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Research Article

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Evaluating the Outcome of Conventional Tese from Clinical Presentation in Patients with Non-Obstructive Azoospermia

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Abstract

Context: Several etiologies have been identified and some clinical factors might predict the probability of finding sperm in non-obstructive azoospermia (NOA).

Aims: Find and correlate the clinical findings to the success of testicular sperm extraction (TESE).

Settings and Design: Retrospective review of patients with NOA undergoing TESE in our department between 2012 and 2015.

Methods and Material: We analyzed the sperm retrieval rate (SRR) based on testicular size, Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), total testosterone and prolactin levels as well as on the karyotype findings. We also report the results of ICSI based on clinical features.

Statistical analysis used: Mann-Whitney U test; chi-square test; receiving operating characteristic and area under the curve; binary logistic regression.

Results: 54 patients, mean age of 34, 57 years. The overall SRR was 51, 9%. Difference between negative and positive SRR regarding mean FSH (p=0,002) and mean LH (p=0,007) was found. No difference in SRR based on karyotype findings (p=0.127) or testicular size (p=0,336) was identified. A multivariate binary regression concluded that only FSH could be used as a predictor for TESE+. A FSH value <11,0UI/L was associated with an OR of 19 for TESE+ result (95% CI 4.1-87.6). With FSH <11,0UI/L the probability of TESE+ increases to 85% and if FSH>11,0UI/L the probability of TESE+ will be 24%. 28 patients proceeded to ICSI with clinical pregnancy and live birth rates of 20,7% and 17,2%.

Conclusions: NOA patients should be fully assessed and informed about the probability of TESE+; FSH levels alone might be useful to do that. Furthermore those with normal FSH and normal testicular size can expect higher rates of sperm retrieval and live birth after ICSI. The probability of sperm retrieval in non-obstructive azoospermia should be predictable and discussed with the patients before further treatment is done, FSH might be a good marker for that. Those patients with normal FSH and normal testicular size can expect higher rates of sperm retrieval and live birth after ICSI.

Keywords

Azoospermia; FSH; Primary infertility; Sperm retrieval rate; Intra-cytoplasmic sperm injection

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Introduction

Azoospermia affects 1% of the male population [1,2]. Non-obstructive azoospermia (NOA) is diagnosed in 60% of azoospermic men and represents the absence of spermatozoa in semen because of minimal or no spermatogenesis [2,3]. Several etiologies for NOA were recognized like genetic disorders, cryptorchidism, testicular torsion, radiation and toxins [4-10]. Testicular volume, FSH and inhibin B levels have been used in prediction models but none was validated externally [11-19]. It would be of great value to estimate an individual's chance of sperm retrieval to empower patients in their decision-making. We report the results of c-TESE and the SRR, correlating them to the anatomical, laboratory and karyotype findings in the patients studied. Additionally we analyze the results of ICSI in the c-TESE positive cases.

Materials and Methods

Patients

The study consisted of a retrospective review of 54 patients with NOA who underwent TESE in our department between January 2012 and December 2015. These cases presented to a single consultant urological surgeon with a single senior clinical embryologist. Each patient underwent physical examination, semen analysis, and endocrinologic evaluation. Physical examination was performed during the first examination of each patient. Due to the lack of orchidometers in every doctor's office of our department, we measured the long axis of the testes as reference. To standardize the evaluation we considered as small testis all the reports where the major axis of testes was less than 4 cm long.

Semen analyses were performed on at least two separate occasions for each patient, and the evaluation was performed according to the methods described in the World Health Organization guidelines of 1999. Endocrinologic evaluation included assays of serum FSH, LH, total testosterone and prolactin levels. Chromosomal analysis was performed using peripheral blood lymphocyte cultures, and following the standard protocol of Giemsa banding. Additional chromosome banding (other than Giemsa banding) and fluorescent *in situ* hybridization were performed to analyze mosaicism. Screening for azoospermia factor (AZF) microdeletions was performed in all patients. We also report the fertilization cycles, clinical pregnancies and live birth rates based on the medical records of the infertility center for these patients. Approval of the ethics committee for the conduction of the present study was obtained.

