Azoospermia: Diagnosis and Management

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6.1 Introduction

Azoospermia is one of the major reproductive disorders which causes male infertility in humans; however, the etiology of this disease is largely unknown. The reliable diagnosis of the absence of spermatozoa in a semen sample is an important criterion not only for diagnosing male infertility but also for ascertaining the success of vasectomy and for determining the efficacy of hormonal contraception (Aziz 2013).

The traditional definition of azoospermia is ambiguous, which has ramifications on the diagnostic criteria. The 5th edition of the World Health Organization (WHO) manual (World Health Organization 2010) defines Azoospermia as: "no spermatozoa are found in the sediment of a centrifuged sample" (Eliasson 1981). The American Urological Association has adopted a more detailed definition: "no sperm after centrifugation at 3000×g for 15 min and examination of the pellet" (Male Infertility Best Practice Policy Committee of the American Urological Association 2006). Thus, the accurate assessment of very low sperm counts is particularly important to avoid labeling severely oligozoospermic men as azoospermic. Some of the important features of analyses of azoospermic semen samples are described in the following section.

Azoospermic samples and those with very low sperm counts appear less opaque. Although a low semen volume is more likely to be due to the incomplete collection of the ejaculate, it may also be due to obstruction of the ejaculatory duct, retrograde ejaculation, or congenital bilateral absence of the vas deferens (CBAVD) (de la Taille et al. 1998; Daudin et al. 2000). A characteristically low pH of 6.8 (normal pH 7.2) could also indicate CBAVD, as a consequence of dysplasia or the absence of the seminal

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K. Gunasekaran, N. Pandiyan (eds.), *Male Infertility*,
DOI 10.1007/978-81-322-3604-7_6

vesicles. Semen volume and pH are important for determining the differential diagnosis of the cause of azoospermia. Therefore, attention to detail is necessary when deviations from the normal value are encountered in the routine semen analysis.

When no spermatozoa are observed in replicate wet preparations, the semen sample should be centrifuged, and the pellet should be examined for the presence of sperm. WHO manual (World Health Organization 2010) recommends centrifugation at 3000 × g for 15 min at room temperature for all samples in which no spermatozoa are detected. The semen analysis should be performed according to the 2010 World Health Organization guidelines, and at least two semen samples, obtained more than 2 weeks apart, should be examined.

6.2 Evaluation of the Azoospermic Male

Complete evaluation of a suspected case of azoospermia should include a complete medical and surgical history, physical examination, and endocrine evaluation. Medical history of childhood illnesses (such as viral orchitis or cryptorchidism) and genital trauma, medications and allergies, and an inspection of past infections are important to ascertain the cause of infertility. Infertility could be due to gonadotoxin exposures and prior radiation therapy or chemotherapy. A general physical examination is an essential part of the evaluation of an azoospermic man. The presence of clinical varicocele should be investigated and correctly classified, as high-grade varicocele could be more frequently related to azoospermia (Cocuzza et al. 2013). Appropriate sexual development and possible androgen deficiency must be assessed. Palpation of the testes and measurement of their size is mandatory, as a decreased testicular size indicates impaired spermatogenic potential (Lipshultz and Corriere 1977). In the vast majority of patients, obstructive azoospermia may be easily distinguished from nonobstructive azoospermia through a thorough analysis of clinical diagnostic parameters, such as FSH levels (Cocuzza et al. 2013).

An endocrinologic evaluation of patients who have severe male factor infertility will be useful to determine specific diagnoses and treatment strategies. Determination of serum testosterone and FSH levels of men with sperm counts of less than 10 million/mL will be sufficient to detect a vast majority of clinically significant endocrinopathies (Sigman and Jarow 1997). The information obtained from a complete endocrine profile may help to elucidate the etiology.

6.3 Etiologies of Azoospermia

The etiologies of azoospermia can be grouped into three general categories: pretesticular, testicular, and posttesticular. Pretesticular causes of azoospermia are endocrine abnormalities that adversely affect spermatogenesis. Testicular etiologies involve intrinsic disorders of spermatogenesis inside the testes. These two categories together constitute the condition termed nonobstructive azoospermia (NOA). The posttesticular causes of azoospermia include obstruction of the ductal system at

any location of the male reproductive tract and are termed obstructive azoospermia (OA). The treatment strategies and success rates for each of these conditions are different and range from correction of the defect to restore fertility to locate and extract sperm for use in assisted reproductive techniques (ARTs).

Pretesticular causes of azoospermia are mostly due to pathological endocrine conditions. It is very uncommon and prevalent to the extent of 3 % of infertile men (Sigman and Jarow 1997). Some of the etiologies include congenital or acquired hypogonadotropic hypogonadism. The pathophysiology involves a defect at the level of the hypothalamic secretion of gonadotropin-releasing hormone (GnRH). Acquired causes include pituitary tumors and trauma and the use of anabolic steroids. Hyperprolactinemia leading to inhibition of secretion of GnRH (Burrows et al. 2002) and androgen resistance due to mutation of the androgen receptor gene (Mak and Jarvi 1996) are the other pretesticular causes.

Testicular etiologies are intrinsic disorders of spermatogenesis. Direct testicular pathology may be due to varicocele-induced testicular damage, undescended testes, testicular torsion, mumps orchitis, gonadotoxic effects from medications, genetic abnormalities, and idiopathic causes. Chromosome alterations responsible for disruption of spermatogenesis are found in 15 % of azoospermic and 5 % of oligospermic men and represent one of the most common genetic defects in infertile men (Pandiyan and Jequier 1996; Peschka et al. 1999).

