Global Andrology Forum (GAF) Clinical Guidelines on the Management of Non-obstructive Azoospermia

Bridging the Gap between Controversy and Consensus

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Global Andrology Forum (GAF) Clinical Guidelines on the Management of Non-obstructive Azoospermia: Bridging the Gap between Controversy and Consensus

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Purpose: Non-obstructive azoospermia (NOA), defined as the absence of sperm in the ejaculate due to testicular failure, is observed in 5% to 15% of infertile men and accounts for two-thirds of azoospermia cases. The management of NOA is marked by significant controversy and global variation in diagnostic and therapeutic approaches, highlighting the crucial need for well-designed and standardized clinical practice guidelines. We present comprehensive graded clinical practice recommendations and statements for diagnosing and treating NOA, aiming to establish standardized strategies that can globally help guide practitioners in their practice.

Materials and Methods: A comprehensive literature review was conducted to gather evidence on the epidemiological, diagnostic, and therapeutic aspects of NOA. The Global Andrology Forum (GAF) recommendations were developed through the collaboration of a global panel of experts using the Delphi method and surveys to achieve consensus. Statements were graded according to the Oxford Centre for Evidence-Based Medicine "GRADE" classification as either "Strong" or "Weak." Statements receiving at least 80% expert consensus were graded as "Strong," while others were categorized as "Weak."

Results: The GAF has formulated a total of 49 recommendations and statements on the diagnosis and treatment of NOA, including 21 for diagnosis and 28 for treatment. The recommendations and statements were evaluated and graded by a panel of 48 GAF experts from 25 countries worldwide. The majority of experts (60.5%) had more than 10 years of clinical experience in managing NOA.

Conclusions: The GAF guidelines address discrepancies in NOA management across diverse clinical settings and provide comprehensive graded recommendations to guide clinicians in its diagnosis and treatment. Developed and graded by a large worldwide panel of experts, the current guidelines present simplified, high-standard strategies that can be seamlessly integrated into the daily global practice, offering practitioners a clear framework for managing NOA.

Keywords: Azoospermia; Guideline; Infertility; Pregnancy; Semen; Sperm retrieval

INTRODUCTION

Azoospermia is defined as the absence of sperm in the centrifuged pellet of ejaculate on two separate semen analyses [1]. Although azoospermia affects 2% of the general population, non-obstructive azoospermia (NOA) is observed in 5% to 15% of men undergoing infertility evaluations and accounts for two-thirds of azoospermic cases [2]. NOA is frequently attributable to genetic factors, testicular trauma, or mumps orchitis,

which are often uncorrectable [3]. It may be less commonly caused by varicocele, secondary hypogonadism, or exposure to gonadal toxins, which are potentially treatable conditions.

A primary objective of NOA management is to address the correctable underlying conditions, such as repairing clinical varicoceles, treating hormonal imbalances, and discontinuing gonadotoxins [4]. These interventions may improve patients' conditions, from azoospermia to the appearance of some sperm in the ejaculate [5,6]. The testicular tissue of men with NOA can exhibit homogenous or heterogenous histologic patterns such as tubular hyalinization, Sertoli Cell Only Syndrome (SCOS), maturation arrest (MA), or hypospermatogenesis (HS). Thus, there may be some focal areas of spermatogenesis within the testicular tissues of men with NOA, with the possibility of successful sperm recovery during surgical sperm retrieval (SSR) [7]. Approximately half of the men with NOA have mature sperm in testicular tissue specimens recovered by microdissection testicular sperm extraction (mTESE) [8].

The Global Andrology Forum (GAF) has recently published a series of two global surveys, demonstrating considerable worldwide controversy and marked variations in the diagnosis and management of NOA [4,9]. The present article discusses and addresses the discrepancies surrounding the diagnosis and treatment of NOA in diverse practices and clinical settings. Therefore, we present graded clinical practice recommendations and statements for diagnosing and treating NOA, aiming to establish standardized strategies that can globally help guide practitioners in their daily practice.

These recommendations and statements were developed by a worldwide collaborative group of GAF experts using the Delphi method [10] to establish a consensus. The statements were then graded according to the Oxford Centre for Evidence-based Medicine "GRADE" classification system [11].

MATERIALS AND METHODS

1. Literature search and review of evidence

To review the contemporary literature and evidence related to the epidemiology, diagnostic, and therapeutic approaches of NOA, the PubMed and Scopus databases were searched for English-language articles under the following terms: "azoospermia," "non-obstructive azoospermia," and "male infertility."

2. GAF's graded expert recommendations statements

The GAF created an international, collaborative panel of senior experts possessing significant academic and clinical experience in different aspects of male infertility, specifically in NOA management. The panel's role was to formulate and draft expert recommendations statements addressing the clinically important and controversial issues related to the diagnosis and treatment of NOA. These statements were adapted from GAF's previous publications [4,9] and also considered guidelines from professional societies and pertinent literature.

These initial statements were then distributed among an invited group of expert GAF members varying in age, academic position, geographical distribution, and subspecialty by creating a Google survey with each recommendation statement listed. An invitation email was sent to the invitees with clear instructions, complete descriptions of the Delphi method [10], and a survey link.

The initial statements were then subjected to a consensus-building process. The invitees were requested to rate each statement on a scale of 1 to 10, with one indicating "strongly disagree" and 10 indicating "strongly agree." A score of 7 or more was regarded as acceptance of the recommendation, whereas a score of 1 to 6 indicated disagreement. A blank space was provided below each statement to allow participants to propose an alternative recommendation statement if they provided a disagreement score of 1 to 6. A passing criterion of scoring seven or more by ≥80% of participants was set as a consensus. Any statement that did not reach a score of 7/10 by 80% of the respondents was revised based on participants' feedback and subjected to further rounds of assessment until consensus was reached for all the recommendations.

