SURGICAL SPERM RETRIEVAL IN AZOOSPERMIA: OUTCOME AND PREDICTIVE FACTORS

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ABSTRACT

Background

Before conducting any invasive surgical sperm retrieval in men with azoospermia, it is worth looking at certain factors to predict the chance of finding viable sperm eligible for Intracytoplasmic Sperm Injection.

Objectives

Evaluating the outcome of percutaneous testicular sperm aspiration in men with azoospermia and the predictive value of serum Follicular Stimulating Hormone (FSH), testicular volume, and testicular strain elastography on the sperm retrieval rate

Methods

Cross-sectional prospective study on 73 men with azoospermia who have had their testicular volume and serum FSH measured before conducting percutaneous sperm aspiration. Fifteen men were also recruited to undergo testicular elastography. Age, FSH, testicular volume, and strain ratio on elastography were correlated with the outcome of sperm retrieval.

Results

Mean age was 36 years (range 19-69), 48% of them have had positive sperm retrieval. Twenty-five cycles of Intracytoplasmic Sperm Injection were performed, 8 (32%) have had positive β-hCG test and 4 (16%) live-birth rates. Serum FSH was the only predictive factor of percutaneous sperm retrieval ($p=0.013$) on multivariate analysis. The strain ratio was shown to be significantly different between men with negative and positive sperm aspirations ($p=0.02$).

Conclusion

Percutaneous testicular sperm aspiration has a satisfactory retrieval rate in men with azoospermia. A combination of serum FSH and testicular strain elastography seems a promising tool that can potentially be used as guidance for predicting the outcome of surgical sperm retrieval.

Keywords: *Azoospermia, Surgical sperm retrieval, Percutaneous TESA, Ultrasound Elastography.*

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INTRODUCTION

Men with azoospermia comprise approximately 10% of male infertility. Almost 60% of men with azoospermia have a primary testicular failure (also known as non-obstructive azoospermia) and the rest (40%) are due to obstructive causes [1].

Surgical sperm retrieval (SSR) combined with intracytoplasmic sperm injection (ICSI) has been a remarkable breakthrough in the management of men with Non-obstructive azoospermia (NOA) since 1994 [2]. However, many patients with NOA have had several attempts of SSR which failed to obtain normal spermatozoa. Therefore, it has soon been evident that it is only possible to harvest healthy sperm for ICSI in approximately half of the patients with NOA [3].

Several factors have been investigated to predict the outcome of SSR including serum FSH, serum LH, testicular size, serum inhibin B, and testicular histology [4,5]. The principal aim was to expect the success of testicular sperm extraction (TESE) before attempting the procedure to avoid the inconvenience and the cost of a potentially unproductive procedure.

Testicular histopathology may be regarded as one of the significant, however invasive, predictive variables for SSR in men with NOA [6, 7]. Therefore, non-invasive methods of predicting the SSR outcome have become the center of interest for many researchers. Y-chromosome microdeletion at AZFa and AZFb regions for instance has been established to be a strong negative predictive factor influencing the success of sperm extraction (even using microdissection TESE) [8]. Testicular volume and serum FSH have also been shown to some extent to correlate with the SSR rate; the higher serum FSH and the lower testicular size the less likely chance of positive SSR [9]. However, none of other non-invasive factors has been proven to be an accurate variable in predicting the outcome.

Ultrasound Elastography

Ultrasound Elastography is a simple and non-invasive technology that has recently been widely used to quantify tissue stiffness on a real-time basis [10]. The basic principle lies in the physical property (Young's modulus E) in which the viscoelasticity of soft tissues in the body changes in dimensions (strain) in response to physical stimuli (force). The tissue stiffness varies not only among different organs but also the pathologic processes within the same organ [11].

Based on the source of physical stimuli, ultrasound elastography can be divided into two main types. The first one is known as Strain Elastography (SE) in which external pressure (often manual) is applied to estimate the tissue stiffness through a change in its dimension (strain) in response to the force.[12]. The strain ratio is then calculated between two areas (namely regions of interest ROI). Stiffer tissues score a ratio of >1, which indicates a relatively low strain value [13].

The other type is called shear wave elastography (SWE) in which the ultrasound probe itself delivers an acoustic wave instead of the manual pressure to cause tissue compression and the velocity is obtained in m/sec which is equal to the square root of the tissue elasticity [14].

Testicular ultrasound elastography has been applied to evaluate testicular cancer, infarction, and torsion[15-18]. Investigating male infertility using testicular elastography however is limited [10, 19, 20]. To the best of our knowledge, there seems to be only one study utilizing testicular elastography in differentiating OA from NOA[20]. However, no similar study is available to use strain elastography in predicting the outcome of testicular sperm aspiration in men with azoospermia.