Posttesticular causes of azoospermia are due either to the obstruction of sperm delivery or ejaculatory dysfunction. The obstruction may be at different sites, such as vas deferens, epididymis, or ejaculatory duct, and depends on the presence of pathological conditions, such as the absence of the vasa deferentia, and disorders of ejaculation. The clinical management of obstructive azoospermia depends on its cause and ranges from surgical correction of the obstruction that may lead to natural conception, to retrieval of sperm directly from the epididymis or testis, followed by the use of ART (Practice Committee of American Society for Reproductive Medicine 2008).

Male factor infertility can result from an underlying medical condition that is often treatable but could possibly be life threatening. It can also be based only on seminal parameters without a physical exam. This behavior may lead to a delay in both the exact diagnosis and in possible specific infertility treatment. In recent years, male factor infertility has been exponentially rising due to a comprehensive evaluation of reproductive male function and improved diagnostic tools. Despite this improvement in diagnosis, azoospermia is always the most challenging topic associated with infertility treatment. Several conditions that interfere with spermatogenesis, reduce sperm production and quality can lead to azoospermia. Azoospermia may also occur because of a reproductive tract obstruction. Optimal management of patients with azoospermia requires a full understanding of the disease etiology (Cocuzza et al. 2013).

Many studies have been conducted to understand the underlying causes of NOA and to develop new therapeutic strategies for patients with NOA. In a recent morphological study, Sertoli cells isolated from NOA patients had a series of abnormal ultrastructural features compared with the normal control Sertoli cells: (i) existence of small and spindle-shaped nuclei, (ii) smaller diameter, (iii) deficient nucleolus or

endoplasmic reticulum, and (iv) more vacuoles. Spectral intensities in Sertoli cells of NOA patients were distinct at four typical Raman peaks compared with the control Sertoli cells. In phenotype, SCF, BMP4, and GDNF transcripts and proteins were significantly lower in Sertoli cells of NOA patients than in the control Sertoli cells (Ma et al. 2013). In a study from Czech Republic, lower concentrations of homocysteine and cobalamin (but not folate) were found in azoospermic seminal plasma than in normozoospermic. Folate and cobalamin were higher in seminal plasma from OA than in NOA patients (Crha et al. 2010).

Comparison of expression of progesterone (PR) and estrogen receptors (ER alpha) in testicular tissue from OA and NOA patients has been studied by immunofluorescence and Western blot (Han et al. 2009). In patients with NOA due to maturation arrest (MA) and Sertoli cell only (SCO) syndrome, the expression of PR was reduced in all cell types as compared to that in the OA patients. ERalpha was expressed principally in the OA testis, but was decreased in MA testis and enhanced in the SCO testis. Thus, PR and ER alpha may be involved in the pathogenesis of MA and SCO phenotype in patients with infertility.

Male fertility problems range from diminished production of sperm, or oligozoospermia, to non-measurable levels of sperm in semen, or azoospermia, which is diagnosed in nearly 2% of men in the general population. Testicular biopsy is the only definitive diagnostic method to distinguish between obstructive (OA) and nonobstructive (NOA) azoospermia and to identify the NOA subtypes of hypospermatogenesis, maturation arrest, and Sertoli-cell-only syndrome. Rare foci of sperm production may be found in up to 60% of men with NOA. Sperm production, if present, is minimal for sperm appearance in the ejaculate. Given that there are no treatment options to restore fertility, sperm retrieval is the only alternative to find testicular sperm that can be used for in vitro fertilization (IVF). Among sperm acquisition methods, micro-surgical testicular sperm extraction (micro-TESE) has higher success rate at obtaining sperm compared with testicular sperm extraction and testicular sperm aspiration. In general, no major differences were noted in short-term neonatal outcomes and congenital malformation rates between children from fathers with NOA and OA (Esteves and Agarwal 2013).

6.4 Obstructive Azoospermia

Obstructive azoospermia can be due to several factors, the most common being congenital bilateral absence of the vas deferens. Other etiologies included an idiopathic cause, an iatrogenic condition due to surgical causes, ejaculatory duct obstruction, trauma, retrograde ejaculation, and vas deferens occlusion. Posttesticular sperm maturation requires a specific luminal environment in the epididymis, which is created, in part, by the blood-epididymis barrier. However, recent microarray studies have shown that epididymal cellular junctions appear to be altered in OA (Dube et al. 2010).

Ejaculatory duct obstruction (EDO) is a rare cause of OA and accounts for approximately 1% of patients presenting with male infertility. It should be suspected when the patient has low-volume, acidic semen that contains no sperm.

Absence of fructose in the semen supports the diagnosis, as fructose is present in the secretions from the seminal vesicles. Occasionally, pain at the time of ejaculation is reported. Physical examination may reveal enlarged seminal vesicles or a midline nodule in the prostate, but frequently, the rectal exam is unremarkable. Testicular volume is usually normal, and vasa deferentia are present. Laboratory studies will confirm normal gonadotropin and testosterone levels. Retrograde ejaculation should be ruled out by examining post-ejaculatory urine for sperm. Transrectal ultrasound is a useful tool for confirming the diagnosis and further defining the causative factor. Sonography can also demonstrate dilation of the ejaculatory ducts, calcifications within the ejaculatory ducts, or prostate, utricle, or Mullerian duct cysts that can occlude the ejaculatory ducts. Traditional treatment consists of transurethral resection of the ejaculatory ducts (TURED) (Yurdakul et al. 2008).

Congenital bilateral absence of the vas deferens (CBAVD) is often caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This condition is suspected based on the absence of palpable vas deferens at the time of physical examination. The caput of the epididymis is present, and the testicles should be of a normal size and consistency; however, the seminal vesicles are absent or hypoplastic in a majority of patients (Kuligowska and Fenlon 1998). Unilateral or bilateral vasal hypoplasia or unilateral absence of the vas may be an indicator of obstructive azoospermia, as a high percentage of these patients will have anomalies of the contralateral seminal vesicle. Surgical reconstruction may be a viable treatment for some patients with unilateral vasal agenesis or hypoplasia. CBAVD is not amenable to surgical reconstruction, but sperm is readily retrievable from these patients via percutaneous (PESA) or microsurgical (MESA) epididymal aspiration, testicular sperm aspiration (TESA), or simple open biopsy (TESE).

