

Management of non-obstructive azoospermia

Koji Chiba¹ · Noritoshi Enatsu¹ · Masato Fujisawa¹

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Abstract Non-obstructive azoospermia (NOA) is defined as no sperm in the ejaculate due to failure of spermatogenesis and is the most severe form of male infertility. The etiology of NOA is either intrinsic testicular impairment or inadequate gonadotropin production. Chromosomal or genetic abnormalities should be evaluated because there is a relatively high incidence compared with the normal population. Although rare, NOA due to inadequate gonadotropin production is a condition in which fertility can be improved by medical treatment. In contrast, there is no treatment that can restore spermatogenesis in the majority of NOA patients. Consequently, testicular extraction of sperm under an operating microscope (micro-TESE) has been the first-line treatment for these patients. Other treatment options include varicocelectomy for NOA patients with a palpable varicocele and orchidopexy if undescended testes are diagnosed after adulthood, although management of these patients remains controversial. Advances in retrieving spermatozoa more efficiently by micro-TESE have been made during the past decade. In addition, recent advances in biotechnology have raised the possibility of using germ cells produced from stem cells in the future. This review presents current knowledge about the etiology, diagnosis, and treatment of NOA.

Keywords Male infertility · Management · Non-obstructive azoospermia · Testicular sperm extraction · Treatment

Introduction

During the past few decades, a decrease of the birth rate has become a growing social problem in Japan. The live birth rate is continuously declining, and has fallen to almost half of that 40 years ago. Several factors have contributed to this trend, with infertility being one of the major problems. It has been reported that approximately 15 % of couples fail to conceive after 1 year of unprotected intercourse, and male factors are responsible for infertility in almost half of these couples [1]. Thus, development of more effective treatment for male infertility is important in this situation.

Several factors can contribute to male infertility, including decreased sperm production, abnormal sperm function, obstruction to the passage of sperm, and erectile dysfunction. Among these, non-obstructive azoospermia (NOA), which is defined as no sperm in the ejaculate due to failure of spermatogenesis, is the most severe form of male infertility. Historically, NOA patients were unable to have their own children and their only options were donor sperm or adoption. In 1978, the first live birth using in vitro fertilization (IVF) was reported [2], followed by successful live birth using the intracytoplasmic injection (ICSI) technique in 1992 [3]. Subsequently, pregnancy was reported after testicular sperm extraction (TESE) and ICSI in NOA patients [4], which allowed these patients to potentially father their own children. These advances in assisted reproductive technology (ART) have dramatically changed the management of NOA. This review

✉ Koji Chiba
kchiba714@yahoo.co.jp

¹ Division of Urology, Department of Surgery Related, Faculty of Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-Cho, Chuo-Ku, Kobe 650-0017, Japan

summarizes current practices and controversies with respect to the diagnosis and management of NOA.

Diagnosis of NOA

Azoospermia is diagnosed when no sperm are found in the ejaculate. It is important to note that at least two semen samples should be examined for accurate assessment [5]. In addition, absence of sperm should be confirmed by centrifugation of the semen specimen. Conducting careful microscopic examination of multiple droplets of sediment from the ejaculate has been reported to result in the detection of sperm in up to 35 % of men who were initially diagnosed as NOA [6]. When a few sperm are found after centrifugation, the condition is defined as cryptozoospermia. TESE might be unnecessary for performing ICSI in these patients, although better implantation rates have been reported using testicular sperm compared with sperm from ejaculates [7].

If azoospermia is diagnosed by semen analysis, the physician must consider whether the patient has obstructive azoospermia (OA) or NOA. The pathological basis of OA is physical obstruction of the post-testicular genital tract, while the etiology of NOA is failure of spermatogenesis due to either inadequate gonadotropin production or intrinsic testicular impairment. Taking a detailed history, physical examination, hormonal evaluation, and genetic testing are employed to establish the diagnosis. A history of factors such as anticancer chemotherapy or undescended testis leads to suspicion that the diagnosis is failure of spermatogenesis. Determining the patient's medications is also important, because some drugs can impair spermatogenesis, including steroids [8] and 5 α -reductase inhibitors [9]. After taking the history, physical examination should be performed. Development of the secondary sexual characteristics is evaluated according to the Tanner stages [10]. When development of the genitalia or pubic hair is poor, this suggests the presence of hypogonadism. Measurement of testicular volume with an orchidometer or by ultrasonography is essential for making a diagnosis of NOA. The size of the testes reflects the level of spermatogenesis, so small testes indicate failure of this process. In patients with NOA, the testes are typically less than 15 cc in volume with a flat epididymis [5].

