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Azoospermia: Etiology, Diagnosis and Management

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Abstract

Male infertility has been on the rise over the past several years, and azoospermia is one of the most common causes. It has two primary subtypes: Non-Obstructive Azoospermia (NOA), where the spermatogenesis is suppressed, and Obstructive Azoospermia (OA) when there appears to be a ductal obstruction while spermatogenesis is normal. Azoospermia is characterized by the absence of sperm in two or more ejaculates. Making the azoospermic man have his biological child is now becoming a reality with the advent of TESE and ICSI procedures, followed by the latest advancements like the combination of imaging, Full-Field Optical Coherence Tomography (FFOCT), stem cell therapy, platelet-rich plasma therapy, and gene therapy. The key aim of this article is to highlight the concept of azoospermia and focus on its evaluation and management through present-day developments in andrology and Medically Assisted Reproduction (MAR). A detailed literature review is performed through PubMed, Science Direct, the Online Library, and Scopus.

Keywords: Azoospermia, Male Infertility, Non-obstructive Azoospermia (NOA), Obstructive Azoospermia (OA), Sperm, Sperm Retrieval (SR) Techniques.

1. Introduction

The International Glossary of Infertility 2017 defines infertility as "a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner"[1,2]. It is a "disease of the reproductive system" and results in disability. It has become a widespread and serious health issue with repercussions on one's ability to have children as well as on their emotional, financial, psychological, family, and societal well-being. About one in six couples have experienced infertility in their lifetime globally according to the International Health Organization (WHO)[3,4].

Male infertility may be due to low production of spermatozoa, blockages that prohibit the delivery of sperm, or abnormal sperm function. Diseases, injuries, chronic health conditions, medications, radiation, genetic and endocrine components, and lifestyle can play a role in the cause of male subfertility. The most intricate problem to solve when treating male infertility is azoospermia. Reduced sperm production, decreased sperm quality, and obstruction of the reproductive canal can all affect spermatogenesis and cause azoospermia.

2. Etiology of Azoospermia

From an etiological perspective, azoospermia can be assigned to three main categories (Figure 1). While testicular causes of azoospermia are challenging to treat, the pretesticular and post-testicular causes are typically treatable. A clinical distinction between Obstructive Azoospermia (OA) and Non-Obstructive Azoospermia (NOA) can be made for azoospermia[5].

2.1 Obstructive Azoospermia (OA)

In OA, sperm is normally produced in the testicle, but due to obstruction or blockage in the reproductive duct, it is prevented from being ejaculated. It comprises 40% of

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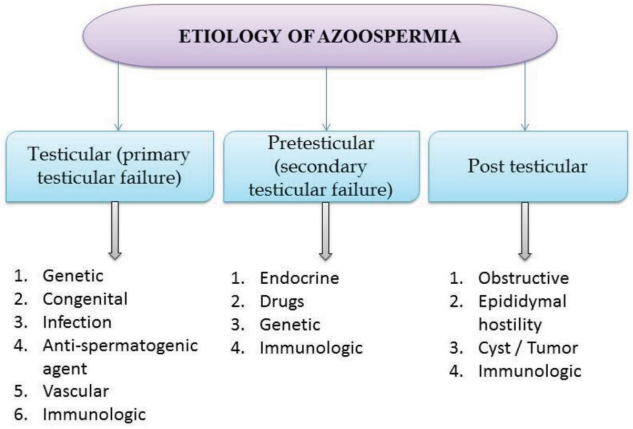

Figure 1. Etiological classification of Azoospermia

azoospermia cases[2]. It is further classified into epididymal (post-infection), vasal (vasectomy, congenital bilateral absence of vas deferens (CBAVD), or ductal (Mullerian cysts)), depending upon the level of obstruction[5].

2.2 Non-Obstructive Azoospermia (NOA)

NOA is a spermatogenesis failure brought on by either insufficient gonadotropin production or intrinsic testicular dysfunction. This will lead to very low sperm production or no sperm at all. It is the cause in about 60% of azoospermic men[5].

2.3 Pretesticular Causes

It comprises a very small proportion of azoospermia cases and includes hypogonadotropic hypogonadism. It can be due to congenital or acquired disorders (endocrinopathies, drug-related, immunological, functional, etc.) of the hypothalamo-pituitary axis.

2.3.1 Hypogonadotropic Hypogonadism (HH)

The hypothalamic-pituitary-gonadal axis (HPGA) is widely recognized to be essential to human spermatogenesis. In summary, with the commencement of puberty, the hypothalamus starts secreting strong pulses of Gonadotropin-Releasing Hormone (GnRH) in neurons that express GnRH. The anterior part of the pituitary gland responds by secreting Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). FSH reaches the testes and triggers the start of spermatogenesis in the Sertoli cells, which support and nourish the sperm cells produced by the testes. Additionally, Leydig cells in the testes are stimulated by LH to produce and release testosterone into the testes and bloodstream. Spermatogenesis is stimulated by testosterone. Anomalies related to HPGA have the potential to cause irregular spermatogenesis or possibly azoospermia. Disturbances in GnRH secretion can lead to non-obstructive azoospermia. About 1%–2% of cases of male factor infertility and 45% of NOA patients are related to HH[6]. It can be congenital or due to acquired disorders of the hypothalamus and pituitary gland. Acquired causes of hypogonadotropic hypogonadism include panhypopituitarism, head trauma, pituitary haemorrhage, pituitary tumours, craniopharyngiomas, trauma, chronic opioid use, infiltrative disorders like hemochromatosis, sarcoidosis, histiocytosis which by their mass effect or local destruction of the gland suppress the levels of gonadotropins released from anterior pituitary.

2.3.1.1 Congenital Causes

Kallman syndrome is a congenital disorder wherein the GnRH-releasing neurons fail to migrate to the olfactory lobe. Several genetic defects have been implicated, amongst which defect in the KAL gene in the Xp22.3 region leads to X linked pattern of inheritance. It has an incidence of between 0.1% and 0.2%, making it the most frequent cause of congenital hypogonadism[7]. Other genes implicated are FGF8, FGFR1, and NELF with autosomal dominant inheritance and PROK2, and PROKR2 with autosomal dominant and autosomal recessive inheritance[8–10]. It is clinically characterized by cryptorchidism, micropenis, issues in secondary sexual characters, decreased libido, anosmia, erectile dysfunction, small testes, cleft palate, congenital deafness, cerebellar dysfunction and renal abnormalities. The presentation is diverse depending on the severity of the defect. With an estimated occurrence rate of 1 in 30,000 males and 1 in 125,000 females, Kallmann syndrome affects men more frequently than women[11]. Mutations in GNRH1, GHNRH-R, KISS1, KISS 1R, LEP, and TAC3 genes can lead to inherited normosmic isolated HH [9,12].

Prader-Willi syndrome is another congenital cause of HH, which involves deletion or uniparental disomy on chromosome 15. It is associated with hypotonia, short stature, hyperphagia, mental retardation and hypogonadism[13]. Similarly, Laurence-Moon syndrome is another rare autosomal-recessive disorder associated with retinitis pigmentosa, spastic paraplegia, mental retardation, and hypogonadism[14].

