Azoospermia

Subjects: Surgery | Health Care Sciences & Services
Contributor: Sandro Esteves

Azoospermia (a-, without + –zoo– » Greek zôion, animal + –spermia– » Greek sperma, sperm/seed) is defined by the absence of sperm in the ejaculate.

Keywords: azoospermia ; diagnosis ; male infertility ; nonobstructive azoospermia ; spermatogenic failure

1. Introduction

Azoospermia (a-, without + –zoo– » Greek zôion, animal + –spermia– » Greek sperma, sperm/seed) is defined by the absence of sperm in the ejaculate. Although the term does not imply an underlying etiology, azoospermia inevitably provokes infertility [1]. According to global estimates, 1 out of 100 men at reproductive age and up to 10% of men with infertility are azoospermic [2][3][4].

Azoospermia is broadly classified into obstructive and nonobstructive. This differentiation is clinically meaningful because it affects patient management and treatment outcomes [4]. Notably, nonobstructive azoospermia (NOA) relates to an intrinsic testicular defect caused by various conditions that ultimately affect sperm production profoundly.

The severe spermatogenic deficiency observed in NOA patients is often a consequence of primary testicular failure affecting mainly spermatogenic cells (spermatogenic failure (STF)) or related to a dysfunction of the hypothalamus-pituitary-gonadal axis (hypogonadotropic hypogonadism (HH)). From this point on, the acronyms STF and HH will distinguish these types of NOA, as appropriate [5]. The above-proposed terminology might be more intuitive for the clinician. It not only indicates the site of the problem (central or local) explicitly, but also makes it clear that the testicular disorder refers primarily to a spermatogenic defect, unlike the indistinct term ‘testicular failure’ that may relate to an isolated spermatogenic defect or such a defect combined with Leydig cell failure.

The differential diagnosis between STF and HH is also essential because the former is linked with severe and untreatable conditions, whereas the latter can be effectively treated with gonadotropin therapy [5][6]. By contrast, obstructive azoospermia (OA) originates from a mechanical block along the reproductive tract, namely, vas deferens, epididymis, or ejaculatory duct [7][8]. Unlike NOA, spermatogenesis is preserved, and both reconstructive procedures and sperm retrieval are typically highly successful in OA patients [7][8][9][10].

Nonobstructive azoospermia can be distinguished from OA using history, physical examination, semen analysis, hormonal assessment, and genetic testing in most patients [4][5][11]. However, in some instances, this distinction is not straightforward, and a testis biopsy is required.

2. Azoospermia Differential Diagnosis: An Overview

The primary goals of the differential diagnosis are the identification of:

- Potentially correctable forms of azoospermia (e.g., by surgery or medication).
- Irreversible types of azoospermia suitable for sperm retrieval and intracytoplasmic sperm injection (ICSI), using own sperm.
- Types of azoospermia in which donor insemination or adoption are the only possibilities.
- Health-threatening illness associated with azoospermia requiring medical attention.
- Genetic causes of azoospermia that may affect the patient or offspring’s health, mainly if assisted reproductive technology is used.

It is critical to evaluate the azoospermic patient using a standardized workup to achieve these goals, as discussed in the next sections.

2.1. Medical History

A thorough medical history is pivotal to help determine the type of azoospermia. It must cover eight critical elements (**Table 1**), which are:

Table 1. Medical history outline. Adapted from Esteves et al. [11], Clinics 66, 691–700, 2011.

Elements	Components
(1) Infertility History	• Age of partners, length of time the couple has been attempting to conceive • Contraceptive methods/duration • Previous pregnancy/miscarriage (current partner/partner/another partner) • Previous treatments • Treatments/evaluations of female partner
(2) Sexual History	• Potency, libido, lubricant use • Ejaculation, timed intercourse, frequency of masturbation
(3) Childhood and Development	• Cryptorchidism, hernia, testicular trauma, testicular torsion, infection (e.g., mumps) • Sexual development, puberty onset
(4) Personal History	• Systemic diseases (e.g., diabetes, cirrhosis, hypertension) • Sexually transmitted diseases, tuberculosis, viral infections, genital and systemic bacterial infections, history of fever
(5) Previous Surgery/Treatment	• Orchidopexy, herniorrhaphy, orchiectomy (e.g., testicular cancer, torsion) • Retroperitoneal and pelvic surgery • Other inguinal, scrotal, or perineal surgery • Bariatric surgery, bladder neck surgery, transurethral resection of the prostate
(6) Gonadotoxin Exposure	• Pesticides, alcohol, cocaine, marijuana • Medication (e.g., chemotherapy agents, cimetidine, sulfasalazine, nitrofurantoin, allopurinol, colchicine, thiazide, α- and β-blockers, calcium blockers, finasteride) • Organic solvents, heavy metals • Anabolic steroids, tobacco use • High temperatures, electromagnetic energy • Radiation (e.g., therapeutic, nuclear power plant workers)