Epididymitis is a common genitourinary condition, and an infectious etiology should always be considered in men with this diagnosis. Gonorrhea, chlamydia, trichomonas, brucellosis, BCG, ureaplasma, mycoplasma, coliforms bacteria, adenovirus, and enterovirus have all been reported as causes of epididymitis. Regardless of the etiology, epididymitis can cause an intense inflammatory reaction, leading to secondary scarring and obstruction of the epididymis. Physical examination may reveal enlarged or indurated epididymides and a transition point suggesting the site of obstruction. Semen volumes are typically normal, and white cells are not necessarily present in the ejaculate. The incidence of postinfectious epididymal obstruction is thought to be low in developed countries due to prompt treatment, but it may account for a disproportionately large percentage of OA in developing countries (Ho et al. 2009). Scrotal exploration and microsurgical reconstruction are a viable option for postinfectious epididymal obstruction.

Iatrogenic injury or injury to the vas during surgical procedures has been well described and presents a unique challenge to fertility specialists. Vasal injury has been attributed to a variety of inguinal, scrotal, and pelvic surgeries, including herniorrhaphy, hydrocelectomy, appendectomy, and renal transplant. Surgical reconstruction is possible in many cases of iatrogenic injury to the vas in the scrotum or inguinal canal. Some factors, such as age and obstructive interval, are likely to impact postoperative outcomes after vasal reconstruction for etiologies of OA.

6.4.1 Treatment of OA

Obstructive azoospermia (OA) is defined as the absence of spermatozoa in the ejaculate despite normal spermatogenesis. It is a common cause of male infertility and can result from infection, congenital anomalies, or iatrogenic injury and accounts for 6.1–13.6 % of patients presenting for fertility evaluation (Aziz et al. 2006; Jequier 1985). Microsurgical vasal reconstruction is a suitable and cost-effective treatment for many cases of OA, this approach may not be feasible or desired in some cases, and such couples will require sperm retrieval paired with in vitro fertilization (Baker and Sabanegh 2013). This process requires several considerations and decisions to be made, including the cause and duration of obstruction, choice of sperm retrieval technique, and whether to use fresh or frozen-thawed sperm. Sperm retrieval can be achieved by percutaneous (PESA) or microsurgical (MESA) epididymal sperm aspiration, testicular sperm aspiration (TESA), or simple open biopsy (TESE).

Use of spermatozoa obtained by PESA in ICSI is a viable treatment for men with OA (Jiang et al. 2013). Spermatozoa can be retrieved from the testis and epididymis of men with OA and used for ICSI. In some cases, the use of testicular spermatozoa altered the embryonic development, and the use of epididymal spermatozoa should be preferred, irrespective of the etiology of OA (Buffat et al. 2006).

Percutaneous sperm retrieval is a highly effective method for collecting sperm in men with OA. Successful sperm retrieval was achieved in over 85 % of the cases using PESA, but more than one aspiration was often required (Esteves et al. 2013). In cases of failed PESA, TESA was adequate to obtain sperm in nearly all cases. Motile spermatozoa were obtained in approximately 73 % of the cases after the first or second PESA aspiration, and TESA has been performed as a rescue procedure after failed PESA in approximately 14 % of the individuals (Esteves et al. 2013). ICSI outcomes using spermatozoa collected by PESA or TESA are similar, suggesting that the reproductive potential of those gametes is independent of their source in OA. However, epididymal spermatozoa are easier to handle in the IVF laboratory compared with testicular sperm, and it is more likely that there will be excess sperm for freezing in case of epididymal retrieval (Miyaoka and Esteves 2013).

Some studies have indicated the risks of using nonejaculated spermatozoa in assisted reproduction techniques. In cases of OA, the epididymal sperm may be immature or senescent because of the long stay in the obstructed epididymis and lead to genetic risks when it is used for fertilization (O'Connell et al. 2002). On the other hand, testicular sperm can have the potential risk of incomplete genomic imprinting, incomplete chromatin condensation, and incomplete protamination (Golan et al. 1996; Tesarik et al. 1998). However, a recent Dutch multicenter study has dispelled some of these concerns. Assessment of children born from ICSI cycles, using retrieved epididymal sperm in comparison with those born from ICSI or IVF using ejaculated sperm, did not reveal any disparities in terms of incidence of stillbirths, malformations, motor performance, or mental-language development (Woldringh et al. 2011).

6.5 Nonobstructive Azoospermia

The initial evaluation of NOA needs to resolve the following issues: (1) confirming azoospermia, (2) differentiating obstructive from nonobstructive etiology, (3) assessing for the presence of reversible factors, and (4) evaluating for the presence of genetic abnormalities. An elevated follicle-stimulating hormone (FSH) level or an absence of normal spermatogenesis by testicular histology in the presence of azoospermia is generally considered sufficient evidence of a nonobstructive etiology. NOA has been identified with the Sertoli-cell-only syndrome. Other etiologies included an idiopathic cause, Klinefelter syndrome, maturation arrest, Y-chromosome microdeletion, cryptorchidism, trauma, exogenous testosterone supplementation, and other genetic disorders.

Hormone analysis forms the cornerstone of further evaluation and management of NOA and serves two important functions. The first function is to identify a distinct subset of men who have hypogonadotropic hypogonadism (low FSH), in which azoospermia results from an inadequate stimulation of the testis by gonadotropins. The second function is to predict the success of medical therapy and of surgical sperm retrieval. The American Urological Association recommends estimation of serum FSH and testosterone as the initial hormonal assessment (American Urological Association 2012).

