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RESEARCH

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Testicular sperm aspiration (TESA) outcome in Middle Eastern patients with non-obstructive azoospermia: a retrospective cohort study

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Abstract

Background Infertility is an important health problem, affecting couples worldwide. Non-obstructive azoospermia is the most severe form of azoospermia, which is mostly idiopathic or caused by different causes such as chemotherapy and genetic disorders. Testicular fine needle aspiration (or testicular sperm aspiration (TESA)) is simple, cost-effective and less invasive than testicular sperm extraction.

Materials and methods Three hundred twenty Middle Eastern patients with NOA were recruited in this study. The patients underwent routine infertility assessment including medical, surgical, and reproductive history, physical examination, semen analysis, and hormonal profile including FSH, Testosterone, and inhibin B in addition to Genetic assessment including karyotype and Y-chromosome micro-deletion.

Results Testicular sperm aspiration was positive in 70 patients (22.18%). Serum FSH levels were clearly elevated in the patients with negative sperm retrieval (mean = 21.39 U/L), while they were reduced in the patients with positive sperm retrieval (mean = 14.61 U/L). Testosterone value did not clearly correlate with the results of testicular sperm aspiration in the two groups of patients, and testicular volume was normal for most of the patients in the two groups. Patients with Y-chromosome micro-deletion were 11.22% of the total patients studied and they had negative TESA results, while 13.12% of patients had Klinefelter Syndrome and their TESA results were negative.

Conclusion We confirmed that there are many factors that negatively affect Testicular sperm aspiration results: high FSH and low inhibin B levels, smoking, and genetic disorders. Despite the absence of sperm in the semen, some NOA patients have a chance to have children by using this technique.

Keywords Infertility, Testicular sperm aspiration, Non-obstructive azoospermia, FSH

Background

Infertility is an important health problem affecting one-fifth to one-sixth of couples at reproductive age worldwide [1]. It can be defined as the disability to fulfill pregnancy after 12 months or more of regular sexual intercourse with no contraceptive measures taken, and 40% of infertility causes are combined between male and female factors [2].

In the female reproductive system: infertility can result from abnormalities in the ovaries, fallopian tubes, or uterus, in addition to hormonal problems. As for the males, it is caused by obstruction of the reproductive

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tracts that may lead to ejaculation problems; disorders in the hormones produced by the endocrine system, in addition to abnormalities in the quality and function of sperm [2].

Environmental factors and unhealthy lifestyles including smoking, heavy alcohol intake, obesity, pollution, and toxins can directly damage the gametes [2].

Azoospermia, which is the most severe form of male factor infertility [3] is defined as the absence of spermatozoa in the semen [4]. It is observed in 1% of the general population and in 10–15% of infertile men [5], and is classified into obstructive azoospermia (OA) and secretory (non-obstructive) azoospermia (NOA) [6].

OA, which is observed in 40% of all azoospermia cases [7] is defined as a pathologic condition in which bilateral distal or proximal genital ducts are obstructed, while the process of spermatogenic is unaffected [8]. Whereas NOA is caused by the failure of spermatogenesis and it is the most severe form of male infertility [9]. The majority of cases of NOA are idiopathic, but there are some known etiologies including genetic disorders, radiation or chemotherapy, developmental or structural abnormalities, and hormonal imbalances [10]. Previously men with azoospermia were not able to be biological fathers, especially men with NOA. However, the development of assisted reproductive technologies made that possible [3].

Historically, both testicular sperm extraction (TESE) and testicular fine needle aspiration (FNA) or (TESA) were used [11].

TESE, which is actually a surgical biopsy of the testis, is an invasive technique and may cause testicular atrophy in the future [12]. On the other side, TESA which was first described in 1930, is considered an easy and safe procedure for obtaining intratesticular fluid for research the physiological and pathological properties of the testis [13]. In comparison with other techniques, FNA is simple, cost-effective, and minimally invasive [7]. The literature contains details of the FNA procedure and other techniques, especially TESE. Indeed, there is not enough information about the results of TESA in men with NOA.

Our objective was to evaluate the results of TESA in Middle Eastern patients with NOA and elucidate its relationship to other parameters such as sex hormone levels.

Material and methods

Study design

A single-center retrospective cohort study.

Setting

We studied patients with non-obstructive azoospermia (NOA) who were referred to the Assisted Reproduction Unit of Orient Hospital in Damascus, Syria, between March 2013 and February 2022. We manually reviewed the recorded data of the screened patients.