A total of 49 recommendations accepted through the Delphi process were then graded by a panel of 48 GAF experts as "Strong" or "Weak", according to the classification of the Oxford GRADE working group [11]. The GAF experts were distributed globally across 25 countries, ensuring worldwide representations of the panel (Supplement Table 1). Furthermore, the majority of experts (60.5%) have had more than 10 years of experience related to the management of NOA, while 39.5%

had 6 to 10 years of experience (Supplement Table 2). Recommendations rated as strong by at least 80% of the experts were graded as "strong," while the others were graded as "weak." Strong recommendations mean that most informed patients would choose the recommended management and that clinicians can structure their interactions with the patients accordingly. Weak recommendations mean that the patients' choices will vary according to their values and preferences, and clinicians must ensure that patients' care is in keeping with their values and preferences [11]. The simplified binary (strong/weak) GRADE scoring system is more straightforward for practitioners to understand and follow.

RESULTS

The GAF have formulated a total of 49 graded recommendations and statements, which were presented in Table 1 (GAF's recommendations and statements on

Table 1. GAF experts' graded recommendations and statements for the diagnosis and treatment of non-obstructive azoospermia

No	Recommendation	GRADE
Part 1. Diagnosis of NOA		
NOA prevalence		
1	The majority of cases of azoospermia are non-obstructive and are due to primary testicular failure.	Strong
Semen analysis		
2	Due to possible fluctuations, one semen specimen might not be enough to diagnose azoospermia.	Strong
3	To establish the diagnosis of azoospermia, at least two separate semen specimens should be examined after being centrifuged and pelleted.	Strong
4	At least one-month interval is preferred between two semen examinations in an azoospermic man, but the physician's clinical judgment should be used to determine the duration between the two tests, depending on individual circumstances or history of any recent medical illness that may affect spermatogenesis.	Weak
Hormonal evaluation		
5	The initial evaluation of a patient with suspected NOA should include serum total testosterone level, FSH, and LH, as these hormones are the primary regulators for spermatogenesis.	Strong
6	When the serum total testosterone level does not match the clinical symptoms or if there is any condition that could dramatically alter the SHBG level, then the calculation of bio-available testosterone (after SHBG assay) is recommended.	Strong
7	In addition to total testosterone, serum estradiol should be measured in obese men.	Weak
8	Serum prolactin should be assayed if there is an associated decrease in libido, and erectile dysfunction in the presence of low total testosterone level.	Weak
Genetic evaluation		
9	Karyotype and Y-chromosome microdeletion tests should be recommended for NOA patients.	Strong
10	*CFTR* gene mutation tests should be done only in cases of vas aplasia or congenital obstruction, or in regions with a high prevalence of carriers of CFTR mutations.	Strong
11	Currently, other genetic tests, such as full exome or genome screening, are not recommended as routine tests.	Weak
NOA with varicocele		
12	The clinical significance of varicocele associating NOA is uncertain.	Strong
Differentiation of NOA from OA		
13	In most cases, clinical findings and serum reproductive hormonal evaluation are sufficient to distinguish NOA from OA.	Strong
14	Scrotal ultrasonography is not required for merely measuring testicular volume to differentiate between OA and NOA - this can be done adequately with a simple Prader orchidometer.	Strong
15	Scrotal ultrasonography has an important role in the assessment of the spermatic cord and epididymis, and in ruling out any testicular pathology or tumors, especially before performing invasive diagnostic or therapeutic procedures.	Strong
16	TRUS and pelvic MRI are not routinely required to distinguish between OA and NOA.	Strong
17	TRUS and pelvic MRI can be helpful in selected patients when azoospermia is associated with low semen volume, to confirm a diagnosis of vas aplasia or ejaculatory duct obstruction.	Strong
18	Scrotal MRI usage to distinguish between OA and NOA has not yet been incorporated into routine clinical practice awaiting future determination.	Weak
19	Diagnostic testicular biopsy should not be performed as a standalone procedure in routine clinical practice.	Strong
20	Diagnostic testicular biopsy should always be combined with SSR, preferably with sperm cryopreservation.	Strong

Table 1. Continued 1

No	Recommendation	GRADE
Predictors of SRR		
21	There are no preoperative biochemical or clinical variables that definitively predict positive sperm retrieval at surgery in patients with NOA. However, close to normal testicular volume, a history of sperm in the ejaculate, and histopathology with hypospermatogenesis predict higher chances of sperm retrieval.	Strong
Part 2: Treatment of NOA		
Medical management		
22	Hormonal therapy is not routinely recommended for men with NOA, but may be considered for selected patients after adequate counseling.	Strong
23	Exogenous testosterone should not be used for hypogonadal men with NOA who are planning testicular sperm retrieval and are interested in future fertility. Instead, SERMs, aromatase inhibitors, or hCG administration can be used to raise testosterone without compromising spermatogenesis.	Strong
Surgical management		
24	Consider performing mTESE as the preferred and most efficient procedure for sperm retrieval in men with NOA, due to its overall higher SRR compared to other procedures such as TESA and cTESE.	Strong
25	TESA is no longer recommended routinely in men with NOA given the low success rates compared with those of TESE.	Weak
26	In some cases, performing cTESE as the first step of mTESE may be acceptable.	Weak
27	Less invasive procedures, such as cTESE, may be tried before mTESE in selected cases with good testicular volume, normal FSH, and/or known favorable testicular histological patterns.	Weak
28	In bilateral symmetrical testes, following a negative mTESE on one side, mTESE can be attempted on the opposite side with a 10% chance of finding sperm.	Weak
29	Testicular biopsy during SSR is useful to establish histological diagnosis and support counseling for future management, and to rule out germ cell neoplasia *in situ*.	Strong
30	Patients should be informed of the small chance that a testicular biopsy may contain spermatozoa despite negative SSR.	Weak
Techniques to optimize SRR		
31	Hormonal therapy can be a definitive treatment for NOA men with hypogonadotropic hypogonadism.	Strong
32	Hormonal therapy for hypogonadism with normal to elevated FSH and LH levels is still controversial. Further research is required to identify the best candidate and the ideal regimen.	Weak
33	FNA mapping prior to SSR is not routinely recommended.	Weak
34	There is currently not enough evidence supporting the use of imaging techniques for identifying areas of spermatogenesis and improving the success of SRR.	Weak
Repeat SSR		
35	The recommended interval between the two TESE procedures is at least six months.	Strong
Special considerations in SSR		
36	The surgeon's experience and the time spent by the embryologist to find sperm can impact the success of sperm retrieval.	Strong
37	It is preferable for the laboratory team to be in proximity to the operating theatre to facilitate continuous transfer and examination of the mTESE specimens.	Weak
38	The samples should be subjected to meticulous scrutiny and examination by the embryologist for at least 60 minutes in an attempt to identify sperm.	Weak
39	Either fresh or cryopreserved sperm can be used depending upon the expertise of the center and the embryologist.	Weak
NOA men with genetic abnormalities		
40	The genetic status of a male has a significant impact on the success rate of SSR.	Strong
41	SSR should not be attempted in men with complete AZFa or complete AZFb deletions. Sperm retrieval may be rarely successful in incomplete, aberrant, or non-classical AZFa and AZFb microdeletions.	Strong
42	There is a reasonable chance of finding sperm in men with AZFc microdeletion.	Strong
43	Genetic counseling should be discussed for the chances of finding sperm, the certainty of transmission of an AZFc deletion to the male offspring, and the option for alternatives such as donor sperm and adoption.	Strong
44	In patients with Klinefelter's syndrome, mTESE can be offered with a 20%–60% chance of sperm retrieval.	Strong
45	Preimplantation genetic testing (PGT) is optional in couples where the man has Klinefelter's syndrome since studies show that most of the embryos obtained from these couples have no chromosomal abnormalities.	Weak
NOA with varicocele		
46	The decision to perform varicocele repair in cases of NOA is a shared decision between the physician and the couple after a detailed discussion of the risks and benefits. The decision may be guided by parameters such as testicular volume, FSH level, testicular histology if available, female partner's age, and overall fertility status.	Strong
47	Varicocele repair for subclinical varicoceles is not recommended.	Strong