6.5.1 Treatment of NOA

NOA is diagnosed in approximately 10 % of infertile men. It represents a failure of spermatogenesis within the testis and, from a management standpoint, is due to either a lack of appropriate stimulation by gonadotropins or an intrinsic testicular impairment. The former category of patients has hypogonadotropic hypogonadism and benefits from specific hormonal therapy. These men show a remarkable recovery of spermatogenic function with exogenously administered gonadotropins or gonadotropin-releasing hormone. This category of patients also includes some individuals whose spermatogenic potential has been suppressed by excess androgens or steroids, and they also benefit from medical management. Hypogonadotropic hypogonadism is one of the few causes of NOA that have shown a consistent response to medical management (Hoffman and Crowley 1982; Belchetz et al. 1978). Gonadotropin therapy is begun at the time the patient wishes to father a child, and 3–6 months of treatment are usually sufficient to induce spermatogenesis (Vicari et al. 1992; Finkel et al. 1985). Therapy is initiated with human chorionic gonadotropin (hCG) at 2,000 IU subcutaneously three times per week or 2,500 IU twice a week and supplemented with FSH (menopausal, purified, or recombinant) at 37.5–150 IU three times a week after 3–6 months. hCG is sufficient to initiate spermatogenesis, but FSH is required to complete the spermiogenesis, particularly in patients with congenital abnormalities (Kumar 2013). An alternative method for treating hypogonadotropic hypogonadism is with a pulsatile injection of 5–20 μg of

GnRH, administered every 2 h subcutaneously. GnRH therapy reliably corrected the hypogonadism, with a reversal of azoospermia (Sykiotis et al. 2010).

The enzyme aromatase, present in the adipose tissue, liver, testis, and skin, is responsible for converting testosterone and other androgens to estradiol in men. Estradiol suppresses pituitary LH and FSH secretion and also directly inhibits testosterone biosynthesis. This results in an imbalance in the testosterone and estradiol (T/E) ratio, which may be reversible. Aromatase inhibitors have the potential to block the conversion of androgens to estradiol. The two types of aromatase inhibitors are steroidal (testolactone) and nonsteroidal (anastrozole, letrozole). Both of these groups of agents have been studied for potential therapeutic roles in NOA. The other, larger category of NOA consists of men with an intrinsic testicular impairment where empirical medical therapy yields little benefit. The primary role of medical management in these men is to improve the quantity and quality of sperm retrieved from their testis for in vitro fertilization. Gonadotropins and aromatase inhibitors show promise in achieving this end point (Kumar 2013).

Locating and retrieving spermatozoa in men with NOA remains a clinical challenge, largely because sperm production in these men can be patchy or focal in nature. Rare foci of sperm production may be found in up to 60 % of men with NOA. Sperm production, if present, is minimal for sperm appearance in the ejaculate. Given that there are no treatment options to restore fertility, sperm retrieval is the only alternative to find testicular sperm. The retrieved sperm can then be used for in vitro fertilization (IVF). Fertilization and pregnancies have been achieved with spermatozoa recovered from the seminiferous tubules. The most common methods for retrieving testicular sperm are testicular sperm aspiration (TESA), or needle/fine-needle aspiration (FNA), and testicular sperm extraction (TESE) by open testicular biopsy. A systematic review of the available sperm retrieval techniques has been published by Donoso et al. (2007) and efficacy of the techniques has been compared (Hauser et al. 2006). The optimal technique for sperm extraction should be minimally invasive and avoid destruction of testicular function, without compromising the chance to retrieve adequate numbers of spermatozoa to perform ICSI.

FNA is highly informative, minimally invasive and is associated with fewer complications than other commonly used approaches for sperm detection. As it is challenging to find foci of sperm production, strategies such as FNA mapping have been developed to find spermatozoa. FNA mapping has gained considerable attraction as an informative, "testis-sparing" technique for sperm detection in NOA. With knowledge of sperm presence and location prior to sperm retrieval, FNA maps can help clinicians tailor sperm retrieval to optimize time, effort, and extent of procedures needed to procure spermatozoa in difficult cases (Beliveau and Turek 2011). Inhibin B and FSH have been evaluated as predictors of the recovery of sperm in testicular fine-needle aspirate in men with azoospermia (Goulis et al. 2008).

Microdissection TESE (micro-TESE), performed with an operative microscope, is widely considered to be the best method for sperm retrieval in NOA, as larger and opaque tubules, presumably with active spermatogenesis, can be directly identified, resulting in higher spermatozoa retrieval rates with minimal tissue loss and low postoperative complications. Micro-TESE, in combination with ICSI, is applicable

in all cases of NOA, including Klinefelter syndrome (KS). In addition, short- and long-term complications of micro-TESE in NOA and KS patients need to be considered (Ishikawa 2012; Everaert et al. 2006).

Among sperm acquisition methods, micro-TESE has higher success rates at obtaining sperm compared with testicular sperm extraction and testicular sperm aspiration. Micro-TESE allowed the identification and extraction of sperm-containing seminiferous tubules with minimum tissue excision and marked reduction in time of processing of testicular specimens for sperm injection (Esteves 2013). Despite the improved success rate of sperm by micro-TESE methods, it becomes necessary to stimulate spermatogenesis in some NOA cases. This has been achieved by hormonal stimulation by using human chorionic gonadotropin (hCG) injections for 4–5 months prior to retrieval. The hCG stimulation was found to be effective in men with hypospermatogenesis (Shiraishi et al. 2012). Use of letrozole (2.5 mg per day) has also been found to improve sperm count in NOA patients with normal serum FSH (Cavallini et al. 2011). Clomiphene citrate has also been administered to enhance the availability of sperm prior to surgical retrieval in NOA patients (Hussein et al. 2005).