Participants

This study included 320 men between 16 and 77 years of age with a diagnosis of non-obstructive azoospermia (NOA). We measured the sample size according to Richards's equation.

Patients were classified according to the results of testicular sperm aspiration (TESA) into two groups:

Group A: performed positive TESA results (presence of testicular spermatozoa) that included 70 patients.

Group B: performed negative TESA results (absence of testicular spermatozoa) that included 250 patients.

The diagnosis of NOA was based on clinical findings and several parameters such as FSH and testicular volume, but no standard method was used.

Both groups underwent routine infertility assessments including medical, surgical, and reproductive history, semen analysis, and hormonal profile including Follicle-stimulating hormone (FSH) and testosterone (T). Serum inhibin B was measured for some patients.

Hormone analysis was done by using enzyme-linked immunosorbent assay (ELISA). Semen analysis was performed according to the WHO laboratory manual for the examination of human semen and semen-cervical interaction, 2010.

All patients in both study groups were screened for age, infertility duration, and smoking habit. Scrotal sonography was performed to detect testicular volume and the presence of varicocele, using a 7.5-MHz probe (GE Voluson E800). Genetic assessment including karyotype and Y-chromosome micro-deletion was documented.

Inclusion criteria

1. Males with non-obstructive azoospermia (NOA)
2. Hormonal profile that includes testosterone (T) and follicle-stimulating hormone (FSH)

Exclusion criteria

1. The presence of sperm in the seminal fluid
2. Obstructive azoospermia cases (OA)
3. Incomplete hormonal profile (does not include T or FSH or both)

Ethics statement

The Reproduction Unit of Orient Hospital approved the use of human subjects for this study.

Clinical assessment

All the patients had been clinically assisted by evaluating testicle volume and the existence of varicocele. Testicular volume was classified into three classes: small (S), medium (M), and normal (N).

The Sarteschi/Liguori classification divides varicoceles into five grades [14]:

Grade 1: characterized by inguinal reflux in non-enlarging vessels while standing during the Valsalva maneuver, Grade 2: characterized by varicosities with reflux only while standing during Valsalva that reach the superior pole of the testis.

Grade 3: characterized by varicosities also around the testis with reflux in standing position and during Valsalva maneuvre. Grade 4: diagnosed if there are varicosities in supine and standing position, which enlarge during Valsalva. Reflux is already present at rest and increases during Valsalva. Testicular hypotrophy may be present.

Grade 5: characterized by enlarged veins in supine and standing positions [14].

Testicular FNA technique (TESA)

All testicular sperm aspiration procedures were done using the procedure described by Jarow et al. [15]. Subjects were placed in the supine position, and draped with a sterile cloth. After adequate anesthesia was obtained, the skin overlying the testis was cleaned with betadine 3 times. A 23-gauge butterfly needle with tubing attached to a 20-ml syringe via stopcock was inserted into the epididymis on each side followed by an immediate microscopic search of the aspirate to rule out the presence of spermatozoa. In the absence of spermatozoa in the epididymal aspirate, multiple punctures (mean of 8, range 5–15 per testis) and aspirations were performed systematically throughout the whole testis on both sides. TESA can cause some discomfort for a few days, as it is a slightly complex procedure, local bleeding or infection may complicate TESA in 1% or less of the procedures. The symptoms will be pain, swelling, or fever. The treatment is with antibiotics. Each testis was divided into three zones: upper (U), middle (M), and lower (L) (Fig. 1).

Data management and statistical analysis

The descriptive analysis of the sample vocabulary was adopted by creating frequency tables, percentages, arithmetic averages, and standard deviations for the variables included in the study.

We used an independent sample Mann–Whitney *U* test in our statistical analysis to differentiate between the group of positive TESA and the one with negative TESA, because the two samples were not normally distributed in the two groups. A p value of less than 0.05 was considered statistically significant for all tests. Statistical analysis was performed with the SPSS (Statistical Package for Social Sciences, version 23).

We have indicated the abbreviations mentioned in this paragraph in a separate table below (Table 1).