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Table 1. Continued 2

No	Recommendation	GRADE
Evolving therapies for NOA		
48	The use of stem cells or PRP to treat NOA is still experimental and is not recommended for routine clinical use.	Weak
49	Gene editing with CRISPR/Cas9 for the treatment of NOA is currently purely experimental.	Weak

NOA: non-obstructive azoospermia, FSH: follicle stimulating hormone, LH: luteinizing hormone, SHBG: sex hormone-binding globulin, CFTR: cystic fibrosis transmembrane conductance regulator, OA: obstructive azoospermia, TRUS: transrectal ultrasonography, MRI: magnetic resonance imaging, SSR: surgical sperm retrieval, SERMs: selective estrogen receptor modulators, hCG: human chorionic gonadotropin, mTESE: microsurgical testicular sperm extraction, cTESE: conventional testicular sperm extraction, TESA: testicular sperm aspiration, AZF: azoospermia factor.

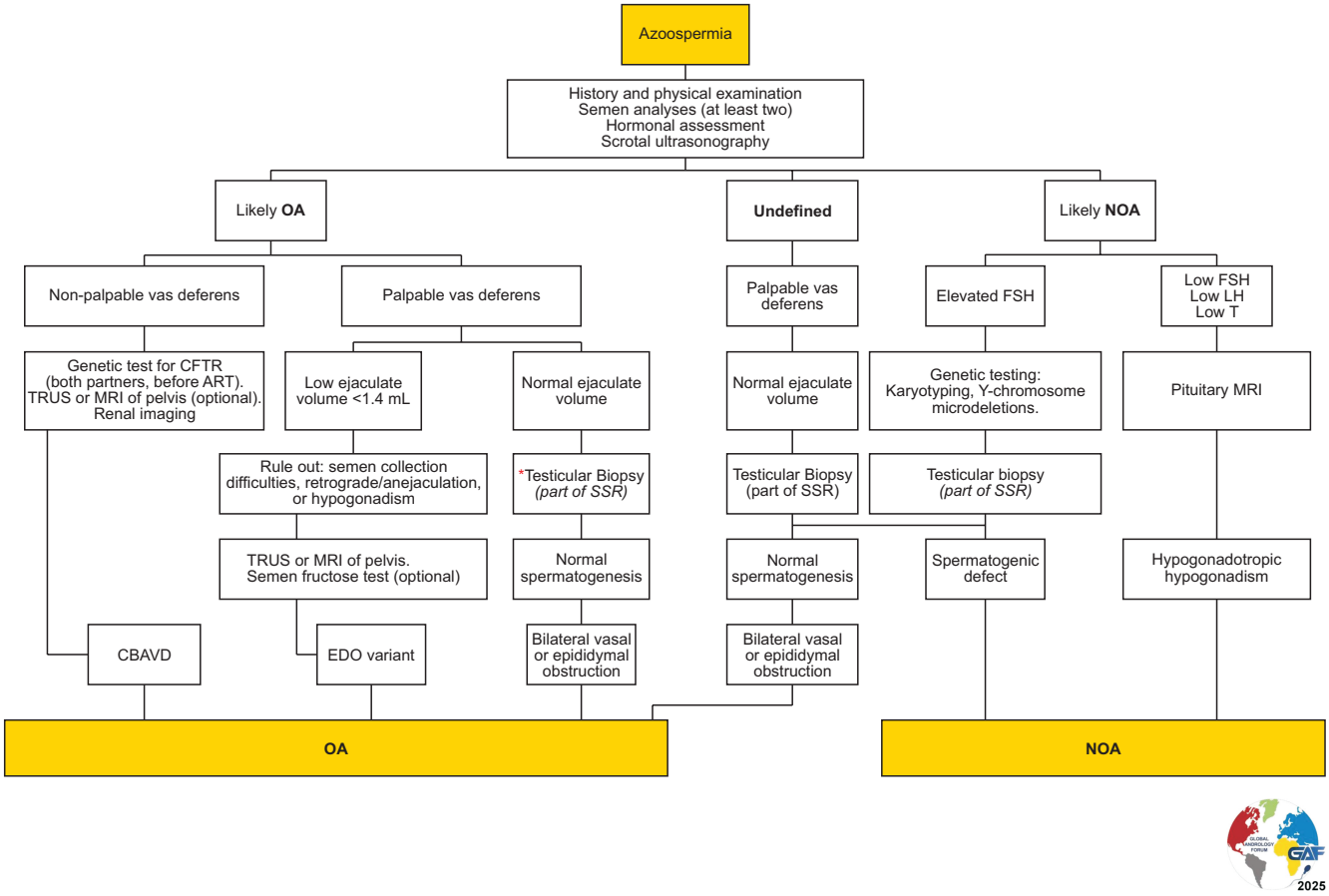

Fig. 1. Algorithm for the evaluation and diagnosis of azoospermia. OA: obstructive azoospermia, NOA: non-obstructive azoospermia, CFTR: cystic fibrosis transmembrane conductance regulator, ART: assisted reproductive techniques, TRUS: transrectal ultrasonography, MRI: magnetic resonance imaging, SSR: Surgical sperm retrieval, CBAVD: congenital bilateral absence of vas deferens, EDO: ejaculatory duct obstruction; FSH: follicle stimulating hormone, LH: luteinizing hormone, T: testosterone. *If the biopsy shows spermatogenesis defect, continue as NOA.

the diagnosis and treatment of NOA).

DISSCUSION

1. Part 1. Controversies in the diagnosis of NOA

1) Clinical evidence

The diagnosis of NOA is a debated issue due to the absence of diagnostic standards. Typically, the evaluation of patients with NOA must meet specific goals, such as distinguishing NOA from obstructive azoospermia (OA), identifying underlying causes, treating potentially correctable causes, and determining the factors that would predict the success of SSR.

The diagnosis of NOA (Fig. 1) relies primarily on the patient's history, physical examination, hormonal profile, and at least two adequately separated semen anal-

yses. The assessment of medical and surgical history should include conditions such as viral orchitis, cryptorchidism, genital trauma, and prior testis surgery. It is also essential to evaluate exposure to different gonadotoxins such as radiation therapy or chemotherapy [6].

To identify different causes of azoospermia, evaluation of secondary sexual characteristics, palpation and measurement of testicular volume, and examination of the condition of both the vas deferens (presence/absence) and the epididymis (normal/full) are crucial. Signs such as tall stature, small testicles, micropenis, gynecomastia, feminine body proportions and hair distribution, as well as visceral obesity, suggest Klinefelter's syndrome (KS). In contrast, undescended testes or a history of orchidopexy indicate the possibility of cryptorchidism-related NOA [12].

Evaluating the seminal fluid volume and pH is useful for identifying OA due to distal obstruction. Men with primary NOA or obstruction in the vas deferens or epididymis have normal seminal fluid volume and pH, indicating properly functioning seminal vesicles and patent ejaculatory ducts. Conversely, a low semen volume (<1.4 mL), acidic ejaculate (pH <7.2), and low/absent fructose levels (<13 μmol per ejaculate) can point to seminal vesicle hypoplasia associated with congenital bilateral absence of the vas deferens (CBAVD) or ejaculatory duct obstruction (EDO) [13].