Current Study

It is worth looking at a combination of non-invasive variables that can easily be applied to predict the SSR and to be utilized during counseling of infertile couples before conducting any invasive procedure. In our study, we aim at evaluating the outcome of percutaneous TESA in men with azoospermia and the predictive value of serum FSH, testicular volume, and testicular strain elastography on the sperm retrieval rate.

PATIENT AND METHODS

This is a cross-sectional study which was conducted prospectively on (n=73) infertile men who presented with primary infertility and were found to have azoospermia at the Sulaimani surgical teaching hospital and the fertility clinic at Royal Hospital, Sulaimaniyah, Kurdistan region-Iraq between October 2018 to November 2019. Ethical approval has been obtained from Research Ethics Committee before conducting the study. Azoospermia was confirmed by analyzing two consecutive semen samples based on WHO guidelines [21]. Following approval by the ethics committee and obtaining informed consent from participants with azoospermia, they were recruited in the study irrespective of the etiology whether obstructive or

non-obstructive. Patients who have had previous open testicular biopsy or open testicular sperm extraction (i.e., conventional TESE) were excluded to avoid bias of changing the elasticity of the testes resulted from previous procedures. Patients with hypogonadotrophic hypogonadism and azoospermia were also excluded from our study and hormonal treatment was offered instead.

All patients underwent clinical history, physical examination including testicular examination and volume determination with orchimetre for those who have not have volume measurement with ultrasound. Hormonal assay (serum FSH, serum LH, serum testosterone, and serum prolactin) was obtained in only 58 patients. The reference ranges were agreed to be for FSH (1.5 to 12.5 mIU/mL), LH (1.5 to 8.5 mIU/mL), testosterone (3-10 ng/mL) and (2.0-15.8 ng/mL) for prolactin.

Real-time Strain Elastography

A group of patients (n=15 i.e., 30 testes in total) agreed to have a testicular ultrasound Elastography using (Phillips iU22 Xmatrix 2015 system) with an L12-5 linear high resolution probe by a single radiologist with more than 4years experience in real-time elastography (RTE). The strain ratio was then calculated between two areas (namely regions of interest ROI in the paranchyma of the testes and surrounding scrotal skin). Testicular parenchyma of each testis was examined by RTE to study its strain pattern; namely high strain (soft) and low strain (hard). Then each strain pattern was compared to the scrotal skin to obtain the strain ratio (20). Moreover, six (6) normal individuals with normal fertility patterns were recruited as a control to compare their strain ratio with azoospermia men.

In addition, using the Lambert formula (Length x Height x Width x 0.71), testicular volumes were calculated on both sides for those who have had ultrasound elastography (22). For the rest of the subjects, an orchimetre was used to determine testicular volume.

Percutaneous testicular sperm aspiration (pTESA) procedure

Patients were provided with written informed consent after explaining the procedure. Similar techniqueby Gottschalk-Sabaget and colleagues (23) were used which were initially described and utilized for diagnostic purposes. Spermatic cord block was performed initially using 10-15 ml local anesthetic (2% Lidocaine) infiltration. The procedure includes percutaneous testicular tissue aspiration from both testicles using an 18G butterfly needle connected to a 20 ml syringe containing a 3-5ml human tubal fluid medium.

Once the needle is inserted into testicular paranchymal tissue a negative pressure is applied by withdrawing the 20ml syringe and an artery forceps is applied to clamp the butterfly cannula and maintain the negative pressure. The testicular tissue was aspirated several times in different directions to aspirate as much testicular tissue as possible. Once satisfied with the aspiration the needle is withdrawn from testicular parenchyma to the exterior and aspirated tissue is further withdrawn with non-toothed dissecting forceps. This procedure is repeated on the upper, mid, and lower poles of each testis until satisfactory tissue is aspirated appropriate for embryology laboratory examination and testicular biopsy when needed.

The testicular tissue is then prepared, washed out, centrifuged, and minced by an embryologist, and then placed on a slide with a coverslip and examined under a phase-contrast microscope looking for mature spermatozoa. If spermatozoa are found the quality of spermatozoa is determined whether suitable for ICSI or not.

The presence of mature spermatozoa with an acceptable morphology and/or motility is regarded as a positive pTESA outcome. Once the embryologist is satisfied with the sperm quality, the patient is informed about the result and the tissue is cryopreserved in liquid nitrogen for future use. In case the spermatozoa were not found at all or dysmorphic and abnormal spermatozoa were found, which are not eligible for ICSI, the remaining tissue was sent for histopathological examination and evaluated by a single experienced pathologist to determine the histological type of the testicular tissue.