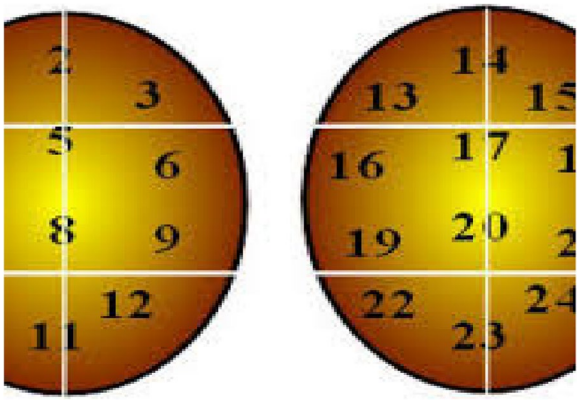

Fig. 1 Testicular mapping-Orient Hospital, Each testicle was divided into three regions: upper, middle, and lower

Table 1 Abbreviations

NOA	Non-obstructive azoospermia
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
E2	Estradiol
T	Testosterone
PRL	Prolactin
TSH	Thyroid stimulating hormone
TESA	Testicular sperm aspiration
SPSS	Statistical Package for Social Sciences, version 23

Results

Spermatozoa were retrieved in 70 patients (22.18%), classified as group A and were absent in 250 patients (77.81%) classified as group B.

Mean serum testosterone ± SD was slightly higher in group A than in group B 13.87 versus 12.87, respectively, but without any significant value ($p=0.566$). The mean serum FSH ± SD was significantly, lower in group A than in group B 14.61 versus 21.39 respectively ($p=0.001$), as illustrated in Table 2.

Demographic characteristics of patients were similar in the two groups in age and infertility duration as shown in Table 2.

Demographic characteristics for all patients are also outlined in Table 3. The percentage of smokers among the studied patients was 43.8% (140\320 patients) (Fig. 2).

Table 2 Demographic and clinical characteristics of patients in both groups (A, B)

Variants	Group A Positive TESA results	Group B Negative TESA results	*P* value
Patient number	70	250	
Age	38.08 ± 11.04	36.98 ± 9.01	NS
Infertility duration	5.23 ± 4.53	6.97 ± 5.86	NS
FSH	14.61 ± 12.79	21.39 ± 14.1	0.001
T	13.87 ± 9.5	12.87 ± 9.05	NS

FSH follicular stimulating hormone (IU/L), *T* testosterone (ng/dl), the numbers expressed for hormone values are the arithmetic mean ± SD

Table 3 Demographic characteristics of all patients

	N	Minimum	Maximum	Mean ± SD
Age (year)	320	16.00	77.00	37.4063 ± 9.1
infertility period (year)	315	1.7	45.00	6.5808 ± 5.41

And 109/140 (77.85%) of smoker patients had negative TESA results.

Serum inhibin B level was documented for 22/320 patients (6.9%) and the mean ± SD value was 48.25 ± 43.52 pg/ml, and 95.4% of them had negative TESA results.

38 of 320 patients (13.12%) who did (KT) test were diagnosed with Klinefelter syndrome (47XXY) and all of them had negative sperm retrieval. Patients with Y-chromosome micro-deletion were 11.22% of the total patients studied and they had negative TESA results (Table 4).

Seventeen patients had varicocele of different stages, and 88.23% of them had negative TESA results as shown in Fig. 3.

The results of TESA according to testicular size are documented in Table 5.

We mentioned chronic diseases like hypertension (HTN) and diabetes mellitus (DM). Patients with a previous diagnosis of HTN were 6 (1.9%) men, whereas those with DM were 15 (4.7%) men. We also mentioned

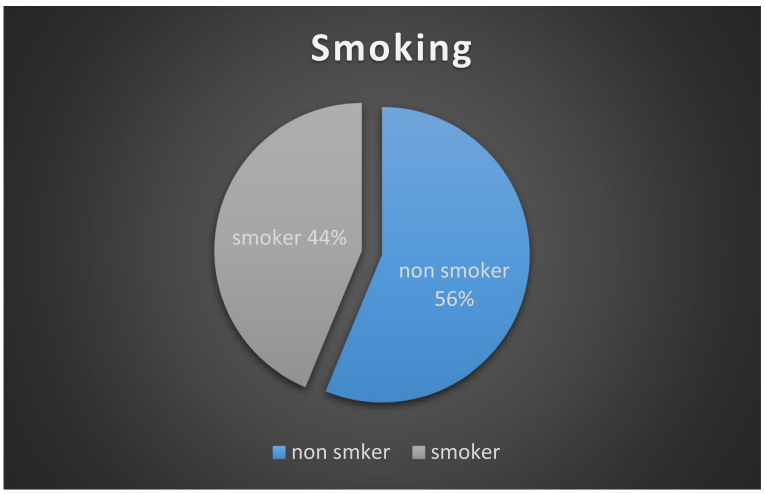

Fig. 2 Classification of total patients into smokers or nonsmokers, so most of the patients were non-smokers