Serum follicle stimulating hormone (FSH) level usually correlates negatively with spermatogenesis [5]. NOA is frequently associated with high FSH and low serum testosterone, while OA is associated with normal values of both hormones. When total testosterone is below normal, repeating the test and measuring free or bio-available testosterone, along with serum luteinizing hormone (LH), prolactin, and estradiol, are usually recommended [6,14]. NOA caused by primary testicular failure is typically associated with non-dilated epididymis with low testicular volume (<15 mL), increased serum FSH levels, and a decreased testosterone/estradiol ratio [15].

Although serum inhibin-B estimation is suggested to be a reliable marker for distinguishing between different categories of azoospermia, it is not superior to FSH. Moreover, neither hormone has been convincingly established as a reliable predictor for the presence of spermatozoa during TESE [6].

Generally, all patients with NOA should undergo karyotyping and screening for Y-chromosome microdeletions [6,14]. KS is the most common karyotypic abnormality in azoospermic men, found in about 10% of cases. Microdeletions in the long-arm of the Y-chromosome are the second most common genetic cause of NOA [16]. On the other hand, OA may be associated with mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Testing for CFTR mutations should be recommended for infertile azoospermic men with anatomical abnormalities of the vas deferens, such as unilateral or bilateral vas agenesis [17]. Men with azoospermia, especially those at risk for inheritable diseases, should receive genetic counseling before assisted reproductive technology (ART) procedures. This involves assessment of the genetic condition of the female partner and discussing the risks and implications of passing the condition to the offspring and also considering preimplantation genetic testing [18].

Despite these recommendations, a recent GAF survey of 367 participants from 49 countries revealed that only two-thirds of the respondents conducted genetic testing for their azoospermia cases despite its possible role in preventing unneeded interventions and ensuring appropriate counseling for couples regarding inheritance of genetic disease [9]. Hence, this study suggested the need to provide clinicians with genetic counseling training [9]. The reasons why some clinicians do not conduct genetic testing are possibly the high cost or lack of availability.

Scrotal ultrasonography is useful for assessing testicular size and ruling out any occult testicular tumor. Ultrasonography can also help diagnosing the varicocele in obese patients, which is less prone to detect during routine physical examination. Sonoelastography, an ultrasonographic modality evaluating the elasticity of biological tissues, has been suggested to provide insights into testicular architecture [19]. In a study using power Doppler ultrasound in men with NOA, Schurich et al [20] reported that areas with increased intratesticular perfusion corresponded to the possible presence of residual spermatogenic areas. Another study used testicular ultrasonography to highlight and measure the diameters of the more prominent seminiferous tubules. Notably, 63% of men with larger seminiferous tubules had a sperm retrieval rate (SRR) *versus* only 11% of men with smaller seminiferous tubules. A seminiferous tubule diameter of ≥250 μm was used to predict SRR during mTESE, with an area under the curve (AUC) of no less than 0.82 [21]. However, all these procedures

depend on the sonographer's experience and have not been supported with further studies; therefore, they are not readily applicable in clinical practice and should not be recommended.