2.3.1.2 Structural Causes

Intracranial injury can occur as a result of pituitary tumours, and infiltrative disorders like hemochromatosis, sarcoidosis, and Langerhans cell histiocytosis which can lead to panhypopituitarism including hypogonadism. A pre-treatment aberration in gonadotropin secretion was present in 13% of the 75 children in a prospective study with different CNS malignancies[15]. The treatment-related decline in gonadotropic hormones can occur in children with CNS tumours with long-lasting effects on fertility. In research that looked back at craniopharyngiomas, just one patient out of 64 exhibited hypogonadism symptoms before receiving therapy[16]. Nonetheless, following adjuvant radiation and surgical resection, 80% of patients assessed at the pubertal age exhibited hypogonadism.

2.3.1.3 Treatment or Drug Related

Chemotherapy and radiotherapy for the treatment of malignancies can lead to variable declines in male fertility. As low as 0.15 Gy of testicular radiation can cause decreased spermatogenesis in males, and as much as 0.3 Gy can cause transient azoospermia[17]. Radiation has an age-dependent influence on testicular function; prepubertal radiation exposure damages Leydig cells substantially more than post-pubertal radiation[18]. Changes in function are also associated with cumulative dosages of alkylating chemicals[17]. Young adult survivors of childhood cancer who took part in a trial comparing three treatment arms for Acute Lymphoblastic Leukemia (ALL) and Non-Hodgkin Lymphoma (NHL) showed a high prevalence of hypogonadism[19]. The preventive cranial radiation treatment with vincristine, prednisolone, l-asparaginase, methotrexate, and 6-mercaptopurine was compared to chemotherapy plus total body radiation and bone marrow transplantation. In the third group of men, 83% exhibited low serum testosterone and high levels of FSH and LH, indicative of primary hypogonadism. In all three treatment groups, 40% of men exhibited abnormalities in spermatogenesis; the most severe malfunction was observed in those who had undergone complete body radiation.

Another cause of HH is exogenous testosterone/anabolic steroid use which suppresses FSH due to a negative feedback loop on the hypothalamic-pituitary axis and consequently intratesticular testosterone levels[20]. Although anabolic drugs can reverse the inhibition of the hypothalamic-pituitary axis, recovery from steroid withdrawal varies and can take four to twelve months[21]. Long-term glucocorticoid therapy can lead to hypogonadism. In one trial, the mean blood testosterone of 16 men with chronic pulmonary illness who received high-dose glucocorticoids for at least one month was 6.9 nmol/L, while the mean serum testosterone of 11 men matched for age and condition was 15.6 nmol/L[21]. The results of this investigation indicate a primarily central mechanism for glucocorticoid-induced hypogonadism, as serum LH levels did not rise. Reversible HH can also be brought on by long-term use of oral and intrathecal opioid analgesics, which inhibit LH secretion[22]. Hyperprolactinemia is a typical side-effect of psychiatric drugs such as risperidone and phenothiazines, which suppress endogenous GnRH release and cause HH[23]. Opioid drug abuse, heroin, naltrexone, and codeine and codeine-like derivatives can cause suppression of the hypothalamus-pituitary axis.

2.3.1.4 Endocrine Abnormalities

Prolactin is produced in the anterior pituitary. An elevated level of serum prolactin inhibits the pulsatile release of GnRH from the anterior pituitary and suppresses both FSH and LH. Overproduction of prolactin secreted in hyperprolactinemia, a kind of HH, affects spermatogenesis and steroidogenesis through prolactin receptors found in Leydig cells and Sertoli cells in the testes. While prolactinoma is not diagnosed clinically until later in males than it is in women, hyperprolactinemia is a prevalent cause of infertility in men that is sometimes overlooked. Endocrinopathies like hyperprolactinemia can be caused by drugs such as tricyclic antidepressants, phenothiazines, imipramine, some antihypertensives like methyldopa and reserpine. It can also be due to concurrent medical illness, pituitary micro- and macro-adenomas[24]. Macroadenomas cause local destruction and compression of gonadotrophs. Macroadenoma will have prolactin levels greater than 250 ng/mL, while in microadenomas, it usually ranges between 100 and 250 ng/mL[25]. Symptoms of prolactinomas include infertility, depressed libido, galactorrhea, headache, fatigue, and erectile dysfunction.

Endogenous excess androgen, as observed in congenital adrenal hyperplasia, may also result in the suppression of gonadotropin stimulation and consequently HH. Glucocorticoids can help treat this illness; however, testicular function may be negatively impacted by the development of pseudotumors in the testes[26].

Patients who are azoospermic or subfertile have been documented to have aberrant estrogen profiles. Shiraishi and colleagues discovered that aromatase was primarily expressed in Leydig cells and that men with NOA (n = 76) had higher levels of both its transcript and protein expression; they also discovered that sperm and aromatase expression were significantly correlated with decreased intratesticular testosterone/increased intratesticular E2 [27].

2.3.1.5 Chronic Illness-Related and Functional HH

It is usually reversible and is due to stressors like excessive physical activity, nutritional deficiencies, obesity, psychological stress, and chronic disorders like diabetes, and Cushing's syndrome[28]. Low testosterone levels can occur during any period of severe chronic sickness or acute illness, including heart attacks, burn injuries, kidney failure, and surgery. Leydig cell function is immediately and directly suppressed in response to acute damage. The reduction of pulsatile LH release is a major cause of hypogonadism that results from prolonged acute stress. Opioids or endogenous dopamine may have a role in the pathophysiology of HH brought on by a serious illness[29,30].

2.3.1.6 Immunological/Autoimmune

Autoimmune pituitary disease leading to HH is rare in men[31]. Men with autoimmune polyendocrine syndrome cluster can lead to pretesticular or testicular deficiency of gonadotropins due to immune dysregulation. The root cause is a homozygous inactivating mutation in the autoimmune regulator gene AIRE, which allows ectopic antigens that are normally expressed only in particular peripheral tissues (like insulin) to be expressed intrathymically. This eliminates T-cells as they mature within the thymus and develop a receptor for the self-antigen (negative selection), preventing autoimmunity[32].

2.3.1.7 Idiopathic HH

Adult-onset idiopathic HH is an entity which shows a significant response to pulsatile GnRH therapy[33]. It has no recognizable CNS abnormalities and men have normal pubertal development. The defect is localized to the parvocellular GnRH neuronal system within the hypothalamus. The cause may be defective biosynthesis or secretion of GnRH. Acquired abnormalities of an autoimmune or toxicologic origin that led to the selective elimination of GnRH secretion-regulating neuromodulators in the hypothalamic arcuate nucleus region remain a possibility. Some patients with idiopathic hypogonadism may have a functional gonadotropin deficiency due to insufficient or inefficient gonadotropin actions. The defect can lie at the level of genetic expression, glycosylation, or function of FSHR. Remarkably, a Single-Nucleotide Variation (SNV) with a G to T nucleotide mutation that is located inside the promoter of the FSHB gene largely regulates the transcriptional activity of the FSH gene[34]. Patients with the T allele may not be able to sufficiently up-regulate circulating quantities of FSH to accomplish full spermatogenesis and consequently deranged semen analysis[35]. Furthermore, a common FSHR SNV that results in an asparagine to serine mutation at position 680 of the protein chain at position 2039 of the transcription start site also affects the amplitude of the Sertoli cell response to FSH[36]. Men with homozygous serine receptor phenotypes may be less responsive to treatment.