Tese procedure

A TESE procedure was performed in 1-day surgery clinic under general anesthesia. A prophylactic antibiotic treatment was given and 1 week later the patients were all rechecked at outpatient clinic. A small (3.5 cm) incision was made longitudinally on the median raphe, and the incision carried down through the fascia over the largest testicle or, in case of equal volume, the testicle with the better consistency. Thereafter, the tunica vaginalis was opened and, if necessary, the testis luxated outside the scrotum. The tunica albuginea was longitudinally incised; the length of each tunica albuginea incision was 0,5cm. A

longitudinal biopsy over the whole length was taken and immediately transported to the fertility laboratory. The biopsy was then subjected to mechanical dissection and cells present in the lumen of the tubules were extracted. The obtained cell suspension was directly examined for the presence of spermatozoa. One other site of testis was similarly biopsied, while the first biopsy has been evaluating.

Second side biopsy was performed if first side sperm harvest was negative. In two patients we found sperm on the second side after a negative first side biopsy. Only with two negative biopsies in each testis allowed us to say that the TESE result was negative. When the yielding spermatozoa biopsy site has been identified, additional tissue was harvested, their number and motility were noted, and the cell suspension was cryopreserved.

Statistical analysis

Statistical evaluation was performed using the Statistical Package for the Social Sciences (SPSS Inc; Chicago, IL, USA) software program version 22.0, and $p<0.05$ was considered to be statistically significant. Data of statistical analysis is presented as mean ± standard deviation (min-max). In pairwise intergroup comparisons between hormones values Mann-Whitney U test was used. In the evaluation of data concerning testicular size and karyotype findings *chi*-square test was used. To find the best cut off value of FSH to predict the TESE result we plotted the variable based on TESE results. Additionally ROC (Receiving Operating Characteristic) and area under the curve were calculated and a binary logistic regression was performed and analyzed.

Results

When patients with obstructive type azoospermia and/or those could not be diagnosed as NOA because of missing data were ruled out, presumptive initial diagnosis of NOA was made and the patients were included in this study. Obstructive azoospermia was ruled out by physical exam: vas deferens gross consistency and diameter; and also by semen analysis based on sperm volume and pH. In our laboratory normal FSH levels were accepted as 1,5-10,0 IU/L, while mean FSH level of the patients in our study group was relatively higher: 15,59 ± 13,71 (1,60-78,7) IU/L. The same was observed in LH, with normal values accepted as 1,7-8,6 IU/L and the mean LH level in our sample lightly increased: 9,00 ± 9,27 (1,8-54,4) IU/L. Mean values for prolactin, and free testosterone were within normal limits.

All demographic data is shown in Table 1. We found statistical difference between negative vs. positive SRR regarding mean FSH (19,88 vs 11,81 IU/L; p = 0,002) and mean LH (10,83 vs 7,39 IU/L; p=0,007) as shown below (Table 2). No statistical difference was observed in other variables. Karyotype analysis and comparative values are shown in Table 3. No statistical significance was found in terms of SRR based on karyotype findings in our patients (p=0.127). There was no difference in sperm retrieval rate between normal and small testes (p=0,336) (Table 4).

Distribution of TESE results with respect to follicle stimulating hormone levels is shown in Graph 1. We plotted the FSH values based on the TESE result (Graph 2): Based on the analysis of this graph we calculated the best cut off value for FSH to predict the positivity of TESE and we obtained 11,0 UI/L. With this cut off value we have a sensitivity of 75% and a specificity of 86% to predict the TESE result with area under the curve equals to 0,807 (Graph 3). Then a binary logistic regression was calculated with FSH variable categorized in ≤11UI/L and >11UI/L with statistical significance explored in the discussion chapter (Table 5). A total of twenty eight patients proceeded to ICSI, and 29 cycles were performed. We divided them in 4 groups based on FSH levels and testes size. There were 6 clinical pregnancies, a clinical pregnancy rate (CPR) per cycle of 26% in the highest SRR group. Two pregnancies (33,3%) resulted in miscarriage. There were 5 live births, including 2 twin births, a live birth rate (LBR) per ICSI cycle of 17,2% (Table 6). In this study, no severe complications, such as acute epididymitis, scrotal haematoma and testicular hydrocele were reported, and, at this time of follow-up, no patients required hormone replacement therapy for post-operative hypogonadism.