For many years, standalone diagnostic testicular biopsies have traditionally been a definitive method for evaluating azoospermic males, to confirm OA for those with normal-sized testes and normal levels of serum gonadal hormones. Recently, however, with the advent of surgical testicular sperm retrieval procedures for men with NOA in conjunction with intracytoplasmic sperm injection (ICSI), the NOA men usually undergo testicular biopsies as part of the SSR procedure [22]. Therefore, in contemporary practice, it is advisable to perform testicular biopsies in facilities equipped for the preservation and storage of testicular sperm for future ART procedures [23-25]. Importantly, the routine use of diagnostic testicular biopsies in azoospermia is not recommended by relevant guidelines and should be reserved for specific cases [26]. Consistent with the European Association of Urology (EAU) guideline recommendations, a testicular biopsy could be taken for histopathology evaluation to confirm the type of azoospermia and combined with TESE for possible sperm cryopreservation using isolated sperm suspensions or tissue fragments [26]. In addition, intratubular germ cell neoplasia *in situ* might be revealed in biopsy specimens taken from those NOA patients who have a history of cryptorchidism and/or multiple foci of testicular microlithiasis. Cautiously, an accurate histological evaluation requires careful handling, fixation in appropriate fluids (*e.g.*, Bouin or Zenker's solutions), and preparation of testicular tissues. Incorrect fixation of the testis specimens in formalin makes subsequent histologic evaluation of the tissue sample difficult and diagnostically unreliable [27].

2) Modalities to differentiate OA from NOA

The etiological factors contributing to both NOA and OA are diverse and include genetic, inflammatory, infectious, environmental, and surgical factors [14]. Determining the cause of azoospermia and distinguishing between NOA and OA are essential for several reasons. Identifying treatable forms of azoospermia can lead to successful interventions such as surgery or medication, offering the possibility of natural conception. For uncorrectable types, sperm retrieval combined with ART using the patient's own sperm provides an alternative route to biological parenthood. In situations where neither corrective measures nor sperm retrieval are feasible, understanding the specific type of azoospermia can help guide patients toward options like donor insemination or adoption. Additionally, recognizing any health-threatening conditions associated with azoospermia ensures prompt medical attention. Lastly, understanding genetic causes is critical, as they can affect the health of the patient and his potential offspring, particularly when using ART [3].

As previously discussed, medical history, physical examination including scrotal and rectal examinations, serum gonadal hormone levels, genetic evaluation, and testicular imaging assessment after at least two adequately separated semen analyses can help differentiate between OA and NOA cases [3]. Evaluations of seminal fluid volume, pH, and possibly fructose levels are also crucial for distinguishing between NOA and OA.

A study showed that FSH levels at or below 7.6 IU/L with longitudinal axes of testis more than 4.6 cm were associated with a 96% probability of having OA rather than NOA [28]. However, it should be noted that while men with NOA typically have small testicles, some men with NOA, due to spermatogenic MA, can have normal-sized testicles. Therefore, testicular size may not be a reliable sole indicator for differential diagnosis [29]. As discussed earlier, in such cases, testicular biopsy during SSR may play a role in differentiation.

Palpating the vas deferens is essential for ruling out CBAVD as a cause of OA. This result from a gene mutation associated with cystic fibrosis. Another key aspect of physical examination is detecting clinical varicocele associated with NOA, which is generally considered a treatable cause [30].

Several imaging techniques, especially scrotal and transrectal ultrasound (TRUS) [31] and magnetic resonance imaging (MRI) [32,33], have been proposed to help differentiate OA from NOA. Scrotal ultrasound is helpful in the determination of testicular volume [31], although this can be performed adequately with a simple Prader orchidometer. Yet, scrotal ultrasound plays an important role in the assessment of the spermatic cord and epididymis and in ruling out any testicular pathology or tumors, especially before performing an invasive diagnostic or therapeutic procedure. TRUS and pelvic MRI are not routinely required, although they can be helpful in selected patients when azoosper-

mia is associated with low semen volume to confirm a diagnosis of vas aplasia or EDO [31]. MRI has been suggested as a useful modality for differentiating OA from NOA and for predicting the histopathologic grade of azoospermia by measuring the testicular volume, apparent diffusion coefficient (ADC), and normalized ADC. The testicular volume is significantly larger, while the testicular ADC and normalized ADC are significantly lower in men with OA than in those with NOA [32]. Furthermore, scrotal MRI was suggested to be a useful marker for predicting the SRR in NOA men undergoing mTESE by measuring the diffusion tensor imaging parameters [34] or using proton magnetic resonance spectroscopy [33]. However, the role of MRI in the management of NOA has yet to be determined. Hence, MRI has not yet been incorporated into routine clinical practice.

A simplified pathway for evaluating and differentiating the causes of azoospermia is shown in Fig. 1. The GAF experts' graded recommendations and statements for the diagnosis of NOA are depicted in Table 1.

2. Part II. Controversies in the management of NOA

1) Clinical evidence

Several controversies exist regarding the medical and surgical management of men with NOA, making it one of the most challenging scenarios in reproductive medicine. The issue with medical and hormonal therapies is that there is no standard approach that can be generally applied to all NOA patients, and there are unclear data, making it difficult to develop strong recommendations. There is also an arbitrary cut-off level for hormones to predict the success of sperm retrieval in men with NOA. A recent multi-center cross-sectional study, including 1,644 men with NOA, reported that only one-third of patients benefit from medical and hormonal therapy before undergoing sperm retrieval procedures [35]. Medical treatments for NOA are generally indicated for patients with hypogonadotropic hypogonadism due to defects, in the hypothalamic-pituitary-testicular axis. This subset of patients is potentially treated with hCG/hMG or pulsatile GnRH.

