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Review of Azoospermia

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Azoospermia is classified as obstructive azoospermia (OA) or non-obstructive azoospermia (NOA), each having very different etiologies and treatments. The etiology, diagnosis, and management of azoospermia were reviewed and relevant literature summarized. Differentiation between these two etiologies is of paramount importance and is contingent upon thorough history and physical examination and indicated laboratory/genetic testing. OA occurs secondary to obstruction of the male reproductive tract, and is diagnosed through a combination of history/physical examination, laboratory testing, genetics (CFTR for congenital OA), and imaging studies. NOA (which includes primary testicular failure and secondary testicular failure) is differentiated from OA by clinical assessment (testis consistency/volume), laboratory testing (FSH), and genetic testing (karyotype, Y chromosome microdeletion, or specific genetic testing for hypogonadotropic hypogonadism). For obstructive azoospermia, management includes microsurgical reconstruction when feasible using microsurgical vasovasostomy or vasoepididymostomy. Microsurgical epididymal sperm aspiration with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) is utilized for those cases not amenable to reconstruction. NOA management includes medical management for congenital hypogonadotropic hypogonadism and microdissection testicular sperm extraction with IVF/ICSI for appropriate candidates based on laboratory/genetic testing. Overall, this important review provides an updated summary of the most recent available literature describing etiology, diagnosis, and management of azoospermia.

Introduction

The absence of spermatozoa in the ejaculate (azoospermia) is identified in 15% of infertile men and can be classified as obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Thorough history-taking and physical examination are critical in the classification of azoospermia etiology and may be accompanied by laboratory and genetic testing. OA and NOA are managed by specific medical and/or surgical options. Recent advances in genetic testing and microsurgical approaches

combined with assisted reproductive technology (ART) are providing options for increasing numbers of patients.

OA, which comprises 40% of azoospermia cases, is typically accompanied by preservation of normal exocrine and endocrine function, and normal spermatogenesis in the testis.¹ OA is the consequence of physical blockage to the male excurrent ductal system and may occur in any region between the rete testis and the ejaculatory ducts.² NOA, the etiology affecting approximately 60% of azoospermic men, includes non-obstructive causes of azoospermia, including toxic exposures or abnormal testicular development.³ NOA results from either primary testicular failure (elevated LH, FSH, small testes affecting up to 10% of men presenting with infertility), secondary testicular failure (congenital hypogonadotropic hypogonadism with decreased LH and FSH, small testes), or incomplete or ambiguous testicular failure (either increased FSH and normal volume testes, normal FSH and small testes, or normal FSH and normal testis volume).

Etiology of OA

Acquired causes of vasal obstruction include vasectomy, iatrogenic injury following inguinal hernia repair, or less commonly vasography performed with improper technique. Obstruction of the epididymis can occur as a result of increased epididymal intratubular pressure from extended vasal obstruction (in more than 50% of men with obstructive interval exceeding 15 y after vasectomy, resulting in microscopic epididymal tubule rupture with sperm leakage causing epididymal sperm granuloma and epididymal obstruction), and following pelvic or scrotal trauma. Iatrogenic epididymal obstruction post-hydrocelectomy, percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm aspiration (MESA), or inadvertent epididymal biopsy may result due to technique.⁴ Congenital OA can be a result of congenital unilateral absence of the vas deferens (CUAVD) or congenital bilateral absence of the vas deferens (CBAVD) usually associated with mutations of the cystic fibrosis transmembrane regulator (CFTR) protein, which lead to absent vasa as well as partial or complete absence of the epididymis. Finally, severe inflammation of the epididymis (epididymitis), prostate, seminal vesicles, or lower genitourinary tract infection may lead to male excurrent ductal obstruction. Ejaculatory duct obstruction (EDO) can result from trauma, surgery, infection, or congenital

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Gonadotropin, Testosterone, and Testis Volume Changes with OA and NOA

Etiology	Subtype	FSH	LH	Testosterone	Testis Volume
Obstructive Azoospermia		↔	↔	↔	↔
Non-obstructive Azoospermia	Primary Testicular Failure	↑	↑	↓	↓
	Hypogonadotropic Hypogonadism	↓	↓	↓	↓

Figure 1. Changes in gonadotropins, testosterone, and testis volume associated with obstructive and non-obstructive azoospermia.

Müllerian duct cysts and is characterized by low semen volume with a crystal clear watery appearance (due to absence of seminal vesicle component), low semen pH, and absent fructose.