Table 4 TESA result according to genetic assessment in studied patients

Test	Number of patients	Percentage	TESA result
Kleinfelter XXY	38	11.87%	N
Kleinfelter Mosaic XXY, XY	4	1.25%	N
Y-Chromosome Microdeletion AZFa	5	1.56%	N
Y-Chromosome Microdeletion AZFb	18	5.6%	N
Y-Chromosome Microdeletion AZFc	12	3.75%	N
Y-Chromosome Microdeletion AZFb + c	1	0.31%	N

N negative sperm retrieval (negative TESA result)

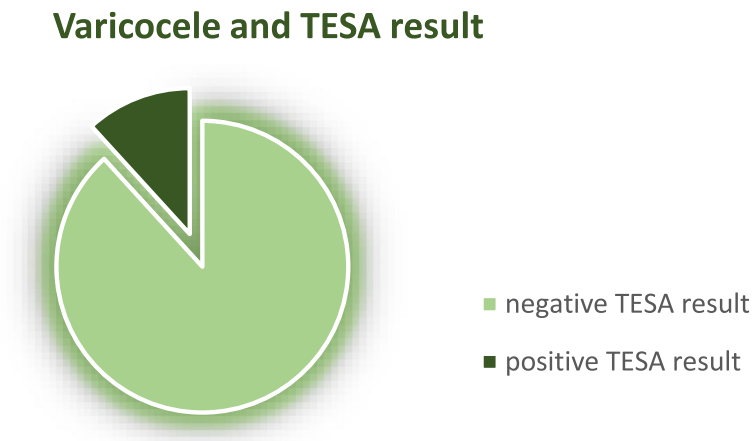

Fig. 3 Illustrated TESA result according to the presence of varicocele, It is noted that the TESA result was negative in most patients who had varicocele

Table 5 TESA result according to testicular volume

Negative result	Positive result	Variants	
25 (10%)	4 (5.7%)	[1–5]	Testicular volume(ml)
65 (26%)	9 (12.8%)	[6–15]	
160(64%)	57(81.42%)	> 15	

[1–5]small volume, [6–15] medium volume, and > *15* normal volume

mumps, especially in childhood and it was documented in 8/320 (2.5%) men.

We included the surgical treatment of varicocele, hernias, and other operations on the testis. Varicocele operations were identified in 74/320 patients (23.1%) and the hernias operations rate was 7.8% (25\320 patients).

We referred to testicular biopsy as a surgical procedure that was performed in 75 me before referring Orient Hospital (23.43%) and their results revealed that 5/75 (6.7%) had a testicular structure consistent with hypo spermatogenesis, 43/75 (57.3%) with maturation arrest, and 27/75 (36%) with Sertoli cell only pattern.

Discussion

About two-thirds of azoospermia cases are caused by severe spermatogenic dysfunction and it is usually called "non-obstructive azoospermia" (NOA) (Caroppo et al. 2022) [16]. Fortunately, the development of intra-cytoplasmic sperm injection (ICSI) and testicular sperm aspiration (TESA) enabled patients to be biological fathers (Ma et al. 2018) [17]. But to find sperm in patients with non-obstructive azoospermia (NOA) is more difficult than it is in those with obstructive azoospermia (OA); this is because sperm production decreases in men with NOA, and as a consequence, spermatogenesis can be patchy," occurring in islands" (Herndon et al. 2022) [18]. However, many factors have been suggested to be predictors of sperm extraction, including serum FSH, Testosterone, inhibin B levels, age, and testicular volume [17]. FSH acts directly to stimulate germ cell number and indirectly to increase androgen production [19]. Severe testicular histopathological patterns in men with NOA are associated with high serum FSH levels and small testicular volumes [20]. In our study, 62.8% of patients had serum FSH levels above the normal range in adults and this is in strong agreement with the previous study [20]. We also found that FSH levels were higher than the normal range in patients with negative sperm retrieval, whereas patients with positive sperm retrieval had lower values. These findings are consistent with the study of Nowroozi, et al. [21] on their study on 385 men showed that positive sperm retrieval in patients with serum FSH < 15 IU/L was 4.8 times higher than those with FSH ≥ 20 IU/l ($P < 0.001$).