Elements	Components
(7) Family History	• Cystic fibrosis, endocrine diseases • Infertility
(8) Current Health Status/Lifestyle	• Respiratory infection, anosmia • Galactorrhea, visual disturbances • Obesity, metabolic syndrome

- Infertility history
- Sexual history
- Childhood and development history
- Personal medical history
- Previous surgery/treatments
- Gonadotoxic exposure
- Family history
- Current health status and lifestyle

The history may reveal the presence of congenital abnormalities, such as cryptorchidism, which could result in NOA-STF. Testicular infections (e.g., mumps orchitis), testicular trauma, testicular torsion, gonadotoxin exposure (e.g., radiotherapy/chemotherapy, anabolic steroid use, testosterone replacement therapy), or a history of brain surgery are informative to help establish a possible etiologic factor for NOA [5]. Hypogonadotropic hypogonadism is caused by congenital (e.g., Kallmann syndrome) or acquired conditions (e.g., prolactinomas, pituitary surgery, or testosterone replacement therapy) [6][12][13]. Notably, testosterone injections—commonly prescribed nowadays to men at reproductive age with signs of hypogonadism—suppress the hypothalamic-pituitary-gonadal axis. Consequently, intratesticular testosterone levels—critical for normal spermatogenesis—remain very low and, therefore, unable to sustain spermatogenesis [14].

On the other hand, a history of hernia repair, scrotal surgery, pelvic surgery, endoscopic urethral instrumentation, or genitourinary infection (e.g., epididymitis) may cause OA. Along these lines, a previous vasectomy is a typical OA etiology [8]. However, in many cases, the etiology cannot be determined, and additional tests are required, as explained in the following sections.

2.2. Physical Examination

The physical exam is critical in the assessment of men presenting with azoospermia. It starts with the appraisal of the overall body characteristics, with a focus on secondary sexual characteristics. Abnormal body hair distribution and gynecomastia may be indicative of hypogonadism or hormonal disturbances [5][11]. Examination of inguinal and genital areas may unveil scars from previous surgeries that could have injured testicular blood supply and the vas deferens. Other physical defects, such as abnormalities of the penis (e.g., hypospadias, epispadias, short frenulum, phimosis, fibrotic nodules), should also be evaluated.

Testicular size, texture, and consistency should be assessed. In routine practice, testicular volume is estimated using the Prader’s orchidometer. The mean testicular volume measured using the Prader’s orchidometer in the general population is 20.0 ± 5.0 mL [15].

Testes of men with OA have a firm texture. About 85% of testicular parenchyma is implicated in spermatogenesis. By contrast, men with NOA usually have small testicles (<15 mL or ≤4.6 cm long axis) [16]. However, it should be noted that there is no threshold for testicular size to completely exclude the possibility of harvesting sperm on a retrieval attempt [17].

Moreover, both patients with OA and NOA-STF due to maturation arrest have normal-sized testicles [18]. Therefore, testicular size may not necessarily be informative for the differential diagnosis. Palpable abnormalities of the testis should be further evaluated with imaging studies because azoospermic men, particularly those with NOA-STF, have increased risks of developing testis malignancy [19].

The presence of the vas deferens and the epididymis' characteristics should always be determined. A normal and healthy epididymis is firm, whereas an obstructed epididymis is ingurgitated (soft) [11]. Patients with NOA typically have palpable vasa deferentia and flat epididymides [5].