2.3.2 Androgen Resistance Syndrome

Androgen resistance affects around 1 in 60,000 births. The androgen receptor gene, which is situated on the X chromosome, has been reported to have more than 300 mutations (Xq11-q12)[37]. It can be complete, partial or minimal and can lead to diverse phenotypical abnormalities. The minimal form of the disease leading to normal virilization but absent or low sperm count is due to mutations in the exons of the androgen receptor gene located on the X chromosome (Xq11-q12) as well as mutations in the promoter region of the gene. Subtle abnormalities of the CAG repeat sequence in exon 1 of the gene have been found in men with idiopathic azoospermia[38]. Serum testosterone values can range from low, to normal or high depending on the severity.

2.4 Testicular Causes

Azoospermia due to the intrinsic disorder of spermatogenesis can be caused by various insults.

2.4.1 Varicocele

It is an abnormal dilatation of the pampiniform plexus of males and is found in about 5% of cases of azoospermia, however, its absolute role in the causation of azoospermia remains to be elucidated[39]. It can cause increased testicular temperature due to turbulent blood flow in scrotal veins, hypoxia, oxidative stress and hormonal changes[40]. A scrotal temperature increase of more than 2°C is linked

to a reduction in the quantity and quality of sperm, which is thought to be the result of heat-induced Leydig and/or DNA and protein damage within the nucleus of spermatic tubule cells[41]. The most common technique for treating varicocele is varicocelectomy, which entails ligating or removing the afflicted veins. In about 5-10% of men, azoospermia is found to be associated with varicocele[42]. In men with oligozoospermia, varicocelectomy may improve semen parameters for natural conception, but its role before assisted reproductive techniques is uncertain. Definitive evidence in favour of surgical varicocelectomy before ART in azoospermia patients is lacking. In a recent meta-analysis by Ramon *et al.*, varicocele repair improved semen parameters like sperm concentration and morphology in patients with azoospermia, however, there was no effect on the FSH and LH levels[43]. In contrast, another meta-analysis evaluating sperm retrieval demonstrated a 20.8% recurrence of azoospermia[44]. According to the study's histopathological results, men with late maturation arrest or hypospermatogenesis have a higher chance of successfully inducing spermatogenesis than men with a Sertoli cell-only pattern (42.1% or 54.5% compared to 11.3% of pregnancy rate, respectively).

2.4.2 Drugs

Certain drugs like chemotherapy agents (cyclophosphamide, sirolimus), colchicine, CCBs, nitrofurantoin, corticosteroids, opioids, radioiodine, and androgens inhibit spermatogenesis; drugs like spironolactone, flutamide, cimetidine, etc. are antiandrogenic, and drugs like SSRIs can cause anejaculation or impaired semen quality. Alpha-adrenergic blockers can cause retrograde ejaculation[45].

2.4.3 Undescended Testes

The most prevalent genital deformity in boys is an undescended testis, which affects 2.7% of infants and up to 0.8% of 1-year-olds[46]. It's critical to distinguish between retractile testes, which are caused by hyperactive cremasteric muscles and occasionally reside in the upper scrotum or intestinal canal, and cryptorchid testes. There are several hypothesized reasons for the subfertility caused by cryptorchidism, including immunologic damage, blockage, endocrine system dysfunction, and testicular dysgenesis[47]. The outcome of treatment depends on the testicle's initial position and may reduce the likelihood of infertility[48]. Before the child turns one, this issue should be addressed surgically, hormonally, or both. Age at orchiopexy and testicular volume are independent factors that predict fertility. After therapy for undescended testes, the incidence of azoospermia is roughly 13% in unilateral cryptorchidism and 34% in bilateral cryptorchidism[47]. However, untreated unilateral and bilateral undescended testes occur in 30 and 80 per cent of cases of azoospermia, respectively[47].

2.4.4 Infections

Infections like pubertal mumps orchitis, and testicular torsion are some of the other testicular causes of azoospermia. Testicular torsion, if corrected within six hours with surgery, will usually salvage the testes. Unilateral torsion can lead to azoospermia, usually when the contralateral testis is previously compromised due to orchiopexy[49].

2.4.5 Oncological Factors

Some of the cancers associated with azoospermia include leukaemia, Hodgkin's lymphoma and testicular germ cell tumours. Patients with testicular germ cell tumours can have azoospermia even before chemotherapy due to apoptosis, reactive oxygen species and sperm DNA damage[50,51].

2.4.6 Genetic Factors

2.4.6.1 Klinefelter Syndrome (KS) (47, XXY)

KS is the most common cause of NOA among all the genetic causes. It is 45 times more frequent among men looking for treatment for infertility[52]. Despite a wide spectrum of phenotypic appearances based on the level of testosterone hormone, all have a few common features like high LH and FSH, reduced testosterone to a varied degree, atrophic testes, and on semen analysis, azoospermia. Clinical presentation of KS depends on age upon diagnosis and severity of the mosaicism[5]. Interestingly, in 50-60% of 47, XXY men rare foci of spermatogenesis may be found, hence micro-TESE is advisable in such cases[53]. These foci appear to arise from 46, XY stem cells, contrary to the belief of complete loss of the spermatogonial stem cells in Y chromosome microdeletion[54].

2.4.6.2 Y Chromosome Microdeletions

AZF microdeletions are regarded as the second most prevalent genetic cause of male infertility, after karyotype

abnormalities[55]. Y chromosome contains several repetitive sequences which makes it prone to changes in the number of gene copies in these sequences[56]. During meiosis, AZF microdeletion often happens de novo via nonallelic homologous recombination between sister chromatids in the father's testis.

The long arm of the human Y chromosome (Yq11) contains the AZF region (called azoospermia factor) which further comprises three genetic domains, proximal AZFa, intermediate AZFb, and distal AZFc. These domains are responsible for proper spermatogenesis. Some authors also consider the fourth domain as AZFd which includes overlapping areas between AZFb and AZFc[57].