Several studies have recommended the use of aromatase inhibitors, such as anastrozol and letrozol, in men with a low testosterone-to-estrogen ratio of less than 10 to inhibit the peripheral conversion of testosterone-to-estrogen, which can affect the total and intra-testicular testosterone levels [3,35]. Additionally, in hypogonadal men with normal LH levels, selective estrogen receptor modulators (SERMs) such as clomiphene citrate were recommended [35,36]. SERMs work by inhibiting estrogen receptors in the pituitary, thus promoting the secretion of LH and FSH and consequently elevating testosterone levels. However, such approaches are not standard, and other studies use different regimens to treat patients with variable outcomes. Other new therapeutic approaches, such as intra-testicular platelet-rich plasma (PRP) [37] and gene therapy with CRISPR/Cas9, have been tested experimentally and have shown promise but are still in preliminary stages.

The debate on SSR procedures still continues regarding which patients have the best prognosis for successful sperm retrieval, how to best optimize men preoperatively, and the intraoperative techniques. The main SSR techniques used in men with NOA include testicular sperm aspiration (TESA), conventional TESE (cTESE), and mTESE. TESA, also known as fine needle aspiration (FNA), involves the use of a fine-caliber needle connected to a syringe to provide negative pressure and pass it in and out of the testicle multiple times through the scrotal skin in an attempt to aspirate sperm and seminiferous tubules [38]. TESA is no longer recommended in men with NOA compared to TESE, given the low success rates and the possible testicular injury [39]. cTESE involves making a small incision into the testicle in an avascular plane to directly remove seminiferous tubules, which are then examined for the presence of sperm [38]. Multiple incision cTESE can also be performed to help increase the SRR. Both TESA and cTESE can be performed under local anesthesia in an office setting. mTESE was first described by Schlegel [40] and is performed in the operating theatre under spinal or general anesthesia [40]. It involves bivalving the testicle and using an operative microscope at 15–40× magnification to identify larger seminiferous tubules, which are most likely to contain sperm; these tubules are selectively extracted and examined for the presence of sperm [38,41]. With a negative mTESE on one side, in bilateral symmetrical testicles, mTESE can be attempted on the contralateral side with a 10% likelihood of finding sperm on the second testis [4].

Some studies have identified that mTESE yields the best SRR [8,38,39,42,43]. However, some men with NOA-

associated with hypospermatogenesis or incomplete spermatogenic arrest can have such success with less invasive options, such as TESA or cTESE. However, there is no accurate preoperative method to predict which patients would benefit more from a mTESE than from TESA or cTESE, and this approach usually translates to subjecting men with NOA to the more invasive mTESE or accepting the possibility of a lower SRR. Some fertility centers can start with a less invasive option and progress to a mTESE in the same setting if no sperm are retrieved. However, this can pose logistical challenges impacting the practicality of this approach for many *in-vitro* fertilization (IVF) clinics.

Another area of debate related to the timing of the female egg retrieval procedure is whether to use fresh or frozen sperm for the IVF cycle. Studies have shown no difference in fertilization, pregnancy, or live birth rates between fresh and frozen sperm [44,45]. However, obtaining fresh sperm on the same day of the oocyte retrieval can often be logistically challenging, mainly when a mTESE is utilized. On the other hand, if only a few sperm are retrieved, this may pose another problem, as some sperm can be lost or damaged during the freeze-thaw process [44]. Thus, the timing of when to perform sperm retrieval rather than oocyte retrieval must be coordinated between the reproductive urologist, reproductive gynecologist, and embryologist.

Testicular biopsy for histopathology at the time of SSR should be considered. As previously discussed, the main reason for obtaining histopathology is to rule out testicular germ cell neoplasia *in situ*, especially if no sperm is retrieved. However, obtaining a biopsy specimen can also help confirm a diagnosis and can provide prognostic value if future sperm retrieval procedures are planned [4,46].

The use of sperm retrieval procedures in men with NOA and genetic abnormalities can be controversial, depending on the type of abnormality. SSR in men with KS can be attempted, with studies reporting sperm retrieval in 20% to 60% of cases [4,12]. Men with chromosome abnormalities other than KS can undergo SSR, but the chances of finding sperm are lower [47]. In this context, patients with AZFc Y-chromosome microdeletions have a reasonable chance of finding sperm, and SSR can be attempted in these patients. However, SSR is not recommended when a patient has a complete AZFa deletion or a complete AZFb deletion [4,46].

2) Optimization of SRR in NOA

Hormonal therapy can be a definitive treatment for NOA men with hypogonadotropic hypogonadism, but its use for hypogonadism with normal to elevated levels of FSH and LH is still controversial. The purpose of hormonal therapy in these subsets of patients is to increase intratesticular testosterone levels and, consequently, improve spermatogenesis. Sperm retrieval was found to be significantly higher in men with normal testosterone than in those with subnormal testosterone [48]. Nevertheless, the results of some studies conflict with the different protocols employed by the clinicians. In a systematic review and meta-analysis comprising 22 studies and including 1,706 subjects, Tharakan et al [49] found that hormonal therapies did not give any benefit to hypergonadotropic hypogonadism, but a benefit to eugonadal men was observed. However, these authors mentioned that the quality of evidence was low, with a moderate to high risk of bias, suggesting the need for caution in the interpretation of these results. With the current state of evidence, no recommendations have been made from the guidelines on the routine use of hormonal therapy for NOA, especially before SSR, and further research will be beneficial in finding the best candidate and the ideal regimen for hormonal therapy.

The optimal levels of hormones for sperm production are still unclear, and most cut-off values reported in the literature are arbitrary [48]. The large heterogeneity of phenotypes displayed by men with NOA adds more confusion; thus, it is obvious that different patients will require different levels of sex hormones, a classic example of "one size does not fit all."