Testis volume and testosterone did not differ between patients with and without mature sperm in TESA samples. [21] We did not find a significant correlation between testosterone levels and TESA results ($p = 0.566$).

As for testicular volume, in the positive sperm retrieval group(A), average testicular volume was significantly greater than its value in the second group, but despite that, the normal testicular volume remained the most common in both groups, so our results agree with the previous study [21]

Inhibin B is an indirect marker of spermatogenesis in men with non-obstructive azoospermia (NOA) [22]. In our study, serum inhibin B level was lower than its value for healthy adult males (IQR 125–215 pg/ml), and 95.4% of them had negative TESA results. Although inhibin B is a valuable index of spermatogenesis, the

measurement of its levels is still limited because of its clinical relevance for individual patients [23]. There is an association between male partner age and the incidence of birth defects and chromosomal abnormalities [24]. In our study, we did not find a clear link between advanced men's age and testicular FNA (TESA) results. As for smoking, 109/140 of smoker patients (77.85%) had negative TESA results, and this agrees with the results of a previous study, as a meta-analysis of 20 observational studies, male smokers were more likely to have low sperm counts [25].

According to genetic abnormalities, Klinefelter syndrome (KS) (47,XXY) is the most common genetic disorder that causes NOA, where it has been estimated in 10–12% of azoospermic subjects [26, 27]. In our study, all of the patients with KS had negative sperm retrieval. On the other hand, some previous studies have shown that more than 200 healthy offspring were born worldwide from KS fathers and only a few cases were reported [28–30]. In addition, it was found that the genes located on the proximal part of the long arm of the Y chromosome play an important role in the process of spermatogenesis. This genetic defect accounts for 14% of patients with non-obstructive azoospermia. [31] In our study, 11.22% of patients had Y chromosome micro-deletion (YCM), and all of them had negative TESA results. This is consistent with a previous study (Alhalabi M, 0.2014) which found that the complete deletion of the AZFa, AZFb, AZFb + c, and AZFb + d areas is inevitably associated with azoospermia in the semen without the possibility of sperm retrieval from the testicles [31].

NOA is caused by testicular failure, the presence of varicocele is one of the reasons, and it is found in 5–10% of men with NOA [32]. In our study, we found that most patients who had varicoceles (88.23%) had negative TESA results.

However, despite the underlying causes of NOA, one of the previous studies suggested that most of these infertile men were candidates for surgical sperm aspiration for later use in intra-cytoplasmic sperm injection (ICSI) [10].

Strength, limitations, and future research

Our study had some limitations in that we did not perform the Inhibin B test for all patients due to the lack of its kit at the time of the study. We focused on the basic criteria affecting sperm production and linked them to testicular FNA results. Therefore, more studies on fertilization, testicular damage, and live birth rates after successful testicular FNA should be done in the future.

Minimal-invasive surgery (TESA) is associated with very few complications, compared to other techniques and it can be performed with simple equipment [17].

The result presented here is a tool that can assist andrologists, gynecologists, and reproductive biologists to help men with NOA conceive.

Conclusion

Non-obstructive azoospermia (NOA) is the most severe form of male infertility, which is caused by the failure of spermatogenesis. Hormonal abnormalities and genetic disorders are among the most important known causes of NOA. In our study, we confirmed that there are many changeable factors that negatively affect TESA results in Middle Eastern patients: high FSH and low inhibin B levels, smoking, varicocele, and genetic disorders such as Klinefelter syndrome, YCM AZFa and YCM AZFb. We did not find a relationship between men's age and FNA results.

Despite the absence of sperms in the semen, we found that there are sperms by testicular FNA in some NOA patients, and this may assist doctors in helping those patients to be biological fathers by using this technique.

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Provenance and peer review

Not commissioned, externally peer-reviewed.

Authors' contributions

The guarantor is Prof. Marwan Alhalabi. The corresponding author of this manuscript is Manal Alquobaili. Marwan Alhalabi and Shahd Hamsho are co-authors. All authors read and approved the final manuscript.

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Availability of data and materials

The data that supports the findings are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The data were opted from studies that were performed in line with the principles of the Declaration of Helsinki. The Ethical Committee of Damascus University approved the studies' protocols.

Consent of publication

We have written to the editorial office of the Middle East Fertility Society Journal and the article is being reviewed.

Competing interests
The authors declare that they have no competing interests.

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