Two single-copy genes, USP9Y and DDX3Y are present in the 1100kb long AZFa region, both of them are lost in the microdeletion[58]. AZFb region is relatively complex consisting of 14 amplicons which are further divided into palindromes, symmetrical arrays of contiguous repeats. Homologous recombination between palindromes P5/proximal P1 can lead to loss of 6.2 Mb region with 32 gene copies. This causes a complete deletion of AZFb[59]. AZFc deletion originates from homologous recombination in P3 and P1 and deletes 21 gene copies in 3.5 Mb[60]. Semen abnormalities can range from mild oligozoospermia to severe oligozoospermia to azoospermia depending on the location of microdeletion. Microdeletions influence the progress of spermatogenesis as well as the development of the testis, which leads to azoospermic and oligozoospermic manifestations in patients. The prevalence of Y chromosome microdeletion is around 8.3% and 5.5% in NOA and severe OA cases, respectively.[61]. Microdeletions, such as AZFc and AZFb partial deletions, have the possibility of sperm retrieval in 50% of cases, whereas there is no possibility of identifying a mature sperm in cases with AZFa and AZFb complete deletions[62]. The most common form of the long arm of Y microdeletions is AZFc deletion (65-70%), followed by deletions of AZFb and AZF b and c and AZF a,b,c regions[63]. AZFa deletions are extremely rare (5%)[63]. AZFa and AZFb deletions lead to Sertoli cell-only syndrome and sperm maturation arrest, respectively, and are a contraindication to surgical sperm retrieval[64]. Microdeletion in AZFd leads to mild oligozoospermia and sperm morphological abnormalities[65]. The most frequently deleted AZF gene includes the DAZ gene family, which is present in the AZFc domain[66]. These patients should be informed of the potential risks associated with assisted reproduction since AZF microdeletions are transmissible to male progeny[65,66]. Since karyotype may not detect subtle Y chromosomal abnormalities, AZF analysis testing can be done in patients with idiopathic azoospermia and a normal karyotype. In the assay, DNA is extracted (often from peripheral blood) and subjected to a polymerase chain reaction (PCR-multiplex) technique using specific markers for AZF microdeletions, or sequence-tagged sites (STS)[57]. Two STS loci should be amplified in each AZF region, to promote sensitivity and specificity of the analysis; for AZFa: sY84, sY86; for AZFb: sY127, sY134; for AZFc: sY254, sY255 (both in the DAZ gene)[59,67].

2.4.6.3 46 XX Male Syndrome

In this disorder, a part of the Y chromosome containing the SRY gene is translocated to the X chromosome during meiotic division which leads to loss of all the AZF genes but normal external and internal genitalia. In 10% of cases, the translocation occurs to an autosomal chromosome or the patient is SRY negative. The incidence of this condition is 1 in 20,000[68,69].

Mixed gonadal dysgenesis patients have 45, X0/46, XY mosaic genotype with cryptorchid testes and contralateral streak gonad which should be surgically removed to avoid the risk of seminoma or gonadoblastoma[70].

2.4.6.4 47, XYY Syndrome

It occurs due to paternal nondisjunction of the Y chromosome with an incidence of about 1 in 1000. These patients are infertile due to Sertoli cell only and maturation arrest morphology on histopathology[71].

2.4.6.5 Other Genes Identified in Sperm Maturation Arrest

TEX 11 (an X-linked coding gene), SYCE1, MEI1, STAG3, TEX 14 and TEX 15 have been identified in men with sperm maturation arrest and consanguineous families (except TEX 11)[72-74]. Stephanie Cheung et al identified certain germline mutations in patients with azoospermia and also correlated their genomic profile with their reproductive potential[75]. NOA men carried mutations in the AP1S2, AP1G2, and APOE genes, which are essential for spermatogenic processes. While NOA-infertile people (n = 3) carried mutations in genes related to early embryonic development (MBD5, CCAR1, PMEPA1, POLK, REC8, REPIN1, MAPRE3, ARL4C) and spermato/spermiogenesis (ADAM29, SPATA31E1, MAK, POLG, IFT43, ATG9B), NOA-fertile men (n = 8) carried

mutations in MPIG6B (stem cell lineage differentiation). Interestingly, they also found that residual spermatogenic foci could be predicted by transcriptome analysis of cell-free RNAs in the seminal fluid of NOA males.

2.5 Post-Testicular Causes

Azoospermia can be due to obstruction of sperm transport or ejaculatory dysfunction.

2.5.1 Congenital Bilateral Absence of Vas Deferens (CBAVD)

CBAVD is detected in approximately 1% of infertile male patients, which includes 2-6% of OA men[76]. Spermatogenesis is normal in these cases, but careful evaluation of concomitant problems of spermatogenesis due to other comorbidities should be excluded. CBAVD will be present in around 95% of men with cystic fibrosis (CF). The most prevalent autosomal recessive illness affecting Caucasians is cystic fibrosis (CF), which affects 1:2500 births and has a carrier frequency of 1:20[77]. The CFTR gene (7q31.2) is 250 base pairs long and has 27 exons. Although more than 800 distinct variants have been identified, the most prevalent mutation in the Caucasian population is a three-base-pair deletion in exon 10 (delta F508)[78,79]. An additional prevalent mutation is the 5T allele in intron 8 abnormality[80]. This area normally contains seven to nine thymidines; a reduction to the five-thymidine variation reduces the efficacy of exon 9 splicing and ultimately results in a 10–50% drop in CFTR mRNA[81]. Ipsilateral renal agenesis should be ruled out in such patients as it is present in about 11% of the patients. Testes are of normal size and caput epididymides are always present, although corpus and cauda are found occasionally.

2.5.2 Vasal Obstruction

Vasal obstruction can happen inadvertently during hernia repair and occurs most frequently in infants. Post-operative inflammation due to mesh entrapment can also obstruct the vas. Obstruction after vasectomy is the most common cause of vas obstruction[82].

2.5.3 Epididymal Obstruction

It can occur due to inspissated secretions in Young's syndrome which is defined as the triad of chronic sinusitis, bronchiectasis and obstructive azoospermia[83].

Ejaculatory duct obstruction can be congenital or acquired. The congenital form is due to utricular and Wolffian cysts. Acquired causes can be due to surgery, seminal vesicle calculi, infections, and prostatic calcifications.

3. Diagnosis of Azoospermia

Around 30% of infertile men have idiopathic sperm abnormalities[84]. Appropriate diagnosis of the male factor is crucial in the identification and management of azoospermia. The initial examination should focus on determining the different parameters, which include verifying azoospermia, distinguishing the causes of OA and NOA, assessing whether any reversible variables are present, checking for genetic anomalies, and evaluating the success rate of sperm retrieval[53]. Evaluation of azoospermic men is a systematic process that includes history-taking, physical examination, semen analysis, biochemical investigation of reproductive hormones, imaging techniques, genetic screening, and surgical procedures. In most cases, based on a thorough analysis of diagnostic parameters, azoospermic men can be assigned to either OA or NOA.

3.1 History

The comprehensive medical background of both male and female partners is mandatory. An initial history of infertility should comprise the duration of infertility, type of infertility, sexual history, sexual dysfunction, personal history and lifestyle, and history of chronic disorders. Further, it includes conditions like trauma, torsion of the testis, a history of childhood diseases (like mumps or cryptorchidism), any disorders that might impair testicular vascularization, vasal patency, or ejaculatory function, and a history of inguinal, pelvic, or scrotal surgery. Additionally, HH might be indicated by the late beginning of puberty. Prior vasectomy and genitourinary conditions such as urethritis and epididymitis can all result in OA, while NOA can be brought on by late pubertal mumps orchitis[5]. The history of genetic disorders like cystic fibrosis and Klinefelter's syndrome also has to be recorded. All these parameters will be useful in tailoring a treatment plan.