Table 1: Demographic data and baseline characteristics of enrolled patients.

Patients (n)	54
Age, years; mean ± SD (min-max)	34,57 ± 4,14 (26-45)
Sperm retrieval rate, %	51,9 (28/54)
FSH, IU/L; mean ± SD (min-max)	15,59 ± 13,71 (1,60-78,7)
LH, IU/L; mean ± SD (min-max)	9,00 ± 9,27 (1,8-54,4)
Testosterone, ng/dL; mean ± SD (min-max)	331,36 ± 192,89 (1,70-900,0)
Prolactine, ng/mL; mean ± SD (min-max)	11,33 ± 6,59 (4,10-33,90)
Patients with history of varicocele; n (%)	3 (5,9%)
Patients with small testes; n (%)	6 (11,1%)
Patients with karyotype abnormality; n (%)	7 (13,0%)

Table 2: Comparison of hormonal levels and age within TESE positive and negative cases.

	TESE Negative	**TESE Positive**	**P**
FSH, UI/L	19,88 ± 9,30 (1,7-37,6)	11,81 ± 15,90 (1,6-78,7)	0,002
LH, UI/L	10,83 ± 7,69 (2,4-35,6)	7,39 ± 10,35 (1,8-54,4)	0,007
Testosterone, ng/dL	295,11 ± 203,00 (4,9-900,00)	362,88 ± 182,22 (1,7-752,0)	0,233
Prolactine, ng/mL	11,34 ± 3,01 (6,6-14,9)	11,33 ± 8,43 (4,1-33,9)	0,261
Age, years	34,31 ± 3,66 (27-40)	34,82 ± 4,60 (26-45)	0,917

Table 3: Sperm retrieval rates based on patient karyotype.

	Patients, n (%) n=54	**Patients with positive SRR, n (%)**	
AZFc microdeletion	1 (1,9%)	1 (100)	X^2=4,130 p=0,127
Klinefelter (47,XXY)	6 (11,1)	1 (16,7)	
Normal (46,XY)	47 (87,0)	27 (56,2)	

Table 4: Sperm retrieval rates based on testes size.

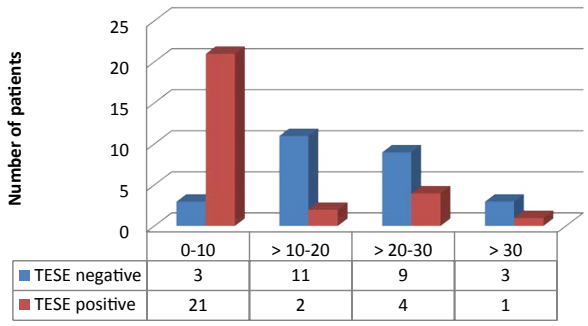
	Patients, n (%) n=54	**Patients with positive SRR, n (%)**	
Atrophic	6 (11,1)	2 (33,3)	X^2=0,927 p=0,336
Normal	48 (88,9)	24 (50,0)	

Table 5: Binary logistic regression with categorical FSH (≤11UI/L vs. >11UI/L).

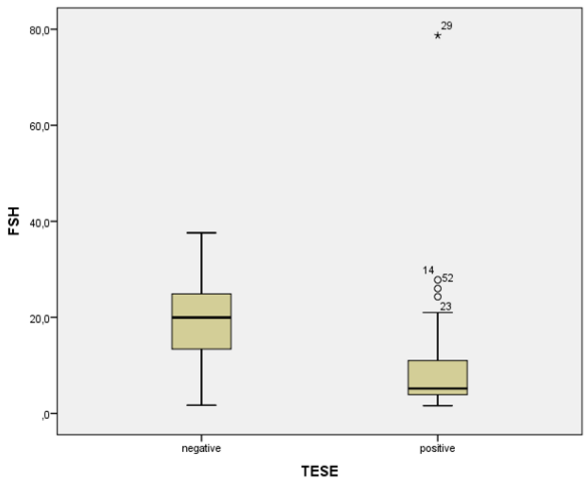
	B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**95% C.I. to Exp(B)**	
							Inferior	**Superior**
FSH	2,944	0,780	14,255	1	0,000	19,000	4,120	87,614
Constant	-1,153	0,468	6,059	1	0,014	0,316		

Table 6: Sperm retrieval rates, clinical pregnancies and live births for clinical subgroups of patients with normal and abnormal FSH or testicular size. Normal testicular size was considered to be ≥ 4 cm, and normal FSH ≤10 IU/L.