Testicular sperm retrieval techniques associated with intracytoplasmic sperm injection are currently used for the treatment of NOA patients, but reliable clinical and laboratory prognostic factors of sperm recovery are still absent. There are no reliable positive prognostic factors that guarantee sperm recovery for patients with NOA. The only negative prognostic factor is the presence of AZFa and AZFb microdeletions (Glina and Vieira 2013).

Numerous studies on NOA have reported that varicocelectomy not only can induce spermatogenesis but can also increase the sperm retrieval rate; however, the value of varicocelectomy in patients with NOA still remains controversial (Inci and Gunay 2013).

Another novel technique used for the identification of spermatogenesis in NOA is the use of ^{1}H magnetic resonance spectroscopy (MRS), a noninvasive imaging tool that can identify and localize spermatogenesis in the testis. Phosphocholine (PC) and taurine tissue concentrations were significantly different between normal and NOA testicular tissue. Mean PC concentrations were three times higher in normal testes compared with NOA (SCO). A predictive model for sperm presence was developed based on tissue concentrations of PC (Aaronson et al. 2010).

6.6 Genetic Studies of Azoospermia

Azoospermia due to obstructive and nonobstructive mechanisms is a common manifestation of male infertility accounting for 10–15 % of such cases. Known genetic factors are responsible for approximately 1/3 of cases of azoospermia. Genetic factors explain 21–29 % of azoospermia (Lee et al. 2011), whereas 12–41 % of azoospermic cases are idiopathic and most likely related to unknown genetic factors (Hernandez Uribe et al. 2001). Azoospermia of a genetic origin is primarily caused by a wide array of genetic disorders, such as chromosomal abnormalities,

Table 6.1 Genetic diseases and abnormalities that result in azoospermia

Obstructive azoospermia of genetic origin
Cystic fibrosis
Congenital bilateral absence of the vas deferens (CBAVD)
Congenital unilateral absence of the vas deferens (CUAVD)
Congenital bilateral epididymal obstruction and normal vasa
Young syndrome
Nonobstructive azoospermia of genetic origin
Genetic pretesticular causes of NOA
Hypothalamic hypogonadotropic hypogonadism
Congenital hypogonadotropic hypogonadism
Adult-onset genetic hypothalamic hypogonadotropic hypogonadism
Pituitary disorders associated with hypogonadism
Generalized anterior pituitary hormone deficiency
Selective gonadotropin deficiency
Genetic testicular disorders affecting spermatogenesis and androgen production
Klinefelter syndrome
XX male syndrome
Mutation in X-linked USP 26
X-linked SOX3 mutation
Bilateral anorchia
Noonan syndrome
45 X/46XY mosaicism (mixed gonadal dysgenesis)
Genetic testicular disorders affecting spermatogenesis
Y-chromosome microdeletion
Autosome translocations
Monogenic disorders
Multifactorial disorders (e.g., cryptorchidism)
Genetic testicular disorders affecting androgen production or action
Androgen receptor mutation
Steroidogenic acute regulatory protein StAR mutation
3BHSD type 2 deficiency
SRD5A2 mutation
Dysfunctional cell regulatory pathways
Epigenetic defects
Genetic abnormities at the primordial germ cell level

Reproduced from Hamada et al. (2013)

monogenic disorders, multifactorial genetic diseases, and epigenetic disorders. These conditions constitute the genetic basis of reproductive failure. Table 6.1 summarizes the genetic basis of azoospermia at the posttesticular (obstructive azoospermia), pretesticular, and testicular (nonobstructive azoospermia) levels.

Among the OA of genetic origin, CBAVD is the most frequent condition (Tuttelmann et al. 2011). Cystic fibrosis (CF) is a life-threatening autosomal

recessive disease in which the failure is due to a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR gene is expressed in the epithelial cell of exocrine tissues, such as the head of the epididymis and the vas deferens. CFTR has a role in sperm maturation in the epididymis, as this protein is necessary for fluid absorption and facilitation of sperm capacitation and fertilization ability (Wong 1998; Chan et al. 2009). Epididymal malformations are common manifestation of CF; seminal vesicles anomalies and obstructed ejaculatory ducts are also common. CBAVD accounts for at least 6–25 % of cases of OA and approximately 2 % of infertility cases (Oates and Amos 1994; Patrizio and Leonard 2000). CFTR is mutated in 60–90 % of patients with CBAVD (Ferlin et al. 2007a). The most common CFTR mutation found in men with CBAVD is a combination of ΔF508/R117H, which accounts for 40 % of the cases (Ratbi et al. 2007; Jezequel et al. 2000). CFTR mutations have also been observed in men with CUAVD.

NOA is the most severe form of azoospermia that can be caused by many factors, such as heat, radiation, drugs, varicocele, infections, and cancer, in addition to genetic factors. Genetic etiologies contribute significantly to the development of this disorder and are responsible for 21–28 % of cases (Lee et al. 2011; Hernandez Uribe et al. 2001; Hamada et al. 2013; Donohue and Fauver 1989). The genetic factors are further classified as pretesticular and testicular causes. The genetic pretesticular etiology encompasses hereditary hypothalamic-pituitary abnormalities resulting in small testes that exhibit an immature histological pattern. In these cases, immature Sertoli cells or spermatogonia type A and the absence of Leydig cells are often observed.

Genetic testicular causes of NOA include the following: (i) chromosomal abnormalities, (ii) Y-chromosome microdeletions, (iii) failure of the primordial germ cells to reach the developing gonads, (iv) lack of differentiation of the primordial germ cells to spermatogonia, and (v) male germ line mutations that affect spermatogenesis.