3.2 Semen Analysis

The semen sample should be analysed thoroughly. First, a wet preparation is made and evaluated; if azoospermia is

indicated, it is further confirmed by centrifugation of the sample at least 3000 x g for 15 minutes and testing again. The total absence of spermatozoa in two centrifuged samples done at adequate intervals as per WHO guidelines is described as azoospermia[85]. Examination of the ejaculated volume and pH is important in the diagnosis of azoospermic men. Men with normal-sized testes with low semen volume may have Ejaculatory Duct Obstruction (EDO) or dysfunction, or CBVAD. Pre-ejaculation details should be thoroughly evaluated, such as abstinence period, alcohol intake, febrile illness, history of recent pelvic surgeries, retrograde ejaculation (assessed by analysing a centrifuged urine sample), etc.

3.3 Physical Examination

To determine potentially treatable reasons for male factor infertility, a physical examination is required. In a warm room, the patient should be evaluated in both a supine and erect position. Evaluation should focus on general examination, BMI (body mass index), secondary sexual features like body physique, the pattern of hair growth and distribution, and gynecomastia (androgen deficiency, Klinefelter's or Kallmann's syndrome), evidence of any scrotal surgeries like scars on the inguinal or scrotal area, penis (phimosis, epispadias, or hypospadias), size of the testis (length a minimum 4.0 cm and width at least 2.5 cm, with the volume approximately 20 mL) and consistency (impaired spermatogenesis), presence and consistency of the vasa deferentia and epididymis (bilateral congenital absence of the vas deferens), and presence of varicocele. Azoospermia is associated with varicocele; thus, varicocele examination is important, and its grade significantly affects the prognosis. A digital rectal investigation is required to evaluate the prostate's size, consistency, and genitourinary masses [5,86].

3.4 Biochemical Evaluation

It is done to rule out any underlying systemic disorders. Studies recommend examination of gonadotropin hormones to diagnose hypothalamic and pituitary disorders with a sperm count <10 million/mL. For the diagnosis of endocrine disorders, serum Follicular Stimulating Hormone and testosterone levels should be checked[87]. Normal luteinizing Hormone (LH), testosterone, and FSH levels are likely in OA; yet, in NOA, LH, testosterone, and FSH levels can be increased or decreased. A high concentration of blood prolactin interferes negatively with spermatogenesis by preventing the release of pulsatile GnRH and, as a consequence, the pulsatile release of FSH, LH, and testosterone. Testosterone levels will indicate testicular failure or HH, which can be congenital or acquired. The most common cause of high prolactin is hypothyroidism, so it is mandatory to examine free T4 and TSH when prolactin is high.

3.5 Surgical Retrieval in Azoospermia

Surgical retrieval of sperm is pivotal in the diagnostic process for azoospermic men. Sperm harvesting techniques comprise Percutaneous Epididymis Sperm Aspiration (PESA), Microsurgical Epididymal Sperm Aspiration (MESA), percutaneous Testicular Sperm Aspiration (TESA), Testicular Sperm Extraction (TESE), and microdissection testicular sperm extraction (MicroTESE). A testis biopsy may be necessary to distinguish OA from NOA and other spermatogenesis disorders.

3.6 Imaging Technique

The first-line imaging modality is ultrasonography of the scrotum to evaluate testicular function[88]. Other tests include transrectal ultrasound (TRUS), abdominal ultrasound, cranial imaging, seminal vesiculography with TRUS guidance, seminal tract washout, vasography, MRI, etc. Doppler US is emerging as a promising method nowadays for assessing azoospermic patients[5]. This technique helps in differentiating OA from NOA, as the latter will show the absence or reduction of testicular vasculature. Newly created 3D-guided transrectal imaging equipment makes vesiculography procedures' visualization and needle guidance easier[5].

3.7 Genetic Evaluation

All azoospermic patients with suspected congenital obstruction (normal testis volume and FSH), primary testicular failure (reduced volume of testis and increased FSH), "incomplete" testicular failure (reduced testis volume and either decreased or high FSH), or HH (low volume of testes, reduced or low-normal LH, and reduced testosterone level) are advised to undergo genetic testing[89]. Genetic defects commonly seen are Klinefelter syndrome, Y chromosome microdeletion, Kallmann syndrome, congenital bilateral absence of vas deference (CBAVD), de la Chapelle syndrome with a 45, XX karyotype, etc. The chromosome alterations are

diagnosed in 15% of azoospermic men and constitute one of the most heterogeneous groups[90]. Obtaining reliable genetic information before ART can prevent unnecessary treatment and the vertical transmission of genetic disorders. Assessment of sperm aneuploidy and Sperm DNA Fragmentation (SDF) rates has been added as extended examination tests in the WHO Laboratory Manual of Semen Analysis. Some of the tests to detect SDF include the comet assay, terminal deoxynucleotidyl transferase dUTP nick end labelling assay, sperm chromatin structure assay, and sperm chromatin dispersion test. With the advent of Next-Generation Sequencing (NGS) and microarrays, the future of genetic testing seems bright. This makes it possible to check for a variety of genetic problems, such as chromosome rearrangements, copy number variations, and single nucleotide abnormalities [91].

4. Management of Azoospermia

Men with OA can be treated through either direct sperm retrieval method from the testis or epididymis, followed by *In Vitro* Fertilization (IVF) or Intracytoplasmic Sperm Injection (ICSI), or surgical repair of the obstruction, which may enable the couple to conceive spontaneously. Except for those with HH and secondary testicular failure, there is currently no therapy that can restore spermatogenesis in the majority of cases of NOA. Therefore, the direct harvesting of sperm from the testicles or epididymis for ICSI is the only option[92]. In a study done by Karavani *et al*., it was found that over time, there is a significant risk of becoming azoospermic in men with testosterone levels ≤ 1 M/mL, particularly in those with low testosterone levels[93]. Sperm banking is therefore advised in these circumstances. Even after three or more years, men with sperm concentrations of more than 1 M/mL have a minimal probability of becoming azoospermic.

4.1 Medical Therapy

Medical management for azoospermic men is an area in need of great exploration. Hypogonadotropic Hypogonadism (HH) has a comparatively good prognosis following hormonal therapy. Human Chorionic Gonadotropin (hCG) is administered as a first line of treatment at 2,000 IU subcutaneously three times per week or 2,500 IU twice weekly with FSH supplementation (menopausal, purified, or recombinant) at 37.5–150 IU three times per week. Three to six months of treatment is beneficial to stimulate sperm production. This period is generally adequate for inducing spermatogenesis. The gonadotropin use in NOA due to HH followed by TESE has yielded success[5,94]. Another study reported an adequate response in HH to initial hCG therapy, with aspermic/azoospermic patients having spermatozoa in their ejaculate[95].