The vas deferens is easily palpable inside the spermatic cord as a firm, round, "spaghetti-like" structure. The vas can be absent at both sides, indicating a congenital abnormality [5][11]. Congenital bilateral absence of vas deferens (CBAVD) is associated with OA, and approximately 10% of these men have concurrent unilateral renal agenesis and should undergo an ultrasound scan to uncover this potentially health-threatening condition. By contrast, most patients (~60%) with congenital unilateral absence of vas deferens (CUAVD) are non-azoospermic [20]. A gene mutation associated with cystic fibrosis causes bilateral vas agenesis; therefore, genetic screening is advisable for the affected couples planning assisted reproductive technology (ART) [11][12]. Mutations affecting the cystic fibrosis transmembrane conductance regulator (CFTR) gene have also been identified in about 10% of men with CUAVD and normal kidneys, and it has been suggested that these patients should undergo CFTR testing as recommended for CBAVD patients.

Assessment of the spermatic cord is mandatory as a varicocele may be found [5][11][21]. Varicocele is a prevalent congenital abnormality linked to infertility, impaired testicular growth, and hypogonadism [22][23][24][25]. Although varicocele is not uncommon in azoospermic men [24], it is debatable whether the vein dilation is coincident or contributory to spermatogenesis disruption in such patients [25]. Nonetheless, spermatogonia, spermatocytes, and spermatids are highly exposed to heat stress caused by varicocele. Furthermore, it was shown that varicocelectomy might ameliorate spermatogenesis and androgen production in azoospermic patients with spermatogenic failure [24][25][26].

The varicocele diagnosis is primarily made by a physical examination in a warm room with the patient standing. Palpable varicoceles are graded as (i) small (Grade 1): palpable during Valsalva maneuver, (ii) moderate size (Grade 2): palpable at rest, and (iii) large (Grade 3): visible and palpable at rest [22]. Scrotal Doppler ultrasound is indicated if a physical examination is inconclusive [11]. A maximum venous diameter of >3 mm in the upright position and during the Valsalva maneuver and venous reflux with a duration> 2 s usually correlate with the presence of a palpable varicocele [27].

2.3. Semen Analysis

The term azoospermia essentially refers to a semen analysis result. The assessment of an azoospermic ejaculate with normal volume (i.e., >1.5 mL) should be followed by examining the pelleted semen after centrifugation to rule out cryptozoospermia, defined by the presence of rare sperm [5][28]. Centrifugation should be carried out at 3000× *g* for 15 min or longer [29]. The finding of live sperm may allow ICSI to be carried out with ejaculated sperm, obviating surgical sperm harvesting. Azoospermia must be confirmed in at least two consecutive semen analyses because temporary azoospermia due to toxic, environmental, infectious, fever, or iatrogenic conditions can take place [30][31]. Assessment of azoospermic ejaculates on more than one occasion is also essential given the biological variability of the same individuals' specimens. However, a limit of semen analyses (e.g., 2–3) might be set from a practical standpoint, although the exact number is difficult to ascertain. An interval between analyses is also advisable (e.g., one month apart) [32], albeit the optimal interval between examinations has not been established.

The state-of-art on how human semen should be assessed in the laboratory is set out by the World Health Organization (WHO), which periodically issues manuals that include standard operating procedures and reference values [29][31]. The lower reference limits (5th centile) for semen characteristics according to the 2010 WHO manual are as follows: (i) Semen volume: 1.5 mL, (ii) Total sperm number: 39 million/mL, (iii) Sperm concentration: 15 million/mL, (iv) Total motility: 40%, (v) Progressive motility: 32%, (vi) Vitality: 58% alive, and (vii) Sperm morphology: 4% normal forms [29].

Ejaculates of men with NOA-STF usually exhibit normal volume and pH (>7.2), indicating functional seminal vesicles and patent ejaculatory ducts [5]. By contrast, hypospermia (ejaculate volume < 1.5 mL) is typical in patients with HH-NOA [5][6]. A combination of a low volume (<1.5 mL), acidic ejaculate (pH < 7.2), with low fructose (e.g., <13 µmol per ejaculate) indicates seminal vesicle hypoplasia or obstruction [11]. Both conditions are associated with OA; the former with CBAVD and the latter with ejaculatory duct obstruction [33][34]. Seminal neutral alpha-glucosidase levels can also be determined as they reflect the epididymal function [29]. It was reported that seminal α-glucosidase levels < 18 mU/ejaculate is a reliable indicator of congenital bilateral absence of the vas deferens [4].