6.6.1 Y-Chromosome Microdeletion

The long and short arms of the Y-chromosome contain many genes that regulate spermatogenesis and testis development, respectively. Microdeletions on the long arm of the Y-chromosome (Yq) are well correlated with male infertility. Yq microdeletions are detected in approximately 13 % of men with NOA and in 5 % of men with severe oligozoospermia (sperm counts lower than 5 million/mL) (Reijo et al. 1995; McLachlan et al. 1998). A microdeletion is defined as a chromosomal deletion that spans several genes but that is small in size and cannot be detected using conventional cytogenetic methods (e.g., karyotyping). The region at Yq11 is referred to as the "azoospermia factor" (AZF) region. The AZF region is further subdivided into three subregions that are termed AZFa, AZFb, and AZFc. The most common aberrations in the AZF region are multiple gene deletions in the AZFb and AZFc subregions (Ferlin et al. 2007b), which can produce a wide range of infertility phenotypes.

Three regions in the long arm of the Y-chromosome, known as AZFa, AZFb, and AZFc, are involved in the most frequent patterns of Y-chromosome microdeletions. These regions contain a high density of genes that are thought to be responsible for impaired spermatogenesis. In 2003, the Y-chromosome sequence was mapped, and microdeletions are now classified according to the palindromic structure of the euchromatin that is composed of a series of repeat units called amplicons. Although it has been shown that the AZFb and AZFc are overlapping regions, the classical AZF regions are still used to describe the deletions in clinical practice (Sadeghi-Nejad and Farrokhi 2007). Y-chromosome microdeletions are among the major causes of male infertility.

Both the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have recommended the use of sY84 and sY86 markers for the detection of azoospermia factor a (AZFa) microdeletion during DNA testing for male infertility (Wu et al. 2011). Detection of various subtypes of these deletions has a prognostic value in predicting the potential success of testicular sperm retrieval for assisted reproduction. Men with azoospermia and AZFc deletions may have retrievable sperm in their testes. However, with ICSI, there is a risk of transmission of these microdeletions to the male offsprings (Mau Kai et al. 2008). There is a high-frequency genetic abnormality, such as Y-chromosome microdeletions in patients of NOA, and a risk of passing the genetic defects to their offspring. Consequently, there is need for genetic testing and counseling of NOA patients prior to ART. The genetic testing may also be useful in prognosis and choice of ART technique. A high prevalence of Y-chromosome microdeletions have been observed in Middle Eastern (28.41 %) (Alhalabi et al. 2013), Ukrainian (35 %) (Pylyp et al. 2013), Brazilian (18.8 %) (Mafra et al. 2011), and Iranian patients (66.67 % of AZFb) (Mirfakhraie et al. 2011), but the incidence seems to be low in Slovak azoospermic patients (Behulova et al. 2011). Genetic anomalies in patients with severe oligozoospermia and azoospermia have also been detected in eastern Turkey. A prospective study detected Y-chromosome microdeletions, especially of the AZFc locus to the extent of 64 % (Ceylan et al. 2010). The most frequent deletions were in the AZFc region (50 %) in Thai men with azoospermia and comparable with infertile men from other Asian and Western countries (Vutyavanich et al. 2007).

About 10 % of cases of male infertility are due to the presence of microdeletions within the long arm of the Y-chromosome (Yq). Despite the large literature covering this critical issue, very little is known about the pathogenic mechanism leading to spermatogenesis disruption in patients carrying these microdeletions. Testicular gene expression profiling of patients carrying an AZFc microdeletion has been carried out by employing a microarray assay techniques. Results indicated a down-regulation of several genes related to spermatogenesis that are mainly involved in testicular mRNA storage. If that several forms of infertility can be triggered by a common pathogenic mechanism, that is likely related to alterations in testicular mRNA storage due to lack of testicular DAZ gene expression (Gatta et al. 2010).

Maturation arrest (MA) refers to failure of germ cell development leading to clinical NOA. Although the azoospermic factor (AZF) region of the human Y-chromosome is clearly implicated in some cases, thus far very little is known

about which individual Y-chromosome genes are important for complete male germ cell development. Stahl et al. (2012) have attempted to identify single genes on the Y-chromosome that may be implicated in the pathogenesis of NOA associated with MA in the American population. Based on the genotype-phenotype analysis of 132 men with Y-chromosome microdeletions, they identified CDY2 and HSFY as the genes for which differences in expression were observed between the MA and OA. Men with OA had 12-fold and 16-fold higher relative expression of CDY2 and HSFY transcripts, respectively, compared to MA. CDY2 and HSFY were also underexpressed in patients with Sertoli-cell-only syndrome. These observations suggest that CDY2 and HSFY are important for sperm maturation, and their impaired expression could be implicated in the pathogenesis of MA.

Genetic mechanisms implicated as a cause of male infertility are poorly understood. Meiosis is unique to germ cells and essential for reproduction. The synaptonemal complex is a critical component for chromosome pairing, segregation, and recombination. Hormad1 is essential for mammalian gametogenesis. Mutational analysis of all HORMAD1 coding regions in Japanese men revealed meiotic arrest in the early pachytene stage, and synaptonemal complexes could not be visualized. By the sequence analysis, three polymorphism sites, SNP1 (c. 163A>G), SNP2 (c. 501 T>G), and SNP3 (c. 918C>T), have been found in exons 3, 8, and 10. SNP1 and SNP2 were associated with human azoospermia caused by complete early maturation arrest ($P<0.05$) (Miyamoto et al. 2012a). In similar studies, SEPTIN12 and UBR2 gene have also been found to be associated with increased susceptibility to azoospermia caused by meiotic arrest (Miyamoto et al. 2011, 2012b). Mutations in PRDM9 (MEISETZ) gene have also been implicated in Japanese NOA patients (Irie et al. 2009; Miyamoto et al. 2008).