Ultrasonography is not only useful for measuring the volume of the testes, but also provides useful information about testicular pathophysiology. Testicular microlithiasis, which is defined as five or more microliths per testis [11], can be diagnosed by ultrasonography. This condition is known to be associated with failure of spermatogenesis [12], and it can be found in patients with testicular dysgenesis syndrome (TDS). Skakkebaek et al. advocated the

concept of TDS, which suggests that poor semen quality, testicular cancer, undescended testis, and hypospadias are features of a single disease entity [13]. Hence, it was thought that testicular microlithiasis might be associated with testicular cancer at the end of the 1990s, but later studies did not confirm such concerns. The European Society of Urogenital Radiology only recommends follow-up ultrasonography when the following risk factors are present: previous germ cell tumor, history of undescended testis or orchidopexy, testicular atrophy (volume of <12 cc), and history of a germ cell tumor in a first-degree relative [14]. If testicular cancer is suspected from the ultrasonography findings, the clinician should consider further examinations such as measurement of tumor markers, MRI, and surgical orchidectomy.

Varicocele is a common condition that can be identified by physical examination. The patient should be examined in both the supine and standing positions, with the scrotum being inspected first and then palpated. Although only 20 % of men with a documented varicocele suffer from fertility problems [15, 16], this condition can cause impairment of spermatogenesis or even azoospermia. Thus, the presence of varicocele should be assessed during diagnosis of NOA patients.

Hormonal evaluation is also useful for making a diagnosis of NOA. Although NOA cannot always be excluded when gonadotropins are within the normal range (especially in patients with germ cell maturational arrest), high serum gonadotropin levels typically indicate primary testicular failure. Testicular biopsy is not usually required to make a diagnosis of NOA, since it has been reported that more than 90 % of patients with azoospermia could be accurately diagnosed as NOA or OA by combined measurement of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testicular volume [17]. Factors associated with azoospermia are summarized in Table 1.

Additional investigations for NOA patients

When NOA is diagnosed, additional investigations such as karyotyping and genetic analysis should be performed. It has been reported that an abnormal karyotype is found in 13.7 % of patients with azoospermia [18], with Klinefelter syndrome being the most frequent abnormality (10.8 %), followed by other sex chromosomal abnormalities (1.8 %) and autosomal anomalies (1.1 %) [18]. Genetic examination may also reveal another condition that is related to NOA. Several genetic defects, such as *KALI* or *FGFR1*, are involved in Kallmann syndrome, which features hypogonadotropic hypogonadism with anosmia [19, 20]. Mutations of the androgen receptor (AR) gene, which is located on the X-chromosome, are responsible for mild-to-

Table 1 Causes of male infertility and associated factors (adapted with permission from Ref. [63])

Diagnosis	Unselected patients (%) (<i>n</i> = 12,945)	Patients with azoospermia (%) (<i>n</i> = 1446)
Undescended testes	8.4	17.2
Varicocele	14.8	10.9
Testicular tumor	1.2	2.8
Klinefelter syndrome	2.6	13.7
XX male	0.1	0.6
Primary hypogonadism of unknown cause	2.3	0.8
Kallmann syndrome	0.3	0.5
Idiopathic hypogonadotropic hypogonadism	0.4	0.4
Pituitary surgery	<0.1	0.3
Systemic disease	2.2	0.5
Obstruction	2.2	10.3
Idiopathic	30	13.3

severe androgen insensitivity [21]. While complete androgen insensitivity typically results in a female phenotype, men who have mild androgen insensitivity are more likely to present with infertility. Several genes on the X-chromosome are known to specifically act on the testis and play an important role in meiosis [22]. Recent studies have frequently detected altered copy number variants (CNVs) of X-chromosome genes in patients with failure of spermatogenesis, although further investigation is needed for clinical application of this finding [23, 24].

The most popular and significant genetic test for management of NOA is a test for azoospermia factor (AZF), which is located on the long arm of the Y-chromosome (Yq) and has three sub-regions (AZFa, AZFb, and AZFc). In Western countries, approximately 8 % of NOA patients have been reported to harbor Yq microdeletions [25]. As described below, microdeletion in the AZF region can predict surgical sperm retrieval, so it is essential to evaluate AZF microdeletion when considering TESE for NOA patients. Recently, a new molecular diagnostic kit was developed that can be used in the routine clinical setting to assess Y-chromosome deletions in Japanese patients [26].