Statistical analysis

Independent t-test and non-parametric (Mann-Whitney U Test) was used to compare means of normally and non-normally distributed data respectively. Spearman test was used to test linear correlations. Multivariate logistic regression analysis was also used to determine the independent predictive significance of the main variables (age, testicular volume, and FSH). Binary logistic regression was utilized for multivariate analysis. SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used and in all tests, a p-value lower than

0.05 was considered statistically significant.

RESULTS

Patient characteristics are shown in Table (1). A total number of men with azoospermia recruited in our study is seventy-three (73) patients with a mean age of 36 years ranging from (19-69 years). The average duration of infertility was 7.2 years (range 1-20 years). Among these almost half 35 (48%) of them have had positive sperm retrieval through pTESA, whereas in 38 patients (52%) no mature sperms were retrieved using the same procedure.

Among those men who have had positive TESA, twenty-five (71%) of them decided to try in vitro fertilization (IVF) through a cycle of intracytoplasmic sperm injection (ICSI). Out of Twenty five (25) ICSI cycles, only 8 (32%) of them had a positive pregnancy test, whereas 10 (40%) of them, unfortunately, failed to achieve embryo implantation and therefore had negative B-hCG test. Moreover, 7 cycles ended up with no transfer for various reasons mainly because of ovarian hyperstimulation syndrome, and embryos were frozen for future transfer. Finally, four cycles out of eight clinically positive pregnancies continue to end up with a live birth (Table1).

We were able to measure bilateral testicular volume of all patients with a mean volume of 12.4ml and a range of 2.8 to 21ml. It is worth mentioning that no significant difference was noted between right and left testicular volumes, therefore average volumes were taken for each man. Serum FSH was measured in only 58 patients with an average of 16.6 mIU/ml ranging from (1-78mIU/ml).

The difference between the two groups of patients with negative and positive pTESA was compared with regards to their age, testicular volume, and serum FSH level (Table 2). The mean age between the two groups was compared using the Mann-Whitney U Test, which showed statistically no significant difference between them (P-value of 0.603). Mean testicular volume was also compared using an independent sample t-Test which shows statistically significant difference between the pTESA positive and negative groups (P-value of 0.035). Finally, the serum FSH level between the two groups of patients was compared again using Mann-Whitney U Test which showed a very highly significant difference (P-value= 0.000). However, when multivariate analysis performed as shown in Table 2, the only factor was the FSH which was shown to be statistically significant (p=0.013). With regards to the fifteen men (i.e. 30 testes) with azoospermia who chose to undergo real-time strain elastography, eleven (11) of them were shown to have negative pTESA, whereas in 4 of them we were able to find sperm suitable for ICSI. As mentioned earlier, six (6) normal men were also used as a control for comparison of their strain ratios. The strain ratio among men with negative and positive pTESA was shown to be significantly different (median 0.27 and 0.7 respectively) and (p-value of 0.02). While the strain ratio of control men (0.8) with those having positive pTESA (0.7) was shown to be almost similar (Table 3).

Using spearman's bivariate correlation test, there seems a statistically significant (p=0.000) negative linear correlation (r=-0.586) between the serum FSH level and testicular volume as is shown in Figure 1.

Testicular histology (Table 4) was examined in 18 men (47%) who have had negative pTESA. Bilateral testicular histology was undertaken in 14 patients only. However, in 4 men only one testis was examined because testicular size was extremely small to perform pTESA (32 testes in total). Comparing the mean age, testicular volume, and serum FSH among different groups of testicular histopathology with hypospermatogenesis, early and late maturation arrest, we have found only serum FSH was different between and among different histological patterns, however, the difference was not shown to be statistically significant (p=0.47).

Table (4) shows various histology patterns in 18 men (32 testes) with azoospermia and negative sperm retrieval by pTESA. Mean age, testicular volume, and Serum FSH were compared among various histopathological groups.

Table 1 .General characteristics of men with azoospermia who have undergone pTESA.

Variables		Value(s)	
Total number of patients		73	
Percutaneous testicular sperm extraction (pTESA)			
Positive n(%)		35 (47.9%)	
Negative n(%)		38 (52.1%)	
ICSI cycle		25	
B-hCG test — **Positive**		8(32%)	
B-hCG test — **Negative**		10 (40%)	
B-hCG test — **No transfer**		7	
Live birth		4 (16%)	
	Number of men (n)	**Mean (±SD)**	**Range**
Age (year)	73	36 (9.5)	(19-65)
Infertility duration (year)	73	7.2 (5.3)	(1-20)
Testicular volume (ml)	73	12.4 (5.1)	(2.8-21.1)
Serum FSH (mIU/ml)	58	16.6 (15.6)	(1-78)

Table 2. Comparing two groups of patients (positive and negative pTESA) regarding their age, testicular volume, and FSH level.