Diagnosis of OA

Differentiation between OA and NOA is usually possible with a good history and physical examination, including testicular volume assessment with OA demonstrating normal testicular volume and indurated epididymides, while NOA with small, soft testes and flat, soft epididymides. Aforementioned surgery, infection, or congenital abnormalities characterize OA along with history of successful prior fertility. Physical examination should focus on vasal gaps, location of vasectomy site (convoluted vas may carry increased risk of epididymal injury since pressure is dissipated over a shorter testicular vasal remnant), presence of granuloma at vasectomy site (a predictor of better microsurgical vasectomy reversal results⁵), and epididymal abnormalities including full or absent portions. Laboratory values, most significantly FSH, is normal in OA and usually elevated in NOA due to lack of normal negative feedback on the hypothalamus and pituitary by inhibin B and testosterone (Fig. 1). Elevated FSH in OA may indicate an increased need for assisted reproductive technology post-vasectomy reversal.⁶ If unilateral obstruction exists, the contralateral non-obstructed side may allow fertility. If partial obstruction is present, severe oligospermia or oligoasthenoteratospermia may result. If the vas is absent unilaterally or bilaterally, CFTR mutation testing with recommended screening panels or DNA sequencing of the full CFTR gene is indicated.^{7,8} In men with unilateral CAVD and negative CFTR mutation testing, renal ultrasound is indicated because at least 20% of these men can have ipsilateral renal agenesis. For men with CFTR mutations, female partner testing and genetic counseling is critical prior to ART. In men with palpable vasa and suspected obstruction, a positive serum anti-sperm antibody indicates active spermatogenesis and eliminates unnecessary testicular biopsy before microsurgical reconstruction.⁹ EDO suspicion should prompt digital rectal examination to rule out midline cysts. Scrotal and transrectal ultrasound can detect structural abnormalities of the epididymis, seminal

vesicles, and prostatic cysts, which can help to confirm etiology of azoospermia.¹⁰ Associated disease with OA (CF symptoms) and NOA (increased risk of cardiovascular disease¹¹ as well as testis cancer and colorectal cancer) should be considered based on physical exam, lab, and genetic abnormalities.^{12,13} For men with unclear etiology of azoospermia, testis biopsy may be indicated in select men with normal volume testes, normal vasa, negative anti-sperm antibody testing, and normal FSH levels.

Management of OA

Optimal management of vasal or epididymal obstruction includes microsurgical reconstruction (vasovasostomy or vasoepididymostomy, respectively) when feasible using the multilayer microdot vasovasostomy technique (Fig. 2A and B)^{14,15} and longitudinal intussusception vasoepididymostomy (LIVE) techniques^{16,17} as described previously (Fig. 2C). Both techniques, pioneered at Cornell, require adherence to standard microsurgical tenets including tension-free, water-tight, precise anastomosis with meticulous preservation of blood supply. For vasoepididymostomy or redo reconstruction surgery, sperm retrieval and cryopreservation for future IVF/ICSI should be performed intraoperatively as an alternative solution if reconstruction proves unsuccessful.¹⁸

Approximately 6% of the 600 000 men undergoing vasectomy annually ultimately desire vasectomy reversal. The decision for reconstruction is multifactorial, based upon number of offspring desired, past surgical history, prior fertility, female partner characteristics (age or infertility), as well as religious and financial background. Outcomes of microsurgical reconstruction have improved over the past four decades and currently include 70–99.5% patency/36–92% pregnancy rates for VV and 30–90% patency/20–50% pregnancy rates for VE.^{19–21} Predictors of microsurgical reconstruction outcomes include intraoperative vasal fluid quality and sperm granuloma presence, vasal obstructive interval, and microsurgeon experience.²² Cost-effectiveness analysis indicates that microsurgical reconstruction is the safest and most financially-sound management option for vasal and epididymal obstruction.^{19,23,24} Transurethral resection of the ejaculatory ducts (TURED), or alternatively with simultaneous microsurgical sperm retrieval, may be completed for ejaculatory duct obstruction.²⁵ However, TURED may not be completed with concomitant VE for secondary epididymal obstruction since this is rarely successful.

For cases not amenable to surgical intervention such as men with CBAVD, microsurgical epididymal sperm aspiration (MESA) combined with IVF/ICSI is the treatment of choice.^{26,27} Retrieved sperm from men with OA may be cryopreserved as data indicate equivalent excellent outcomes with fresh or frozen sperm used for IVF/ICSI.^{28,29} Although MESA yields far more sperm typically than testicular sources, testicular and epididymal sperm from men with OA have been identified to yield similar outcomes with IVF/ICSI.³⁰