Management of NOA

Retrieval of testicular sperm

At the present time, there is no treatment that can restore spermatogenesis in the majority of NOA patients, apart from those with secondary testicular failure. Therefore, the

only way for the affected couples to achieve pregnancy without involving a donor is to retrieve spermatozoa directly from the testes for ICSI. An ideal surgical technique would achieve efficient retrieval of sperm while causing minimal trauma to the testes [27]. Several sperm-retrieval techniques have been developed, including TESE and fine-needle aspiration (FNA). TESE has been performed with multiple biopsies to increase the sperm retrieval rate (SRR) [28, 29], but removal of large amounts of tissue could lead to testicular atrophy after surgical intervention [30]. FNA is another possible technique. It was initially used for diagnostic purposes and is a less invasive method of sperm retrieval compared with TESE, but most studies have shown a significantly lower SRR with FNA than TESE [31–34]. The technique of microdissection testicular sperm extraction (micro-TESE) was first described by Schlegel in 1999 [35]. If an operating microscope (magnification of 15–25×) is employed during TESE, seminiferous tubules containing spermatozoa can be visualized. Micro-TESE has several advantages, including a higher yield of spermatozoa per biopsy, removal of less testicular tissue, and identification of blood vessels to minimize vascular injury [35]. This procedure has been widely suggested to be a better method of sperm retrieval in patients with NOA, and several studies have supported the superiority of micro-TESE for testicular sperm retrieval. In NOA patients, the sperm retrieval rate is reported to be 43–63 % when micro-TESE is employed [35–42]. It should be noted that the SRR of micro-TESE is influenced by the surgeon's experience, especially in patients with Sertoli cell-only syndrome (SCO) [43]. Experienced andrologists as well as embryologists are required to treat these patients with severe infertility.

NOA with varicocele

Treatment of NOA patients with varicocele is still controversial. Varicocele is the most common correctable cause of male infertility and surgical varicocelectomy is an important treatment for restoring fertility. Although a systematic review that included patients with subclinical varicocele or normal semen parameters concluded that there was insufficient evidence to support the efficacy of varicocelectomy for increasing the likelihood of conception [44], there have been several other reports about the efficacy of varicocelectomy in patient populations excluding men with subclinical varicocele or normal semen parameters [45, 46]. Varicocele is associated with NOA in 5–10 % of patients. Although this issue remains controversial, several articles supporting the efficacy of surgical varicocelectomy for these patients have been published [47, 48]. However, recent reports have indicated that even if there is some improvement of

spermatogenesis, the postoperative sperm concentration is still quite low and ART such as ICSI will be required [49]. Thus, it is important to decide whether to offer varicocelectomy or sperm retrieval without varicocele repair for these patients. According to a report from Cornell, even if patients have sperm in the semen after varicocelectomy, <10 % will have viable sperm at the time of ICSI and be able to avoid TESE [50]. That study also indicated the SRR was not influenced by whether the patient underwent varicocelectomy or not [50]. At the same time, improvement of the SRR [51] or improvement of the clinical pregnancy rate and live birth rate [52] have also been reported among NOA patients with varicocele. Furthermore, a meta-analysis of 233 NOA patients with varicocele showed a spontaneous pregnancy rate of 6 % following treatment of varicocele [53]. Thus, although treating varicocele shows limited efficacy in NOA patients, some of them may benefit and the physician should counsel couples with care.

NOA with undescended testis

Undescended testis is a frequent congenital disease that is usually diagnosed and treated during childhood. Its prevalence is 30 % in preterm infants and 3 % in term infants worldwide [54, 55]. When the testis is in an abnormal location (e.g., abdominal or inguinal), there is a risk of the development of testicular malignancy as well as impairment of spermatogenesis [56, 57]. This condition was thought to be associated with a 35- to 50-fold greater risk of malignant testicular tumors compared with the normal population [58], although later studies suggested a somewhat lower risk of malignancy (five- to tenfold elevation) [59, 60]. The higher temperature to which the undescended testis is exposed has a detrimental effect on spermatogenesis [61]. Given that spontaneous testicular descent cannot be expected more than 3–6 months after birth [56], early orchidopexy is recommended to promote normal testicular development in adulthood [62]. Even after the testes are relocated to the proper position, infertility is still an issue, although its frequency may be reduced. Among patients with bilateral undescended testes undergoing orchidopexy, azoospermia is still found in approximately 40 % [63]. For these patients, retrieval of testicular sperm needs to be offered as a fertility treatment. Raman et al. investigated the SRR of TESE in NOA patients with a history of orchidopexy. Sperm was retrieved in 35 of 47 attempts (74 %), which was a higher rate than in other NOA patients. The authors also reported that the age at orchidopexy was an independent predictor of SRR in these patients [64]. Unfortunately, undescended testis is sometimes not diagnosed until adulthood, and may even be found during physical examination for assessment of