2.4. Hormonal Evaluation

Assessment of reproductive hormones' serum levels may add relevant information to establish azoospermia type. Follicle-stimulating hormone (FSH) and testosterone are the essential hormones driving spermatogenesis [5][11]. Testosterone is produced by the Leydig cells under luteinizing hormone (LH) stimulation. Adequate levels of intratesticular testosterone are critical for sperm maturation [35]. By contrast, FSH is mainly responsible for increasing sperm production, and it collaborates with intratesticular testosterone to promote cell proliferation [36].

In general, there is an inverse relationship between FSH levels and spermatogonia quantity [37][38]. When spermatogonia number is absent or remarkably reduced, FSH levels increase; when spermatogonia number is normal, FSH levels are within normal ranges. FSH levels also relate to the proportion of seminiferous tubules exhibiting Sertoli cell-only on testicular biopsies [39]. Nevertheless, for patients subjected to sperm retrieval, FSH levels do not precisely predict whether spermatogenesis is present [40]. It is, therefore, possible to find focal sperm-producing areas in the testes of men with NOA-STF and elevated FSH levels during testicular sperm extraction [5][40][41][42][43].

Low FSH levels (e.g., <1.5 mIU/mL), combined with low LH (e.g., <1.5 mIU/mL), and low testosterone levels (e.g., <300 ng/dL) indicate primary or secondary HH [5][11]. In such cases, azoospermia is the result of an absence of testicular stimulation by pituitary gonadotropins. Pharmacotherapy using exogenous gonadotropins is highly effective in inducing sperm production in patients with congenital or acquired HH forms, with reported pregnancy rates of up to 65%, which are achieved naturally or with medically assisted reproduction [6][44].

Typically, patients with NOA-STF present with elevated FSH (>7.6 mIU/mL) and low testosterone (<300 ng/dL) levels, whereas those with OA show normal FSH and testosterone levels. Other hormones can also be assessed, including inhibin B, prolactin, estradiol, 17-hydroxyprogesterone, and sex hormone-binding globulin (SHBG) [11]. In particular, prolactin levels should be measured in patients with HH because prolactinoma may be the causative factor [11]. Inhibin-B levels reflect Sertoli cell integrity and spermatogenesis status [45]. However, its diagnostic value seems to be no better than that of FSH, and its use in clinical practice for azoospermia differentiation or sperm retrieval success prediction has not been broadly advocated [30][40].

2.5. Genetic Analysis

Azoospermia may have a genetic origin. The frequency of numerical autosomal and sex chromosome abnormalities, single-gene mutations, and partial or complete microdeletions of the Y-chromosome is increased in azoospermic patients [12][46]. Indeed, the incidence of genetic abnormalities increases as the sperm output decreases [47][48]. For instance, approximately 15% of men with NOA present with chromosomal anomalies, in contrast to ~5% of those with sperm concentration between 1 and 10 million/mL and <1% of men with >19 million/mL [49].

As a general rule, azoospermic men should undergo karyotype and Y chromosome microdeletion studies [5]. Exceptions apply to conditions in which azoospermia has an evident obstructive origin (e.g., vasectomy, ejaculatory duct obstruction) or a non-genetic-related etiology (e.g., post-chemotherapy/radiotherapy, post-orchitis). Karyotype and Y chromosome microdeletion tests are broadly available and are based on the screening of genomic deoxyribonucleic acid (DNA) taken mainly from peripheral blood samples.