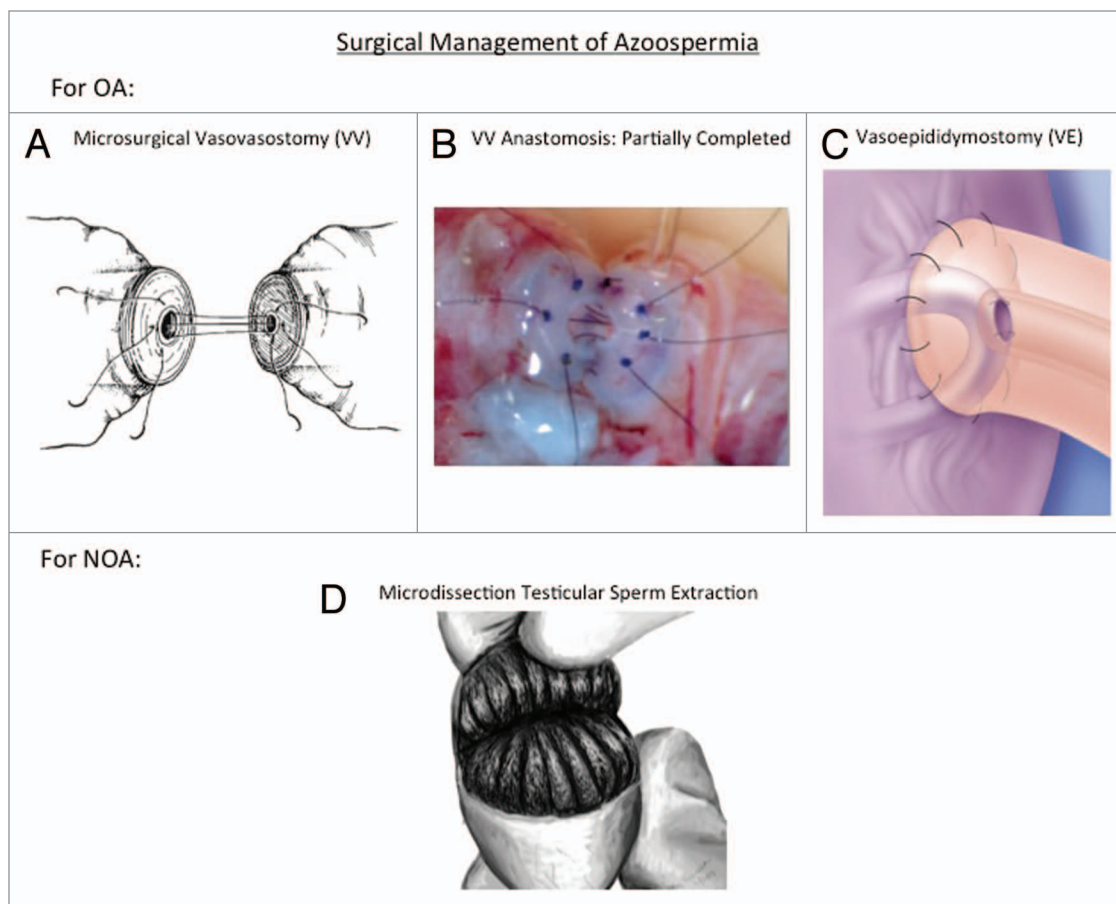


Figure 2. (A) Vasovasostomy (VV) with mucosal stitches placed prior to tying. (B) Vasovasostomy (VV) anastomosis partially completed: the posterior side has been completed and anterior 3 mucosal sutures have been placed according to the microdot technique and prepared for tying. (C) Completed VE anastomosis using longitudinal intussusception VE technique (LIVE). The vasa lumen reaches the desired epididymal tubule without tension after completed LIVE procedure. (D) Microdissection testicular sperm extraction (MicroTESE) with testis opened along equatorial plane at mid-portion revealing seminiferous tubules.

Etiology of NOA

NOA includes primary testicular failure (elevated LH, FSH, small testes affecting up to 10% of men presenting with infertility), secondary testicular failure (congenital hypogonadotropic hypogonadism with decreased LH and FSH, small testes), and those with an incomplete or ambiguous picture of testicular failure (either increased FSH and normal volume testes, normal FSH and small testes, or normal FSH and normal testis volume) (Fig. 1). For example, maturation arrest is often associated with normal FSH and/or testis volume and is associated with genetic abnormalities including MLH1 mutations, which are characterized by decreased recombination frequency.³¹ Serum testosterone, LH, FSH, and prolactin can distinguish between NOA and OA. Patients typically demonstrate small soft, atrophic testes unlike normal volume testes in OA. NOA may result from prior toxic exposures (i.e., chemotherapy, radiation) or history of abnormal development, cryptorchidism, or large varicoceles.