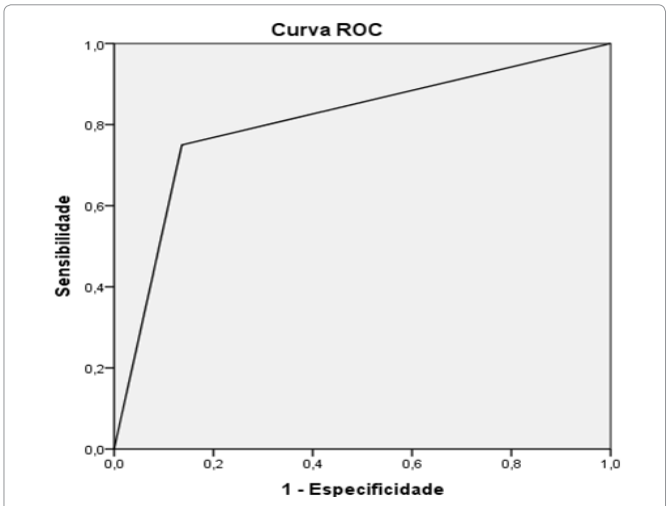
	Patients	Adequate sperm retrieval	ICSI cycles	Clinical Pregnancy	Live births
Normal FSH, normal testes	23	87,0% (20/23)	19	5	5
Increased FSH, normal testes	25	24,0% (6/25)	6	1	0
Normal FSH, atrophic testes	1	100% (1/1)	2	0	0
Increased FSH, atrophic testis	5	20% (1/5)	2	0	0
Total	54	51,9% (28/54)	29	6	5



Graph 1: Distribution of TESE positive and negative cases based on FSH levels (UI/L).



Graph 2: Box plot of FSH levels in positive and negative TESE.



Graph 3: ROC curve for FSH = 11, 0 UI/L.

Discussion

To date, NOA subjects may retrieve spermatozoa through TESE, giving the chance for an assisted reproductive technology process [20-22]. TESE combined with ICSI is the first-line treatment in NOA patients. In various studies performed, an average sperm retrieval rate of 59% has been reported using TESE similar to our study (51, 9%) [23]. Micro-TESE was developed with the aim to minimize testicular tissue loss and increase the rate of sperm retrieval [24]. Successful SRR in TESE ranges between 16.7 and 62%, whereas in micro-TESE positive SRR varies between 43 and 63% [25]. Therefore, there is still a debate in literature between these techniques, and, to date, no significantly robust data sustain the micro-TESE superiority. There are different SRRs between c-TESE and micro-TESE based on testis histology with significant difference for micro-TESE in hypospermatogenesis [19]. However, in that group sperm can be found by conventional biopsy since there are sperm distributed throughout the testes. In the Sertoli Cell only group mTESE has a significant advantage because spermatogenesis may be very focal.

Nevertheless, it should be considered that mTESE requires magnification equipment, which means higher costs, longer operative times and it is associated with a significant learning curve. Therefore, considering the overall SRR, multiple c-TESE might still represent the first-line approach to selected NOA patients. The most appropriate number of biopsies to be performed still remains controversial. To increase the chance of finding a focus of sperm production, it is advisable to take multiple samples from different sites of the testis. Furthermore, the multiple TESE approach is related to a significantly higher SRR, when compared with single TESE (49% vs. 37.5%) [26]. As no specific location in the testis was more likely to contain spermatozoa, multiple TESE has been recommended, as we perform in our center, with two biopsies of each testis [22]. Nowadays, there is still no possibility to predict the TESE outcome in NOA on the basis of simple clinical non-invasive parameters [27]. At the initial consultation, couples often want to know the preoperative likelihood of SRR success achieved by TESE [4-6]. In a recent meta-analysis, inhibin B was investigated as non-invasive marker of active spermatogenesis. Inhibin B resulted the most predictive of the spermatozoa presence, as higher level of specificity and sensibility, but not enough to be considered as one independent marker of spermatogenesis in men with NOA [28]. Diagnostic testicular biopsy has been the prognostic marker with the highest predictive value. Presence of mature spermatids has been defined as the best marker for the presence of mature spermazoa [29]. As histology is rarely obtained prior to treatment in current practice, there is a need for clinical parameters that can be used to predict the success of SSR.