Suppression of spermatogenesis may also result from excess androgens by exogenous administration or tumours of the testicular, pituitary, or adrenal glands. This group of acquired or iatrogenic NOA is also considered amenable to medical management using clomiphene and human chorionic gonadotropins[96]. The retrospective study by Ledesma et al. reported improved semen analysis and natural pregnancy[96]. Treatment with exogenous dexamethasone in many studies has been suggested for overturning the condition of azoospermic reversal in congenital adrenal hyperplasia cases[97,98]. The type and dose of steroid administered may influence azoospermia reversal.

In idiopathic azoospermia, men treated with Human Follicle-Stimulating Hormone (hFSH) for 3 months and subjected to Assisted Reproduction Techniques (ARTs) with TESE had a considerable sperm retrieval rate[99,100]. FSH increases spermatogenesis in humans both independently of testosterone and through a process that shares some characteristics with testosterone[101]. The following are the main functions of FSH in spermatogenesis: i. Sertoli cell number determination; ii. Spermatogonial proliferation as well as metabolic and structural support; iii. Stimulation of meiotic progression until the spermatid stage; and iv. Metabolism and transport of nutrient materials to germ cells. This summarizes that FSH treatment has greater efficacy in azoospermic patients. A Cochrane meta-analysis revealed an increase in the overall pregnancy rates in couples in which men are treated with FSH, however, due to the small number of trials further research is needed to reach definite conclusions[102]. Esteves et al have proposed a novel APHRODITE criteria to classify male infertility in patients with testicular dysfunction[103]. Based on evidence from limited studies, five subgroups were delineated as "(1) Hypogonadotropic hypogonadism (acquired and congenital); (2) Idiopathic male infertility with lowered semen analysis parameters, normal serum FSH and normal serum total testosterone concentrations; (3) A hypogonadal state with lowered semen analysis parameters, normal FSH and reduced total testosterone

concentrations; (4) Lowered semen analysis parameters, elevated FSH concentrations and reduced or normal total testosterone concentrations; and (5) Unexplained male infertility in the context of unexplained couple infertility." A Gonadotropin regimen was suggested for all five subgroups; HCG (± FSH) for group 1; FSH alone for group 2; FSH (± HCG) for group 3; HCG (± FSH) for group 4; and FSH alone for group 5. Response to treatment can be variable based on genetic polymorphisms. In one study, Simoni and colleagues discovered that carriers of the homozygous N polymorphism of the FSHR (p.N680S) showed a greater improvement in semen quality as measured by sperm chromatin damage than did those with the S allele (p.N680S)[36]. Patients with the FSHB −211G>T genotype were the most sensitive to medication, suggesting that the single-nucleotide polymorphisms affected the observed effect. In a different study, Selice and associates noted that only men with common allelic variants in the FSHR gene, specifically Ala307-Ser680/Ala307-Ser680 homozygosis or Thr307-Asn680/Ala307-Ser680 heterozygosis, showed a statistically significant improvement in the sperm parameters upon using FSH therapy[35].

4.2 Surgical Treatment

The surgical approach depends on the level of obstruction. The two obstructive conditions where surgery can be successfully performed are ejaculatory duct obstruction and vasectomized patients seeking recanalization of the vas. Microsurgical reconstruction, which is a vasectomy reversal with microsurgical reconstruction or vasoepididymostomy, can be done in patients with vas deferens or epididymis obstruction. A study observed the return of sperm in their ejaculation among 70% to 95% of patients after surgery; of these, 30% to 75% of couples could achieve natural pregnancy with vasovasostomy, while 70%–90% patency restoration and 50% fertility with vasoepididymostomy[104]. Similarly, Transurethral Resection of the Ejaculatory Ducts (TURED) resulted in 59% and 94% increases in semen quality and patency, respectively; natural pregnancy rates were reported to be 12–31% after performing TURED[105]. The above results show the promising success rate of such procedures.

4.2.1 Sperm Retrieval Techniques and IVF/ICSI

Sperm Retrieval techniques (SR) are beneficial for both OA and NOA. It can be applied for both diagnostic and therapeutic management and is performed under anaesthesia. Testicular Sperm Extraction (TESE), Percutaneous Testicular Sperm Aspiration (TESA), Microsurgical Testicular Sperm Extraction (Micro-TESE), and Microsurgical Epididymal Sperm Aspiration (MESA) are common techniques used in SR operations[106]. The success of surgical retrieval is concluded with the collection of several motile or immotile sperm that can help perform ICSI[107]. Vasal sperm aspiration and seminal vesicle sperm aspiration with transrectal ultrasound guidance are two less common procedures[108,109]. The choice of procedure is primarily influenced by the preferences and experiences of the embryologist and the surgeon[110]. Techniques of sperm retrieval with their indications, pros, and cons are presented in Table 1.

4.2.1.1 Microsurgical Epididymal Sperm Aspiration (MESA)

For men with OA, MESA is considered the gold standard and preferred procedure for sperm retrieval. MESA is essentially an open surgical sperm retrieval treatment that employs operative microscopes to locate specific epididymal tubules so that a significant amount of sperm can be retrieved. It involves incising the epididymal tunica and aspirating fluid from the tubule. This procedure can be repeated at multiple sites of the ipsilateral (from cauda to caput) or contralateral epididymis. If enough motile spermatozoa are not retrieved, alternate options such as TESA or TESE can be performed simultaneously[111-113]. In OA cases, MESA was found to be more significant than TESE, with a 39% vs. 24% Live Birth Rate (LBR)[114]. In another study, MESA reported a better clinical pregnancy rate (96%) and normal fertilization rate (74%) than the combined MESA/TESA group, which had 72% and 78% success rates, respectively[115]. Among both groups, a higher number of healthy deliveries was reported in the MESA group. MESA (single procedure) has provided a higher volume of sperm, which can be stored and used in the future as well.

4.2.1.2 Percutaneous Epididymal Sperm Aspiration (PESA)

PESA involves the removal of sperm cells from the epididymis through a 19G needle fitted to a syringe. A 26-G needle attached to a tuberculin syringe filled with 0.1 mL of sperm-wash medium is used to puncture the scrotal skin after palpating the head of the epididymis and

stabilizing it between the index finger and thumb. It can also be performed using a butterfly needle attached to a 20-mL syringe. The droplets of aspirated fluid can be easily visualized in the tubing. The presence of spermatozoa in the fluid has a definite diagnostic value. In patients with congenital absence of vas deferens, PESA has given a better outcome when fertilizing human oocytes *in vitro*[116]. Different studies on PESA, it has provided an average Sperm Retrieval Rate (SRR) of 83%[117]. Despite its success rate, complications like swelling, hematoma, pain, and infection were also observed in some cases.