Diagnosis of NOA

Karyotype and Y chromosome microdeletion (YCMD) tests are warranted in men with NOA without history of prior fertility, obstruction of male reproductive tract, or toxic exposures.³² Karyotype analysis identifies both numeric and structural abnormalities of chromosomes, which are identified to be abnormal in up to 19% of men with NOA. The most frequently identified karyotype abnormality is Klinefelter syndrome (47,XXY; found in 1/600 men), while Robertsonian translocations (fusion of long arms of two acrocentric chromosomes 13, 14, 15, 21, or 22) are a frequent structural abnormality. Declining spermatogenesis is correlated with increasing number of X chromosomes. YCMD of three azoospermic factor (AZF) regions located in Yq11 (long arm of the Y chromosome) encode proteins influencing spermatogenesis mapped initially in 1976 with additional ensuing characterization.^{33,34} PCR amplification using sequence-tagged sites (STS) identifies abnormalities in the AZF regions (AZFa, AZFb, AZFc) in 10–20% of azoospermic men, which

Table 1. Microdissection TESE outcomes at Cornell

Condition	Overall sperm retrieval rate	Pregnancy rate
	per micro-TESE cycle	
Cryptorchidism	64%	50%
Post-chemotherapy azoospermia	48%	40%
KS (classic and mosaic)	65%	40%
AZFc deletion (Y chromosome microdeletion)	72%	46%
Uniform Maturation Arrest	50%	29%
Sertoli cell only	44%	46%

have prognostic implications. AZFa, AZFb, and AZFb+c have not previously been associated with successful sperm retrieval, while AZFc microdeletions are associated with successful sperm retrieval in up to 80% of cases; however, male offspring will also manifest AZFc microdeletion and have the same fertility problem as their fathers.^{35,36}

For men with congenital hypogonadotropic hypogonadism, the most common form is Kallmann syndrome, which manifests with a spectrum of symptoms, including anosmia/hyposmia due to faulty migration of GnRH-secreting neurons from the nasal olfactory epithelium to the basal hypothalamus during embryogenesis.³⁷ Due to absent hypothalamic GnRH, the pituitary is not stimulated to produce LH and FSH, although pituitary gonadotrophs are present and normal. Therefore, both spermatogenesis and testosterone production by the testes are not stimulated. Specific genetic testing is warranted and varies depending on the inheritance pattern (X-linked or autosomal) for KAL1 (Xp22.32), FGFR1 (8p11.2–11.1), and other genes.^{38,39} KAL1 mutations are noted in 15–50% of familial X-linked and 10% of sporadic cases. Patients present with variable clinical picture including absent or incomplete pubertal development, severe hypogonadism, anosmia, and occasionally impaired hearing or oral anomalies. Therapy includes pulsatile GnRH administration, or more practically, hCG (to replace LH), recombinant FSH, hMG.⁴⁰ Sperm production may be induced with up to 1–2 y of treatment in the vast majority of patients with excellent pregnancy rates even with low sperm counts. NOA patients with ambiguous clinical picture and low semen volume should undergo CFTR mutation (otherwise typically reserved for OA patients with suspected CUAVD, CBAVD) testing since this could be related to seminal vesicle atresia. In applicable cases, preimplantation genetic diagnosis may be considered given potential risk of increased pregnancy loss and offspring abnormalities.⁴¹ Genetic counseling is requisite before and after any genetic testing.

Management of NOA

Prior to microsurgical testicular sperm retrieval techniques and IVF/ICSI, donor insemination was the only option available to men with NOA. Microdissection testicular sperm extraction (microTESE) in combination with IVF/ICSI, for appropriate azoospermic candidates based on laboratory and genetic testing (i.e., Klinefelter syndrome, AZFc microdeletion patients),

yields sperm in approximately 60% of cases with increased sperm retrieval rates and decreased structural alteration of the testis,^{42,43} even with elevated FSH levels or decreased testicular volume,⁴⁴ compared with non-microsurgical approach (Table 1; Fig. 2D).^{45,46} Additionally, in hypogonadal men with NOA, there is no relationship between preoperative testicular volume, FSH levels, or testosterone response to hormonal therapy and TESE sperm retrieval outcomes.⁴⁷ Klinefelter patients, however, do demonstrate improved sperm retrieval outcomes when preoperative testosterone increased to greater than 250 ng/dl with medical therapy (77% with testosterone response vs 55% without response).⁴⁸ The benefits of microTESE have also been demonstrated for men with history of chemotherapy-related NOA (mean SRR of 43%) with best results for patients with testicular cancer and worst results for patients with sarcoma history.⁴⁹ Aside from men with hypogonadotropic hypogonadism or oligospermia, there is minimal evidence of any benefit to providing supplemental gonadotropins for men with NOA due to primary testicular failure.^{50,51} Additional genetic understanding of LH and FSH receptors and aromatase could select men who may derive benefit.