Variables	pTESA (Mean+/-SD)		P-value	pTESA n(%)		p-value multivariate analysis
	Positive	Negative		Positive	Negative	
Age (year)	36(10)	37(9)	0.60	35(48%)	38(52%)	0.514
Testicular Volume (ml)	13.7(4.9)	11.2(5.1)	0.03	35(48%)	38(52%)	0.482
Serum FSH (mIU/ml)	9.1(8.4)	22.4(17)	0.00	24(41%)	34(59%)	0.013

Table 3. Shows strain ratio, testicular volume, FSH of 15 patients who have undergone real time elastography.

Variables n(15)	Negative pTESA n(11)	Positive pTESA n(4)	Control n(6)
	Median	**Median**	**Median**
Strain Ratio	0.27	0.7	0.8
Volume (ml)	10.5	13	n/a
Serum FSH (mIU/ml)	25.7	7.4	n/a*

n/a: Not applicable

Table 4. Comparing the mean age, testicular volume, and serum FSH among different groups of testicular histopathology with hypospermatogenesis

Histology type	Normal	Hypo spermatogenesis	Late maturation arrest	Early maturation arrest	Sertoli cell-only	P-value
Number of testesn(%)	3 (9%)	15 (47%)	5 (16%)	7 (22%)	2 (6%)	
Mean age (year)	n/a	37	39	38	n/a	**0.786**
Mean testicular Volume (ml)	n/a	11	10	11	n/a	**0.772**
Mean Serum FSH (mIU/ml)	n/a	23	29	20	n/a	**0.47**

n/a: Not applicable

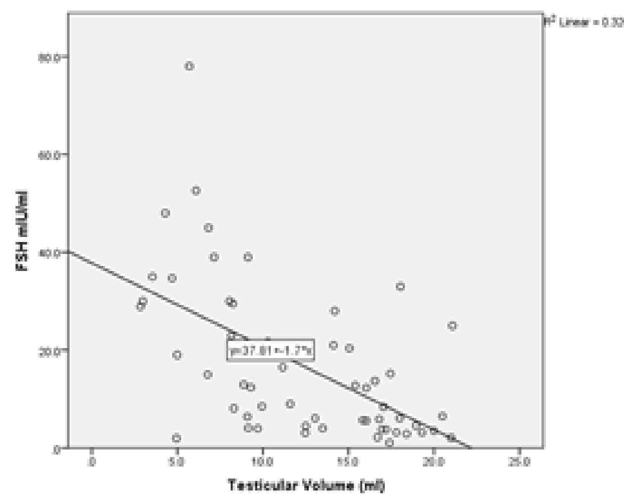

Figure 1. Scatter plot with a line graph showing a correlation between serum FSH (mIU/ml) and testicular volume (ml) in 73 men with azoospermia.

DISCUSSION

Our study is an attempt to investigate the outcome of a simple non-invasive procedure namely percutaneous TESA and the prediction value of combined pre-operative factors namely FSH, testicular volume, and strain elastography in estimating the individual's chance of obtaining a healthy sperm eligible for ICSI. This is of crucial importance during counseling regarding the chance of SSR success to aid in patient's decision making.

We have been able to retrieve mature sperm in approximately 48% of our men with azoospermia using the simplest technique described in the literature. Our results are equivalent to the majority of the previous success rates described in an update by Tournaye (2010), in which there is virtually a fifty percent chance of successful sperm retrieval in men with NOA [24]. However, men who have had positive pTESA were not investigated histologically whether having obstructive or non-obstructive azoospermia. Therefore, the retrieval rate might also be attributed to those men with OA.

Regarding age, our finding seems to show that patient age is not a predictor of SSR outcome. In a prediction model by Cissen et al and colleagues in 2016, they demonstrate an opposite finding in which combined men's age, low FSH and LH, and high testosterone along with the absence of AZFc regions can be a predictor of successful sperm retrieval [25]. A similar finding was also observed by Ramasamy et al (2013) in a logistic regression analysis, in which male age along with Klinefelter syndrome and cryptorchism appear to be a significant predictor of SSR [26]. The difference between our results and literature might be due to the variation in the population age among our community

and western society, in which the age range in men seeking fertility help in our community generally seems to be younger.