Specimens from testicular biopsies of men with NOA have been used to investigate the expression of spermatogenesis-related genes MND1, SPATA22, GAPDHS, and ACR. Analysis of the expression of spermatogenic genes in human testes with abnormal spermatogenesis showed different expression patterns in patients from the three groups: hypospermatogenesis (HS), maturation arrest (MA), and Sertoli-cell-only syndrome (SCO) groups. Fertilization rates were similar at 70 %, but pregnancy rates for ACR and GAPDHS genes were low at 6–8 % (Dorosh et al. 2013).

A genome-wide association study in Chinese population has revealed that variants within the HLA region are associated with risk for NOA (Zhao et al. 2012). They have detected variants at human leukocyte antigen (HLA) regions, HLA-DRA, rs3129878, and rs498422 to be independently associated with NOA.

Recently, a separate Chinese genome-wide association study (GWAS) (Hu et al. 2012) identified four autosomal single-nucleotide polymorphism (SNP) loci as being significantly associated with risk factors for NOA: rs12097821, rs2477686, rs10842262, and rs6080550. Although not significant, three of four SNPs (rs12097821, rs2477686, and rs10842262) have also showed associations in Japanese men. However, further larger case–control studies are required to establish whether the SNPs are genetic risk factors for NOA in these populations (Sato et al. 2013). C677T in the methylenetetrahydrofolate reductase (MTHFR) gene is also suggested as a genetic risk factor in Chinese men (A et al. 2007).

A genome-wide gene expression study by Okada et al. (2008) demonstrated that SNPs (rs6836703) of the ADP-ribosyltransferase 3 gene (ART3) were associated with NOA with highest significance. These findings clarify a molecular pathophysiology of NOA and suggest a novel therapeutic target in the treatment of NOA. MicroRNAs (miRNAs) are a class of small noncoding RNA molecules. The expression of miRNAs is altered in testicular tissues of patients with NOA, suggesting a role of miRNAs in regulating spermatogenesis (Lian et al. 2009).

Nonetheless, at least 40 % of cases are currently categorized as idiopathic and may be linked to unknown genetic abnormalities. It is recommended that various genetic screening tests are performed in azoospermic men, given that their results may play vital role in not only identifying the etiology but also in preventing the iatrogenic transmission of genetic defects to offspring via advanced assisted conception techniques (Hamada et al. 2013).

6.7 Prognostic Factors of Sperm Retrieval

Introduction of intracytoplasmic sperm injection (ICSI) has brought hope for men with severe male infertility and provided a chance for them to become biological fathers. ICSI and other assisted reproduction techniques require testicular spermatozoa to be extracted to fertilize oocytes. Sperm retrieval is conducted with testicular aspiration or biopsy for testicular sperm extraction (TESE). Despite the current use of TESE, reliable clinical and laboratory prognostic factors of sperm recovery are still absent. Currently, several prognostic factors such as testis size, follicle-stimulating hormone (FSH), inhibin beta, the etiology of infertility, and genetic alterations are utilized; however, the histological testicular pattern remains the best predictor of sperm retrieval, but is associated with an invasive procedure (Glina et al. 2005).

Measurement of follicle-stimulating hormone (FSH) levels has been used as a predictor of sperm recovery, but its use remains controversial. Inhibins, anti-Mullerian hormone (AMH), and activins are glycoproteins that are transforming growth factors (TGF). Plasma levels of inhibin fraction B and seminal levels of AMH can be used as predictive parameters for sperm recovery in NOA (Deffieux and Antoine 2003). Other tests include the genetic detection of chromosome alterations. Y-chromosome microdeletions have also been used as a prognostic factor for sperm recovery. This possibility is based on the absence of mature sperm in azoospermic men with AZFa and AZFb microdeletions who underwent sperm retrieval techniques. Fortunately, AZFc is the Y microdeletion most often found in azoospermic men (60 %), and sperm can be retrieved for these patients. Therefore, the presence of AZFa or AZFb is a negative predictive factor for sperm retrieval in azoospermic men (Shefi and Turek 2006). The following section describes recent attempts toward development of prognostic markers that predict sperm retrieval in azoospermic patients.

Cell-free seminal mRNA (cfs-mRNA) exists in human ejaculate at high concentrations and with high stability and contains many tissue-specific transcripts secreted

from the male reproductive system. Such cfs-mRNAs have been evaluated as candidates for noninvasive biomarkers for the presence of germ cells and of physiopathological condition of complete obstruction in men with azoospermia. Li et al. (2012) have used the highly sensitive mRNA technology, to amplify the germ cell-specific (DDX4), seminal vesicle-specific (SEMG1), and prostate-specific (TGM4) mRNAs from cfs-mRNAs. TGM4 was detected in all participants. Consistent with their diagnosis, DDX4 was detected in all patients with MA or incomplete Sertoli-cell-only patients, but was absent in cases of complete Sertoli cell only, vasectomy, and congenital bilateral absence of the vas deferens (CBAVD), indicating absence of sperm. These results suggest that cfs-mRNA could be used as noninvasive biomarkers with high sensitivity.

More recently, the study of the seminal plasma proteome appears to offer the potential to identify biomarkers that may aid in the diagnosis of the causes of azoospermia. Many of the proteins in the seminal plasma are expressed in the testis and epididymis and are linked to fertility. Some of these proteins may be useful as noninvasive biomarkers to discriminate NOA from OA (Batruch et al. 2012). Drabovich et al. (2013) have identified two proteins, epididymis-expressed ECM1 and testis-expressed TEX101, which differentiated OA and NOA with high specificities and sensitivities. The performance of ECM1 was confirmed by enzyme-linked immunosorbent assay. On the basis of a cutoff level of 2.3 μg/ml derived from the current data, we could distinguish OA from normal spermatogenesis with 100 % specificity and OA from NOA with 73 % specificity, at 100 % sensitivity. Immunohistochemistry and an immunoenrichment mass spectrometry-based assay revealed the differential expression of TEX101 in distinct NOA subtypes. TEX101 semen concentrations differentiated Sertoli-cell-only syndrome from the other categories of NOA. They have proposed a simple two-biomarker decision tree for the differential diagnosis of OA and NOA and, in addition, for the differentiation of NOA subtypes. ECM1 and TEX101 clinical assays have the potential to replace most of the diagnostic testicular biopsies and facilitate the prediction of outcome of sperm retrieval procedures, thus increasing the reliability and success of assisted reproduction techniques (ART).