Approximately 15–20% of men with varicoceles experience fertility issues.^{52,53} For men with NOA and varicocele, microsurgical varicocelectomy has been reported to improve spermatogenesis (and decrease scrotal temperature⁵⁴ and DNA fragmentation⁵⁵) in the testis leading to return of sperm to the ejaculate in 22–55% of men, with 10–40% of men avoiding TESE.^{56–58} Recovery of sperm to the ejaculate may be dependent on testicular histology, with hypospermatogenesis and late maturation arrest demonstrating this favorable outcome, while early maturation arrest and Sertoli cell-only histology consistently require TESE.⁵⁹ Some studies demonstrate that the chance for successful sperm retrieval in men with NOA undergoing varicocelectomy is equivalent to those without varicocele repair, while other studies show improvement in sperm retrieval rates (Table 2).^{56,60} Cost-effectiveness analysis indicates that varicocelectomy may be cost-effective for only some patients when compared with upfront testicular sperm extraction with ICSI for men with NOA. Overall, decision for varicocelectomy in men with NOA must take into account the age of the female partner, grade of varicocele, along with the best available data from clinical trials.⁶¹

Additional genetic testing in the future, including analysis of sperm small non-coding RNA (sncRNA), mRNA, microRNA (miRNA), piwi-interacting RNA

Table 2. Varicocelectomy outcomes for men with non-obstructive azoospermia

Study	Number of patients	Mean post-operative sperm concentration (x10 ⁶ /ml)	Return of sperm to ejaculate	Pregnancy rate
Matthews, 1998 [57]	22	2.2	12/22 (55%)	3/22 (15%)
Kim, 1999 [66]	28	1.2	12/28 (43%)	2/28 (7%)
Kadioglu, 2001 [67]	24	0.04	5/24 (21%)	0/24 (0%)
Schlegel, 2004 [56]	31	N/A	7/31 (22%)	0/31 (0%)
Cakan, 2004 [68]	13	0.7	3/13 (23%)	0/13 (0%)
Esteves 2005 [69]	17	0.8	8/17 (47%)	1/17 (6%)
Gat, 2005 [70]	32	3.8	18/32 (56%)	4/18 (12%)
Poulakis, 2006 [71]	14	3.1	7/14 (50%)	2/14 (14%)
Pasqualotto, 2006 [72]	27	0.87	9/27 (33%)	1/33 (3%)
Lee, 2007[58]	19	0.36	7/19 (36%)	1/19 (5%)
Ishikawa, 2008 [73]	6	0.2	2/6 (33%)	0/6 (0%)
Cocuzza, 2009 [74]	10	5.5	3/10 (30%)	N/A
Youssef, 2009 [75]	51	3.56	14/51 (28%)	2/51 (4%)
Abdel-Meguid, 2012 [59]	31	2.3	10/31 (32%)	N/A
Overall	325	1.89	117/325 (36%)	16/276 (6%)

Adapted from: Schlegel and Goldstein, 2011.⁷⁶

(piRNA), epigenetic modifications (histones/protamines), post-translational protein-modified gene products,⁶² DNA damage repair gene SNPs,⁶³ and copy number variation affecting spermatogenic failure may further refine diagnosis and treatment of azoospermia.^{64,65}

Conclusion

Differentiation of OA from NOA is required to formulate diagnostic and management options for azoospermic patients. OA occurs secondary to obstruction of the male reproductive tract, and is diagnosed through a combination of history/physical examination, laboratory testing, genetics (CFTR for congenital OA), and imaging studies as needed. If vasal or epididymal obstruction are present, microsurgical reconstruction, if feasible based on multifactorial decision analysis, provides efficacious, safe, and cost-effective outcomes using the most recent available techniques. If

obstructive etiology or the patient is not amenable to microsurgical reconstruction, microsurgical sperm aspiration provides excellent outcomes in conjunction with ART since normal spermatogenesis is usually present in OA. Patients with NOA (which includes primary testicular failure and secondary testicular failure) is differentiated from OA by clinical assessment (testis consistency/volume), laboratory testing (FSH), and genetic testing (karyotype, YCMD, or specific genetic testing for HH). Management includes microdissection testicular sperm extraction and IVF/ICSI. In the case of varicocele with NOA, varicocelectomy may prove beneficial. Future study of biologic and genetic mechanisms will optimize diagnosis and prognosis for azoospermic patients.

Author: Please include in-text citation for references 66-75.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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