Other controversial issues include varicocelectomy for men with NOA. Several studies indicate the benefit of varicocelectomy in improving spermatogenesis, with some patients recovering sperm in the ejaculate postoperatively [50]. However, if sperm are recovered in the ejaculate after varicocelectomy, sperm cryopreservation is advised due to the increased risk of relapse back to the azoospermic state [30]. The success rate of SSR has also been shown to improve in patients who underwent varicocelectomies than in untreated patients [51]. Nevertheless, such a success rate depends on several factors, including histopathology [50]. Given such uncertainty and the low to moderate quality of evidence, it should be offered only after shared decision-making between the patient and physician.

mTESE has become the gold standard for sperm re-

trieval in men with NOA [46]. However, some clinicians prefer a stepwise approach with a less invasive technique to create a more effective and efficient procedure [42,48,52,53]. FNA mapping prior to TESE has also been controversial due to the concern about testicular injury, despite studies showing its success in localizing sperm "hot spots" after failed mTESE [54]. The use of advanced technologies, such as Raman spectroscopy, narrow band imaging, multiphoton microscopy, surgical digital exoscopes, ultrasonography, full-field optical coherence tomography, and artificial intelligence have also been evaluated to assist in improving visualization and success rate during mTESE [55,56]. However, these technologies are currently not available to most centers, and their effectiveness needs to be confirmed by further studies.

Furthermore, laboratory aspects such as sperm handling and selection are highly important to optimize the outcome [57].

3) Repeat SSR

When mTESE fails, a redo procedure may be the only option for NOA patients. Studies have shown that a longer interval from the first to the second procedure could increase the success rate [58]. However, Tai et al [59] conducted a retrospective study involving 146 patients with NOA who underwent two mTESE procedures from the same testis. The patients were divided into three groups based on the time interval between the two surgeries: 44 patients had a repeat mTESE within 3 months, 60 patients between 3 and 6 months, and 42 patients after more than 6 months following the first procedure. Overall SRR did not differ among the three groups (93.2%, 90.0%, and 88.1%, respectively; p=0.719), nor did fertility outcomes, including the rates of fertilization, biochemical pregnancy, clinical pregnancy, and cumulative live births. Several other factors should be considered before such a procedure is repeated, especially if the initial sperm retrieval fails [58,60]. Clinicians may consider evaluating the testicular histopathology, giving more hormonal treatment, or even initiating a discussion with the patients to consider sperm donors or adoption if the possibility of successful repeat mTESE is very low.

4) Predictive factors of sperm retrieval by cTESE/mTESE

Although several parameters have been studied to predict SSR outcomes, the conclusions are inconsistent. The current data do not recommend the use of a single predictor; thus, scoring systems and artificial intelligence have even been developed to improve prediction. Along with their study, Boitrelle et al [61] developed a predictive algorithm that combines testicular volume, serum FSH, and inhibin-B levels to forecast cTESE outcomes, which produced a positive likelihood ratio of +3.01 for predicting successful cTESE. Although no specific recommendations exist on the routine use of a specific factor(s) for prediction, it seems that incorporating non-invasive biomarkers into the diagnostic and treatment plans for NOA patients is highly promising [62]. With thorough research, technological development, and ethical oversight, these biomarkers could significantly transform sperm retrieval strategies, leading to more individualized, informed, and patient-centric approaches in managing male infertility [62].

(1) Testicular volume: Typically, close to normal testicular volume correlates with OA or the possibility of retrieving spermatozoa in NOA cases; however, this correlation is not always present [63].

(2) Prior semen analysis: The previous presence of sperm could suggest an increased likelihood of success in sperm retrieval [64].

(3) Previous sperm retrieval: A successful previous TESE procedure might also indicate a higher probability of successful sperm retrieval [65].

(4) FSH: The common belief is that elevated serum FSH levels are typically linked to impaired spermatogenesis and the histopathological patterns of SCOS and tubular hyalinization. Some studies suggest that serum FSH levels and testicular size might predict SSR outcomes, though no definitive threshold has been established [66,67]. Additionally, it has been demonstrated that there is no significant difference in age, testicular volume, or hormonal levels between the TESE-positive and TESE-negative groups [68,69]. A meta-analysis by Yang et al [70] found that serum FSH was a moderate predictor of the SRR before cTESE/mTESE. Conversely, Corona et al [71] did not correlate between serum FSH levels and SRR in their analysis of 117 studies, possibly due to differences in surgical techniques, with mTESE being more successful and the most common procedure in their studies.

(5) Anti-Müllerian hormone (AMH) and inhibin-B: AMH is a Sertoli cell-secreted glycoprotein responsible for Müllerian duct regression in male embryos. The

seminal AMH levels of most fertile men are higher than their serum AMH levels (mean concentrations 153 pmol/L *vs.* 10.7 pmol/L). Seminal AMH is proposed to be of testicular origin, as its concentrations are undetectable in all OA patients. In NOA patients, the seminal AMH concentration is lower (mean 17 pmol/L) than that in fertile men and is not correlated with the plasma FSH level [72]. Additionally, a comparison of seminal AMH concentrations and the histological patterns of testicular biopsies from NOA men has revealed that undetectable AMH was associated with a lack of spermatozoa in 11 of 14 males, while detectable AMH concentrations were associated with persistent spermatogenesis in seven of nine patients [73]. Conversely, although it was shown that seminal plasma AMH is an absolute testicular marker, it has demonstrated poor predictability for successful testicular SRR in NOA patients [73].

On the other hand, Puia et al [74] showed that patients with successful SSR had significantly higher mean serum inhibin-B levels (134.6 *vs.* 72.4 pg/mL).

(6) Cell-free seminal mRNAs (cfs-mRNAs): cfs-mRNAs exist in human ejaculate at high concentrations and contain many tissue-specific transcripts secreted from the male reproductive system such as germ cell-specific (DDX4), seminal vesicle-specific (SEMG1) and prostate-specific (TGM4) transcripts. Li et al [63] detected DDX4 in all patients with MA and incomplete SCOS, but it was absent in most patients with complete SCOS (75.0%) or non-CBAVD (85.7%) and in all men with vasectomy or CBAVD. The presence of DDX4 in some men with complete SCOS or non-CBAVD suggests the presence of germ cells in the testis and incomplete obstruction. SEMG1 was undetectable in patients with CBAVD with bilateral absence of seminal vesicles. Goel et al [75] evaluated the role of the cell-free seminal markers DDX4, PRM1, and PRM2 in differentiating between OA and NOA. DDX4 was more sensitive to NOA than OA. Among the various subtypes of NOA, DDX4 positivity was greater in patients with MA and HS compared with SCOS.

