three distant regions of the testis was resected. The testicular tissue was placed in HEPES 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 examination fixed 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 compare mean 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 group are 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 spermatozoon 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 TEFNA 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 spermatozoon 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 spermatozoon collected by TEFNA vs. TESE and the mean number of spermatozoon retrieved by 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 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 predict the chance of sperm retrieval with TESE. A proportion of 12.5% of patients with unsuccessful TEFNA was found to have detectable sperm for ICSI with TESE at the contralateral side by the same method in 95% of cases.

Overall, the sperm retrieval rate of TEFNA was 65.5 % for any spermatozoon and 34.4% for enough spermatozoon for one IVF-ICSI cycle (1spermatozoon/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 that 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 chances of sperm retrieval with TESE. A proportion 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 procedures less 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 yield 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. (iii)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 cycle are found; the search for sperm may be continued with TEFNA on the other testis based on TESE numeral superiority. 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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References
1. Craft I, Bennett V, Nicholson N (1993) Fertilising ability of testicular spermatozoa. See comment in PubMed Commons below Lancet 342: 864.
2. Schoysman R, Vanderzwalmen P, Nijs M, Segal L, Segal-Bertin G, et al. (1993) Pregnancy after fertilization with human testicular spermatozoa. Lancet 342: 1237.
3. Devoey P, Liu J, Nagy Z, Goossens A, Tourneay H, et al. (1995) Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. See comment in PubMed Commons below Hum Reprod 10: 1457-1460.
4. Tourneay H, Camus M, Vandervorst M, Nagy Z, Joris H, et al. (1997) Surgical sperm retrieval for intracytoplasmic sperm injection. See comment in PubMed Commons below Int J Androl 20 Suppl 3: 69-73.
5. Tourneay H, Verheyen G, Nagy P, Ubaldi F, Goossens A, et al. (1997) Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? See comment in PubMed Commons below Hum Reprod 12: 80-86.
6. Tourneay H (1999) Surgical sperm recovery for intracytoplasmic sperm injection: which method is to be preferred? See comment in PubMed Commons below Hum Reprod 14 Suppl 1: 71-81.
7. Lewin A, Weiss DB, Friedler S, Ben-Shachar I, Porat-Katz A, et al. (1996) Delivery following intracytoplasmic injection of mature sperm cells recovered by testicular fine needle aspiration in a case of hypergonadotropic azoospermia due to maturational arrest. Hum Reprod 11: 769-771.
8. Lewin A, Reubinoff B, Porat-Katz A, Weiss D, Eisenberg V, et al. (1999) Testicular fine needle aspiration: the alternative method for sperm retrieval in non-obstructive azoospermia. See comment in PubMed Commons below Hum Reprod 14: 1785-1790.
9. Friedler S, Raziel A, Strassburger D, Soffer Y, Komarovsky D, et al. (1997) Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. See comment in PubMed Commons below Hum Reprod 12: 1488-1493.
10. Fasouliotis SJ, Safran A, Porat-Katz A, Simon A, Lauffer N, et al. (2002) A high predictive value of the first testicular fine needle aspiration in patients with non-obstructive azoospermia for sperm recovery at the subsequent attempt. See comment in PubMed Commons below Hum Reprod 17: 139-142.
11. Ezeh UI, Moore HD, Cooke ID (1998) A prospective study of multiple needle biopsies versus a single open biopsy for testicular sperm extraction in men with non-obstructive azoospermia. See comment in PubMed Commons below Hum Reprod 13: 3075-3080.
12. Rosenlund B, Kvist U, Ploen L, Rozell BL, Sjöblom P, et al. (1998) A comparison between open and percutaneous needle biopsies in men with azoospermia. See comment in PubMed Commons below Hum Reprod 13: 1266-1271.
13. Van Peperstraten A, Proctor ML, Johnson NP, Phillipson G. (2008) Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. Cochrane Database Syst Rev16: CD002807.
14. Matsuyama K, Namiki M, Takahara S, Kondoh N, Takada S, et al. (1994) Clinical study of azoospermia. See comment in PubMed Commons below Int J Androl 17: 140-142.
15. Haimov-Kochman R, Imbar T, Lossos F, Nefesh I, Zentner BS, et al. (2009) Technical modification of testicular sperm extraction expedites testicular sperm retrieval. See comment in PubMed Commons below Fertil Steril 91: 1167-1171.
16. Tournaye H (2012) Male factor infertility and ART. See comment in PubMed Commons below Asian J Androl 14: 103-108.