infertility. Previously, we reported 10 patients with bilateral undescended testes diagnosed in adulthood, all of whom had azoospermia. Micro-TESE was performed in four of these patients, but no sperm could be retrieved, indicating the severe effect on spermatogenesis when undescended testis is not treated until adulthood [65]. Because testicular function is severely impaired, orchidopexy for bilateral undescended testes in adulthood was once considered to be cosmetic and unlikely to have any effect on spermatogenesis. However, case reports have been published documenting fertility after bilateral orchidopexy [66, 67]. We also experienced a patient who achieved pregnancy by TESE with ICSI at 7 years after bilateral orchidopexy as an adult [68]. Although it is rare, it seems that improvement of spermatogenesis can be achieved by orchidopexy in some adult patients with bilateral undescended testes. After orchidopexy, self-examination of the scrotum is highly recommended for these patients to detect testicular malignancy.

NOA with chromosomal/genetic abnormalities

As described above, Klinefelter syndrome is the most frequent chromosomal abnormality among NOA patients. Men with Klinefelter syndrome tend to have small testes, less muscle, less body hair, low sex drive, and gynecomastia. Usually, the diagnosis is made during evaluation of male infertility. Approximately 95 % of men with Klinefelter syndrome have a 47, XXY chromosomal complement [69]. Micro-TESE combined with ICSI is the only approach that can be offered to NOA patients with Klinefelter syndrome. In these patients, the SRR is reported to be approximately 40–50 % [70], with a range of 21 to 72 % [71–76]. While development of micro-TESE and ICSI has allowed some of these patients to have their own progeny, it should also be noted that the spermatozoa of patients with Klinefelter syndrome may have a higher aneuploidy rate of sex chromosomes and autosomal chromosomes [77, 78]. When a patient is diagnosed as having NOA with Klinefelter syndrome, sufficient information should be given to the couple, and options such as prenatal diagnosis or preimplantation genetic screening should be presented [79, 80].

AZF microdeletion is also important when considering the fertility of NOA patients. This region on Yq has an important role in germ cell development and differentiation, and it is divided into three sub-regions which are AZFa, AZFb, and AZFc [81, 82]. The AZF region contains multiple genes required for different stages of spermatogenesis. For instance, *USP9Y* and *DBY* are located in the AZFa region, *RBMY* is in the ABFb region, and *DAZ* is in the AZFc region. Deletions affecting the AZF region have been reported in 8–12 % of NOA patients [63]. The most

frequently deleted region is AZFc (80 %), followed by deletion of AZFb (1–5 %), AZFa (0.5–4 %), and AZFb+c (1–3 %) [83–85]. Evaluation of microdeletion in the AZF region is clinically important because it can predict SRR during micro-TESE. Typically, complete deletion of the AZFa region is associated with the SCO phenotype, while complete AZFb deletion or AZFb+c deletion is associated with maturation arrest. Accordingly, when NOA patients have these deletions, the SRR will be virtually nil if micro-TESE is attempted [86]. On the other hand, patients with AZFc deletion, which is the most frequent abnormality, are known to have residual spermatogenesis. In these patients, the SRR is reported to range from 50 to 70 % [87, 88], although embryonic development may be impaired even if sperm are retrieved [89]. It is important to note that such Yq micro deletions will be inherited by male offspring. Therefore, genetic counseling is mandatory to provide information about the risk of conceiving a son with infertility and possibly other genetic abnormalities [86].

Hypogonadotropic hypogonadism

Hypogonadotropic hypogonadism (HH) is a condition in which secondary testicular dysfunction is caused by either hypothalamic or pituitary disease. The hyposecretion of gonadotropins results in low testosterone production by the testes and impaired spermatogenesis. HH can be classified as congenital or acquired (Table 2). Mutations of *KALI* (X-linked recessive), *FGFR1* (autosomal dominant), and *GNRHR* (autosomal recessive) are reported to be associated with congenital HH [90], but the etiology remains unknown in approximately 70 % of patients. Although the diagnosis of congenital HH is usually made before adulthood because of the lack of puberty, a rare type of congenital adult HH has been reported, which occurs in otherwise healthy men who have completed normal pubertal development and often have proven fertility [91].