FSH and testicular size are both associated with maturation arrest, and testicular failure [30]. FSH concentration has been shown

to be inversely proportional to the spermatogonial population and is associated with NOA [31]. It is, therefore, not surprising that patients in this study with either small testes or elevated FSH had a significantly worse outcome than those with normal parameters.

SRR was poorer in small testes group and elevated FSH, although not formally recommended; we could suggest that this group may benefit to underwent mTESE instead of conventional biopsy. The hormonal relationship with spermatogenesis is certainly not straightforward, with neither FSH, nor inhibin, able to predict successful sperm retrieval [32]. This is manifest by the range of levels of FSH seen in our study, and in particular the successful retrieval in 1 patient with an FSH of over 30 IU/L. Conversely, men with both normal testicular volume and normal FSH and yet late stage maturation arrest may be clinically indistinguishable from those with rete testis obstruction, particularly as FSH is normal in almost a third of those with defective spermatogenesis [4]. We calculated the best FSH level to predict the TESE result. Based on the analysis of the distribution of FSH levels in TESE positive and negative cases, and defining ROC curve we achieved 11, 0 UI/L as the best cut off value. A binary logistic regression was then taken considering the categorized FSH variable (greater or less than 11, 0 UI/L) and was statistically significant (p <.001). The model explained 45% (Nagelkerke R2) of the variance, correctly classifying 80.4% of the cases. An FSH value < 11, 0 UI/L was associated with an OR 19-fold higher for TESE+ result (95% CI 4.1-87.6). In practice and according to this model, an FSH value <11, 0 UI/L has a probability of TESE+ of 85% and if FSH> 11, 0 UI/L the probability of a TESE+ will be 24%. Further prospective studies with larger samples are needed to confirm this finding. Detection of Y chromosome micro-deletion occurred in 1, 9% of the cases and klinefelter karyotype in 11, 1% of patients. In the literature micro-deletion rates change between 8 and 18 percent [29]. It is known that the best TESE outcomes have been obtained in the AZFc group [33]. In compliance with this information, also in our study, in the only case with this deletion spermatozoa could be retrieved using TESE. As previously described in the literature patients with non-mosaic Klinefelter syndrome have sperm recovery and pregnancy rates comparable with patients having non-obstructive azoospermia and normal karyotype [34]. In our study only 16, 7% of patients with Klinefelter syndrome had sperm retrieved compared to 50% in the normal karyotype group. There was no statistical significance because the number of patients enrolled was small. We observed a clinical pregnancy and live birth rates of 26% in the best prognostic group (normal FSH and normal testis) in accordance with the previously published [35,36]. Unfortunately we weren't able to achieve any live birth in the other prognostic groups, probably due to the restricted number of patients enrolled. Although the retrospective design and the restricted number of patients involved in the study we consider it as a useful example for all the urologists involved in the treatment of azoospermic patients. The possibility to advise patients about the positive sperm retrieval based on the FSH level (greater or less than 11, 0 UI/L) is an important clinical tool presented in this study. As goals to future studies in our center we plan to confirm these findings with an increased sample, have a detailed volumetric evaluation of testis, as well as inhibin B measurement in all patients.

Conclusion

In this study we achieved a SRR with multiple c-TESE of 51, 9% supporting this low-cost technique associated with ICSI as the first line treatment of NOA patients. These patients should always be fully assessed, and informed about the probability of positive TESE. FSH levels alone might be predictive as presented in this study. Furthermore those with normal FSH and normal testicular size can expect higher rates of sperm retrieval and live birth after ICSI.

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