(7) Testis-expressed 101 (TEX101): Drabovich et al [76] pointed out that the combined detections of the TEX101 and ECM1 proteins could result in a diagnostic output with high sensitivity and specificity. Similar results were observed for the combined detection of TEX101

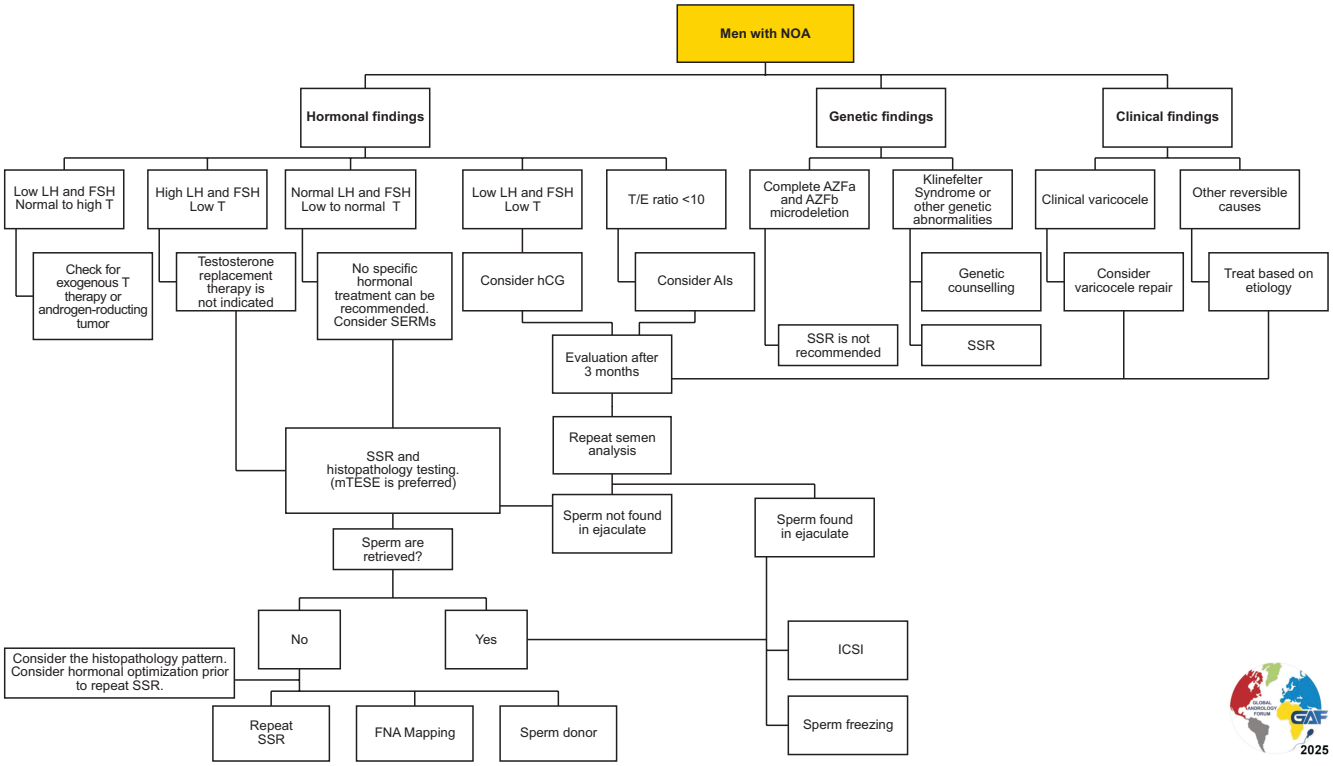

Fig. 2. Treatment algorithm of NOA. NOA: non-obstructive azoospermia, LH: luteinizing hormone, FSH: follicle stimulating hormone, T: testosterone, T/E ratio: testosterone/estradiol ratio, AZF: azoospermia factor, hCG: Human chorionic gonadotropin, mTESE: microsurgical testicular sperm extraction, FNA: fine needle aspiration, ICSI: intracytoplasmic sperm injection, AI: aromatase inhibitor, SSR: surgical sperm retrieval.

and sperm-associated antigen 1 (SPAG1) [77].

(8) Proton MR spectroscopy: Ntorkou et al [78] showed that proton magnetic resonance spectroscopy can be used to assess metabolic information within the NOA testes to assess spermatogenesis before TESE.

A simplified pathway for the treatment of NOA is presented in Fig. 2. The GAF experts' graded recommendations and statements for the treatment of NOA are depicted in Table 1.

CONCLUSIONS

A global survey of the evaluation and management of NOA highlights significant controversies and discrepancies in diagnostic and treatment approaches across clinical settings. This underscores the need for standardized, evidence-based guidelines to improve the management of NOA globally.

The GAF has developed and presented graded recommendations and statements, which were formulated by a large, worldwide panel of experts with substantial academic and clinical experience in NOA management, ensuring the highest level of scientific rigor.

To enhance usability, the GAF adopted a simplified binary (strong/weak) GRADE scoring system, making the guidelines more accessible and easier for practitioners to interpret and implement.

The GAF clinical practice guidelines provide streamlined, high-standard strategies designed to seamlessly integrate into the daily clinical workflows of practitioners, offering clear guidance for the effective and consistent management of NOA across diverse settings. The current guidelines aim to bridge existing gaps in the global practice, support informed clinical decision-making, and ultimately improve outcomes for patients with NOA.

Conflict of Interest

The authors have nothing to disclose.

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Supplementary Materials

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