4.2.1.3 Testicular Sperm Aspiration (TESA)

Since TESA is a minimally invasive operation, it has largely replaced other options as the treatment of choice since it carries a low risk of parenchymal and testicular tissue loss and injury. After the testis is pierced through the scrotal skin, the sample is aspirated using either a fine (#23) or large-bore (#18) needle. After that, testicular parenchyma samples are submerged in a medium containing human tubal fluid, and mechanical disruption is carried out[118]. In a study, TESA was performed in 208 azoospermia patients, including 82 men with OA and 126 men with NOA; the overall SRR observed in OA and NOA men on prognostic TESA was 100% and 30%, respectively[119]. According to Lewin *et al.*,[120] and Khadra *et al.*,[121], 85 men and 84 men with NOA, underwent SR via TESA and found an SRR of 58.8% and 53.6%, respectively. Interestingly, cryopreserved sperm obtained through TESA have resulted in good fertilization rates using ICSI[122].

4.2.1.4 Fine-Needle Aspiration (FNA)

Fine Needle Aspiration Cytology (FNAC) is now being recognized as a diagnostic and treatment modality for selected cases of male infertility. It aids in the visualization of all types of testicular cells without a biopsy. The Sperm Index, Sertoli Cell Index, and Sperm-Sertoli Cell Indexes are good indicators to examine the extent of spermatogenesis and to identify the causes of azoospermia[123]. It can provide detailed information on the exact sites of spermatogenesis and confirm the diagnosis of ductal obstruction. It also minimizes deleterious effects while performing biopsies and simplifies sperm retrieval options in NOA cases. The technique is often performed under local anaesthesia. Percutaneous punctures are performed at different sites into the testes using a fine (#23) 1-inch butterfly needle connected to a 20-mL syringe with an aspiration handle at 5 mm apart from each template to aspirate parenchyma tissues from the testis at different locations. It is reported to have a 68% SRR among heterogeneous groups[120]. For spermatozoa, up to 18 template-guided locations are analysed and sampled. Any aspirate positive for spermatozoa enables successful SR at that site[124]. Through this, multiple blind attempts and other adverse effects can be minimised, and thus, directed open testicular biopsies can also be minimised. In a study, FNA mapping in sperm identification among NOA patients was found to be around 58.8%, using 10-20 aspiration sites per testicle[120]. In another study analysing fine needle aspiration mapping in 22 patients with obstructive azoospermia, 100% of aspiration locations showed sperm[125]. TESA, TESE or microTESE can be done based on the distribution of sperms in FNA mapping[126]. If only few sites show evidence of active spermatogenesis, TESE can be offered with a success rate of about 90%[127]. This procedure is well tolerated by the patients without major side effects, so it may be considered the preferred method for sperm recovery in NOA men. Furthermore, it is quick, minimally invasive, and simple. Already, there are a few studies positively supporting the use of FNA in combination with ICSI for a better success rate. Still more cohort studies and meta-analyses are needed to ascertain the importance of FNAC in the treatment of male factor fertility.

4.2.1.5 Testicular Sperm Extraction (TESE)

The preferred method for treating NOA is TESE, and performing TESE with microsurgery appears to increase SRR. Compared to TESA, TESE is more invasive and requires scrotal exploration. The testis is gently compressed, causing the parenchyma to protrude after a little incision is made into the tunica albuginea without disturbing adjacent vessels. Similar to TESA, the extruded seminiferous tubules are removed and processed. The highest SRR was seen in the group with hypospermatogenesis (79%), followed by Maturation Arrest (MA) (47%), and Sertoli Cell-Only syndrome (SCO) (29%), in research that included 81 men who underwent TESE[128]. Another study reported 62% of SRR in the overall cohort of 87 patients[129]. A study concluded that TESE is preferred over micro-TESE in OA patients, as the result was 100% in the case of the former. Micro TESE can be reserved for non-obstructive azoospermia cases[130]. Multiple biopsies conducted during the TESE to enhance the SRR carry the usual risks associated with

an invasive procedure, scrotal hematomas and rarely testicular atrophy[131]. They can also develop temporary hypogonadism, which may be insignificant clinically[132]. Notably, amongst cancer survivors, it was found that the combination of TESE and ICSI delivered better results, with SRR being the same as that of non-cancer azoospermic men[133].

4.2.1.6 Micro-Dissection Testicular Sperm Extraction (MicroTESE)

MicroTESE was developed to reduce the amount of tissue excision. According to Schlegel, surgeons can lessen testicular trauma, maximize exposure for a comprehensive examination and dissection of the seminiferous tubules, and pinpoint specific locations that could support active spermatogenesis by bivalving the testis and using optical magnification[134]. According to a meta-analysis, as compared to single or multi-site TESE, microTESE has a better SRR[135].

In NOA patients, microTESE has displayed good SRR in Klinefelter syndrome (KS) patients (66%)[136]. It has been observed that microTESE is highly effective for hypospermatogenesis[137]. In contrast to good SRR in the above cases, it was found to be less effective in SCO (40%)[138] and Y chromosome microdeletion on the AZFc region (60–70%)[139]. It is however not effective in AZFa and AZFb patients. In a prospective controlled study by Verza Jr. *et al*, microTESE had 45% overall SRR as compared to 25% with TESE[140]. Furthermore, in all testicular histological categories of hypospermatogenesis (92.9% micro-TESE; 64.3% TESE), MA (63.6% micro-TESE; 9.1% TESE), and SCO (20% micro-TESE; 5.7% TESE), micro-TESE success rates were significantly greater than TESE ($P<0.01$). It is preferred in cases with increased FSH, atrophic testes, or when SCOS with elevated FSH are anticipated. Microscope assistance gives this technique a better visualisation of the seminiferous tubules; it facilitates the location of areas of active spermatogenesis and enlarged tubules for extraction more easily compared with other procedures. For the majority of patients with NOA, spermatogenesis cannot be re-established through any procedure; thus, SR in NOA currently relies heavily on micro-TESE. With all the available literature, it can be concluded that in NOA men, microTESE has a significantly higher SRR than conventional TESE.

4.3 Intracytoplasmic Sperm Injection (ICSI)

Intracytoplasmic Sperm Injection (ICSI) is a procedure wherein a single male gamete (sperm cell) is injected directly inside the ooplasm of a female gamete (oocyte). Even azoospermic men with focal spermatogenesis in the testis may benefit from ICSI. It leads to better fertilization in patients after surgical sperm retrieval. Numerous studies have indicated that when employing ICSI, fertilization rates per injected oocyte range between 45% and 75%, clinical pregnancy rates (CPR) between 26% and 57%, and delivery rates between 18% and 54%[110].

4.4 Varicocelectomy in NOA

Varicocele is found in 5–10% of NOA patients. It is the most common reversible cause associated with male subfertility, and it can be successfully treated by microsurgical subinguinal varicocelectomy. According to a meta-analysis by Esteves et al., SRR was higher in patients who received varicocelectomy than in people who did not have the procedure[141]. Another meta-analysis by Agarwal et al. also reported significant positive effects on semen parameters in patients with clinically palpable varicocele[142]. However, the value of varicocelectomy in azoospermic men remains controversial. Though its role in the pathophysiology of azoospermia is not fully established, surgical treatment has been attempted to achieve better rates of sperm retrieval. A consensus is warranted on a rational approach to infertile NOA men with varicocele.