Table 2 Classification of hypogonadotropic hypogonadism (adapted with permission from Ref. [63])

Congenital
Kallmann syndrome
Idiopathic hypogonadotropic hypogonadism
Acquired
Tumors of the hypothalamus and pituitary gland
Granulomatous disease
Empty sella syndrome
Hemochromatosis
Obesity
Anabolic steroids
Aging

Although HH is a rare condition, fertility can be improved by medical treatment in these patients. When fertility is the issue, standard medical therapy is administration of gonadotropins. Human chorionic gonadotropin (hCG), with later addition of human menopausal gonadotropin (hMG) or recombinant FSH, is usually administered to rescue spermatogenesis. Detection of sperm in the ejaculate and even natural pregnancy can be expected with this treatment. If fertility is no longer an issue, administration of testosterone instead of gonadotropins could be a treatment option. Interestingly, reversal of idiopathic HH has been documented [92, 93], although lifelong hormone therapy was believed to be necessary for these patients. According to Raivio et al., 10 % (5/50) of idiopathic HH patients showed sustained reversal of their condition after discontinuation of hormone therapy [92]. Thus, brief discontinuation of hormone therapy to assess reversibility may be a reasonable approach in a subset of patients.

Future prospects

Since there is no treatment that can restore spermatogenesis in the majority of NOA patients, retrieval of testicular sperm is currently the main method of achieving pregnancy. However, spermatozoa cannot be retrieved in a certain number of patients even if surgery is performed. Various attempts to retrieve spermatozoa more efficiently have been made during the last decade. Administration of gonadotropins to NOA patients (except those with HH), particularly patients who have elevated plasma gonadotropin levels, has generally been accepted to be ineffective. Nevertheless, this treatment may have some benefit for NOA patients, although the exact mechanisms/potential effects are unclear. One possible explanation is that exogenous gonadotropins increase intra-testicular testosterone, after which spermatogonia are stimulated, leading to DNA synthesis and spermiogenesis in patients with residual spermatogenic activity [94–96]. Shiraishi et al. reported that in 20 NOA patients whose sperm could not be retrieved by micro-TESE, treatment with hCG and recombinant FSH after TESE led to sperm retrieval in 21 % (6/28 patients) during the 2nd micro-TESE attempt [97]. In that study, none of the patients who did not receive hormone therapy after the first micro-TESE attempt had successful sperm retrieval during the second micro-TESE attempt [97]. Although a definite conclusion cannot be made due to lack of well-designed clinical trials, various methods are being tried to enhance sperm retrieval.

Technical improvements using newer instruments are also being made to increase the chance of sperm retrieval during micro-TESE. Ramasamy et al. conducted a study in rodents using multiphoton microscopy (MPM) and

reported that there was a significant difference between seminiferous tubules with and without sperm [98]. The potential concern with this procedure is increased sperm DNA fragmentation by the MPM laser, but no increase was seen at the laser intensity used for imaging of the tubules [98]. The same group published another rodent study using full field optical coherence tomography (FFOCT) to identify the presence of spermatozoa in testicular tissue [99]. Because the light source for FFOCT is a halogen lamp, there is no concern about increased physical or genetic damage to sperm [99]. Recently, we reported a study performed in rodents using a narrow-band imaging system (NBI), which allowed us to distinguish spermatogenically active regions through visualization of blood vessels [100]. Although further studies need to be carried out, these new approaches could lead to better identification of spermatogenesis in humans.

Recent advances in biotechnology have shed light on possible innovations in the treatment of NOA. Successful in vitro production of spermatozoa in cultured neonatal mouse testes was reported by Sato et al. [101]. They then performed ICSI with the spermatozoa and produced healthy offspring [101]. Induction of germ cells from human-induced pluripotent (iPS) cells is also an encouraging technique in this field. It has been reported that generation of haploid round spermatids from human iPS cells can be achieved in vitro [102]. Further progress will contribute to the development of novel therapeutic techniques for NOA patients in the future.

Conclusions

Because there is no treatment that can restore spermatogenesis in the majority of NOA patients, micro-TESE is currently the mainstay for the management of NOA. Chromosomal and genetic testing should be performed in these patients because of the relatively high incidence of such abnormalities in NOA, and sufficient counseling should be provided to couples about these issues. Although various attempts have been made to establish a better sperm-retrieval system with micro-TESE, there is no other option available for patients to get their own progeny if spermatozoa cannot be retrieved. Further studies, including stem cell research, may contribute to novel therapeutic techniques for NOA.

Compliance with ethical standards

Conflict of interest Koji Chiba, Noritoshi Enatsu, and Masato Fujisawa declare that they have no conflicts of interest to declare.

Human/Animal studies This article does not contain any studies with human or animal subjects performed by any of the authors.

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