Looking at the serum FSH, our results showed a significant difference between two groups of patients, namely negative pTESA and Positive pTESA. This was also the case in multivariate analysis in which FSH was the only predictive factor for the success of SSR. This implies that the lower the FSH level the more likely to retrieve mature sperm.

There seems a wide range of discrepancies in the predictive value of FSH in literature. In a meta-analysis by Yang and colleagues [27], serum FSH in men with NOA has been shown as an independent predictive factor for SSR particularly in young patients and certain geographical areas (East Asia). Similarly, the prediction model of Cissen et al (2016) showed the predictive value of FSH combined with aforementioned factors [25]. However, the metanalysis was unable to demonstrate a clear cut-off value for FSH to predict the outcome.

As far as the testicular volume is concerned, first, our data demonstrated a negative correlation between Serum FSH and testicular volume as shown in the scatter plot, which entails that the smaller the testicular volume, the higher FSH level in men with azoospermia, which is a well-known fact. Second, although the testicular volume has individually been shown to be significantly different between pTESA positive and negative patients, it was not shown to be a significant predictor of the outcome when combined with other factors in the multivariate analysis.

Regarding the testicular volume in literature, in multivariate logistic regression analysis in 149 men with NOA, only total testicular volume, serum FSH and inhibin B have been shown to affect the outcome of SSR [28]. On the contrary in the model investigated by Ramasamy et al, (2013) recruiting 1026 patients, neither FSH nor testicular volume was shown to predict the outcome of SSR [26].

In brief, our findings support the argument that FSH level, in particular, could potentially predict the outcome of pTESA, which was also reiterated by a recent study conducted on 170 patients [29]. It is difficult to conclude with regards to a certain threshold beyond which confidently assume the negative or positive outcome.

Although limited numbers of men (15 in total i.e. 30 testes) were recruited for strain elastography, the preliminary data demonstrated promising results in which there is a significant difference in the stiffness of testes with negative pTESA compared with those who have had positive sperm retrieval. The only study we have found in literature was the one conducted by Li et al (2012), which revealed a significant difference between strain elastography of men with NOA as opposed to OA [20]. In addition, they found different strain patterns among various testicular volumes and histological models of the testes.

Our results, however, are not precisely comparable to Li et al (2012) since the difference was revealed among two different groups of men; namely positive and Negative pTESA. Those with positive TESA were not diagnosed histologically whether obstructive or non-obstructive. Nonetheless, the strain ratio of men with positive TESA was similar to control men, which implies that those who have had positive sperm harvest will be expected to have a strain ratio similar to normal fertile men. We, therefore, suggest the predictive value of strain elastography is yet to be determined by recruiting a larger number of men with the non obstructive azoospermia group.

Finally, the histological pattern of men with negative sperm retrieval was mainly hypospermatogenesis followed by maturation arrest (MA) and Sertoli cell-only syndrome (SCOS). In a retrospective analysis of 111 men with NOA, it has been shown that hypospermatogensis is associated with a higher rate of positive sperm retrieval compared with MA and SCOS [7]. This is contrary to our findings in which the majority (47%) of our histology results in men with negative pTESA showed hypospermatogensis. This is possibly due to the fact that men with positive sperm retrieval in our study did not have testicular histology which is expected to show hypospermatogensis pattern in the majority. Therefore, the real picture will have only been evident if both arms would have had testicular histopathology. Furthermore, a significant number of our patients who have had negative pTESA(38%) were shown to have MA, which is consistent with the findings in the relevant literature [7, 29].

The limitations of our study are, firstly, small sample size particularly in those who have had strain elastography which made it difficult to draw a strong conclusion regarding its predictive value. Secondly, we were not able to evaluate and correlate the result of testicular

histopathology of patients with negative SSR to their testicular strain ratio results on strain elastography due to limited number of cases. Finally, although pTESA is a simple and non-invasive method of SSR we chose in our study, the sperm yield is not comparable with other methods of SSR such as microTESE. Therefore, it seems prudent to combine elastography and other non-invasive factors to predict the outcome of more invasive procedures such as micro-TESE in the future.

In conclusion, the current study has demonstrated that using simple, minimally invasive percutaneous testicular sperm aspiration (pTESA) technique can yield a satisfactory retrieval rate in men withazoospermia. Moreover, serum FSH seems to be the most significant predictor of pTESA compared with other factors such as testicular volume and mens age. Lastly, testicular strain elastography can potentially be used as guidance for predicting the outcome of SSR combined with serum FSH.

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