5. Future Perspectives

Micro-TESE has become the preferred choice over conventional testicle biopsies for retrieving sperm for ART/ICSI in men with NOA. The main technical challenge encountered in micro-TESE is the low magnification. Out of several studies conducted to overcome the problem, few have shown positive outcomes. A study conducted on rodents used Multiphoton Microscopy (MPM). The main concern associated with this was the risk associated with the MPM laser causing sperm DNA fragmentation[144]. Another investigation made use of Full-Field Optical Coherence Tomography (FFOCT), which quickly produces high-resolution tomographic pictures of fresh, untreated, and unstained tissue using white light interference microscopy. This technique has proven more reliable because it uses a non-laser light source (a 150 W halogen lamp), which prevents sperm from getting mutagenized or destroyed. FFOCT can aid in micro-TESE for infertile men and enable real-time visualization

Table 1. Techniques of sperm retrieval with their indication, advantages and disadvantages[143]

Techniques	Indication	Advantages	Disadvantages
PESA	OA cases only	A surgical incision is not required, is simple and quick, is low cost, can be repeated multiple times in one sitting, and requires few instruments and materials.	Sperm-containing ductule may be missed as a blind procedure, with few sperm retrieved, a risk of hematoma or spermatocele, fibrosis, and obstruction at the aspiration site, and limited cryopreservation.
MESA	OA cases only	The higher the volume of sperm retrieved, the higher the chances for sperm cryopreservation, the lower the risk of hematoma, and reconstruction can be done.	Open surgery is needed, expensive, and time-consuming; microsurgical instruments and expertise are necessary; postoperative uneasiness.
TESA	Failed PESA in OA, Epididymal agenesis in CAVD, favourable testicular histopathology in NOA, previous successful TESA in NOA	Fast and inexpensive, can be repeated, no microsurgical expertise needed, no surgical incision required, fewer instruments and materials, minimal postoperative complications.	Comparatively low success rate and few sperm retrieved in NOA; cryopreservation limited; risk of hematoma or testicular atrophy.
TESE	Failed PESA or TESA in OA, NOA cases	No microsurgical expertise is needed; it is quick and repeatable.	Compared to the low success rate and low sperm retrieved in NOA, there is a possibility of testicular atrophy with multiple biopsies. Postoperative uneasiness.
Micro-TESE	NOA cases only	Better success rates in NOA, a greater number of sperm retrieved, maximizing the chance of sperm cryopreservation, and minimal complications.	Surgical incisions are necessary, expensive, and time-consuming; microsurgical instruments and expertise are needed; and postoperative uneasiness.
FNA	Azoospermia cases	Quick, easy, and minimally invasive.	Blind puncture technique. Possibility of progressive testicular damage caused by TFNA.

of human spermatogenesis[145]. By taking advantage of enhanced ergonomics, Robot-Assisted Vasectomy Reversal (RAVR) may displace the traditional operating microscope[146].

Proteomics and RNA sequencing have also been widely applied to male infertility. Men who are fertile and infertile appear to have varied sperm RNA contents. Certain proteins found in seminal plasma, such as ECM1, TEX101, and LGALS3BP, seem to be reliable indicators of the outcome of TESE[147,148]. The role of oxidative stress associated with stasis has been explored in patients with azoospermia. There have been reports of functional polymorphisms in genes like superoxide dismutase (SOD) and nuclear factor erythroid 2-related factor 2 (NRF2) (SOD); however, the specific role of antioxidants is yet to be defined[149,150].

The current advancements in the field of artificial reproductive technology have resulted in improvements in the diagnosis and management of azoospermia. Healthy progeny was produced by successful *in vitro* sperm generation in cultured neonatal mouse testes after ICSI[151]. Human-Induced Pluripotent Stem (iPS) cells are also being used to induce germ cells. The production of round haploid spermatids from human induced pluripotent cells has been reported to be possible *in vitro*[152]. Innovations in the future can potentially open up novel therapeutic techniques for NOA patients.

Viral gene therapy has been used recently to repair impaired sperm production linked to AR gene abnormalities in SCARKO mice using adenovirus-based gene therapy in the testis. The AR mutants that can activate the expression of the classical and non-classical pathways in Sertoli cells can regulate specific mRNA levels in spermatogonia germ cells inside the testis, which may preserve spermatogenesis and male fertility. Results were encouraging, with fertilization and normal offspring[153]. It is a positive step ahead, with more such types of studies warranted before making it an option in the treatment of non-obstructive azoospermic men.

Furthermore, the excellent regenerative potential of Platelet-Rich Plasma (PRP) has been reported in various studies. Though PRP therapy has given good results in treating female infertility, the benefits of PRP in men have not been widely studied. A retrospective study on 91 patients with PRP injection has been reported, among whom 2.2% had total normal sperm function restoration[154]. In a different study using rats as the model, PRP greatly enhanced the number of spermatogenic stem cells, count, motility, and length of the sperm's tail in animals treated with busulfan (BUS). It concluded that PRP has the potential to enhance the structure and function of the testis in the treatment of BUS[155]. PRP therapy is at a very early stage, and since it is non-harmful to the patients, further studies are warranted to reveal its potential for increasing spermatogenesis and clinical management of male infertility.

Other techniques like 3D printing and the creation of artificial testes for cancer patients, genetic editing by replacing the defective locus using the CRISPR/Cas9 system, and the rapidly evolving artificial intelligence in the domain of male infertility may revolutionize the management of male infertility associated with azoospermia in the future[148].

6. Conclusion

Proper diagnosis and appropriate management can be the keys to addressing the problem of azoospermia in male infertility. Identifying genetic causes and referring them for genetic counselling should be emphasized. Azoospermia is a major factor in male infertility, and proper diagnosis and appropriate management can be the keys to resolving the problem. Thus, an accurate etiological diagnosis of azoospermia is important to plan treatment. Imaging, hormonal analysis, and testicular biopsies play a major role in the process of evaluating male infertility; the latter plays a significant role in distinguishing OA from NOA. In current practice, semen analysis as per the WHO 2021 standard remains the foundation for the initial diagnosis but is not adequate for the proper diagnosis of the different etiological factors; thus, male genetic factors should be given equal priority. Microsurgical reconstruction and ICSI can be effective treatments for OA, whereas no treatment can restore spermatogenesis in most NOA patients, except those who have secondary testicular failure (HH). Among the surgical procedures, microTESE is more effective with a high SRR in NOA. Thus, direct sperm retrieval from the testes for ICSI remains the chief treatment for azoospermia cases to achieve pregnancy without involving any donor programme. Medical management is beneficial only for HH cases. The arrival of stem cell therapy is the futuristic treatment modality for the management of non-obstructive azoospermia, for which more research is mandated.

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