Genome-wide microRNA expression profiling of three validated seminal plasma miRNAs (sp-miRNAs) was examined in testicular tissues of patients with NOA and of fertile controls. miR-141, miR-429, and miR-7-1-3p are significantly increased in seminal plasma of patients with NOA compared with fertile controls. These sp-miRNAs could provide a novel noninvasive, semen-based test for NOA diagnosis, as they show reproducible and stable expression levels (Wu et al. 2013). In another study from Japan, expression levels of VASA, outer dense fiber-1 (ODF1), ODF2, and sperm mitochondria-associated cysteine-rich protein (SMCP) mRNAs in testicular tissue specimens were found to be significantly high in successful micro-TESE cases, compared to failed ones. Of these mRNAs, VASA mRNA expression was independently related to micro-TESE outcome and could be a useful adjunct parameter to predict sperm retrieval in NOA (Ando et al. 2012).

In NOA, testicular sperm extraction (TESE) is successful only in about 50 % of cases. A parameter for predicting TESE quality and pregnancy rates after ICSI of

testicular spermatozoa has been devised by Boitrelle et al. (2011), based on multivariate analysis of total testicular volume (TTV), FSH and inhibin B levels, and TESE quality from a retrospective study. This score has been found to be a predictor of successful TESE, with a positive likelihood ratio of +3.01. When the score was <18.5, TESE was successful in 77.4 % of cases and "sperm-rich" (i.e., yielding >100 spermatozoa) in 91.1 % of cases; 42.8 % of couples took a baby home. Such a score can be useful in improving patient case and pre-ICSI counseling in cases of NOA.

In a novel attempt, Ma et al. (2011) have used leptin and artificial neural networks (ANNs) to predict the accuracy of sperm retrieval in NOA. Twelve factors, grouped into four sets, were recorded as the input variables for ANNs: (1) testicular volume, (2) semen volume, seminal pH, seminal alpha-glucosidase and fructose, (3) serum hormones including FSH, LH, total testosterone (TT), prolactin, estradiol, and (4) serum and seminal leptin. Different ANN models were constructed and their prediction accuracy was compared by receiver operating characteristic (ROC) curve analysis. ANN consisting of all four sets of factors had the largest area under the curve (AUC = 0.83) and demonstrated significantly greater accuracy compared to FSH (AUC = 0.63, $P < 0.01$) and leptin (AUC = 0.59, $P < 0.01$).

Optimal cutoff value for FSH has been used to predict the presence of spermatogenesis in patients with NOA. In a comparative study of FSH levels in NOA patients with spermatogenesis and successful sperm retrieval, mean serum FSH was significantly higher than those failed in sperm retrieval (Chen et al. 2010). A cutoff value of 19.4 mIU/mL discriminated between the two groups with a sensitivity of 70 %. The positive predictive value for failed sperm retrieval could reach 100 %. Elevated plasma levels of FSH could be used as a reliable criterion for a trial of sperm retrieval from testes in artificial reproductive techniques. On the other hand, a meta-analysis of inhibin B as an indirect marker of spermatogenesis in NOA concluded that it cannot serve as a stand-alone marker (Toulis et al. 2010).

The detection of seminal haploid cells by flow cytometry (FCM) has been evaluated for the prognosis of TESE results (Koscinski et al. 2005). FCM was found to be more sensitive (100 versus 59 %) but less specific (67 versus 83.5 %) than cytology. FCM will provide another noninvasive technique to predict TESE results and improve the management of NOA patients.

Conclusions

An accurate diagnosis of the etiology of azoospermia is important prior to the initiation of the appropriate treatment. Nonobstructive azoospermia remains the most challenging diagnosis for andrologists. Thousands of single or multiple genes are involved in establishing the male fertility potential, and many others are yet to be revealed. In the current era of ART, however, genetic testing has emerged as tools of paramount importance in helping clinicians not only to explore the specific genetic background of a disease but also to take the necessary precautions to prevent the transmission of the disease to the offspring via ART.

Incorporating novel techniques, such as genomics, proteomics, and metabolomics, into infertility research may assist in the creation of a complete portrait of

the genes that are involved in infertility and would allow for improvements in ART and the development of more targeted solutions (Hamada et al. 2012). Microarrays are emerging as valuable tools for the determination of the gene expression profiles of infertile phenotypes and the examination of spermatogenesis (Lin et al. 2006). Gene expression microarray studies could be used to characterize the gene expression signature for normal human spermatogenesis, which can be a valuable diagnostic marker. Proteins identified using 2D electrophoresis and mass spectrometry techniques could be used to create proteome maps in relation to sperm and seminal plasma (Johnston et al. 2005; Pilch and Mann 2006). The identification of protein biomarkers for male factor infertility will allow for unbiased comparisons of fertile and infertile males and will clarify the pathophysiology of the disease. An advantageous characteristic of genomic and proteomic technology is that the results provide a definitive characterization of infertile phenotypes.

Metabolites are small biomarkers that indicate the functionality of a cell and characterize certain diseases or physiological states. Determination of metabolite profiles for normal and infertile phenotypes may be useful in diagnosis and treatment of male factor infertility.

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