The most common abnormal karyotypic finding in azoospermic men is Klinefelter syndrome (KS), detected in ~10% of cases [12]. Azoospermia in KS men is associated with reduced testicular growth, pre-pubertal degeneration of primordial germ cells, or spermatogenic maturation arrest. For this reason, all azoospermic KS men have NOA-STF. Two karyotypic patterns are typically noticed: non-mosaic (47,XXY; ~85% of cases) and mosaic (47,XXY/46,X; ~15% of cases) [12]. Residual foci of active spermatogenesis is found on microdissection testicular sperm extraction (micro-TESE) in about 30–50% of KS men [12][40][50]. The retrieved sperm may be used for ICSI and generate a healthy child [28][40]. However, KS patients seem to be at an increased risk of having aneuploid gametes, which might increase the chance of producing offspring with a chromosomal abnormality [48]. Although the finding of an extra X chromosome is confirmatory, KS is suspected during the initial workup stages. These patients classically present with extremely small (1–8 mL) testes, gynecomastia (~40% of cases), and hypogonadism (e.g., scanty facial and pubic hair, poor libido, and erectile dysfunction) [5][11][12]. Reduced testosterone levels are commonly noticed (~80% of cases) and are attributed to decreased Leydig cell population due to the small testicular size.

Microdeletions in the long arm of the Y chromosome are the second most common genetic cause of azoospermia [12]. This region aggregates 26 genes involved in spermatogenesis regulation, located in an interval named "AZF" (azoospermia factor); microdeletions at this interval are usually associated with azoospermia [38]. The AZF interval has

three subregions, named AZFa, AZFb, and AZFc, each enclosing vital genes for spermatogenesis control. Approximately 10% of men with NOA-STF have microdeletions within the AZF interval that justify their condition [12][51].

The Y chromosome microdeletion study is based on a multiplex polymerase chain reaction (PCR), which amplifies Y chromosome sequences using specific sequence-tagged site primers [51]. Y-chromosome microdeletion testing allows detecting almost all clinically significant deletions. Hence, it helps identify the male infertility etiology, but it also provides information about treatment prognosis. Sperm retrieval success is determined by the type of Y microdeletion detected. Among men with AZFc deletions, sperm may be occasionally found in the ejaculate, or through testicular sperm extraction in at least 50% of individuals [5][51]. By contrast, patients with complete AZFa and/or AZFb microdeletions are not eligible for surgical sperm retrieval because large deletions involving these subregions are virtually incompatible with any residual spermatogenesis [5].

Notwithstanding the observations above, case reports showed sperm in the ejaculate of men with partial AZFb deletions [52][53]. While the AZFa region is relatively small and contains only two single-copy genes (*USP9Y* and *DDX3Y*), the AZFb and AZFc regions span over several megabase pairs and contain multiple relevant genes [51]. Notably, deletions usually remove more than one gene, but testing as currently performed only determines the presence or absence of a set of primers rather than gene-specific deletions.

Azoospermic patients with AZFc microdeletions in whom testicular sperm are successfully retrieved can father a child through ICSI [5][40][54]. The probability of biological parenthood by ICSI appears to be not affected by the microdeletion. However, the male offspring of such fathers will inherit the genetic defect and, consequently, be infertile [54]. Genetic counseling is, therefore, recommended before sperm retrieval. Preimplantation genetic testing may be proposed for embryo sex selection to couples undergoing ICSI with testicular sperm retrieved from patients with AZFc microdeletions to avoid transmitting this form of infertility to the offspring.

Cystic fibrosis transmembrane conductance regulator gene mutations usually result in CBAVD and, consequently, the affected patients have OA [12][55]. Over 2000 mutations have been discovered in the CFTR gene [56]. About eight out of ten patients with CBAVD harbor two CFTR mutations, usually in compound heterozygosity [57]. CFTR mutations were also implicated in bilateral epididymal obstruction in patients with palpable vasa. According to the 2020 European Association of Urology (EAU) guidelines on sexual and reproductive health, testing for CFTR gene mutations should be recommended for men with infertility and anatomical abnormalities of the vas deferens (unilateral or bilateral vas agenesis) when associated with normal kidneys [30]. In such cases, testing should be carried out in both partners of an infertile couple and has to include common point mutations (e.g., deltaF508, R117H, W1282X) and the 5T allele.

Screening for CFTR mutations is carried out in clinical molecular genetics laboratories. Methods for CFTR testing typically apply semiquantitative PCR analysis (e.g., multiplex ligation-dependent probe amplification) or quantitative fluorescent multiplex PCR [57]. The test report should be interpreted with prudence as not all mutations are implicated in disease. However, findings of mutations with clinical relevance confirm a genetic cause of OA [12]. In such patients, spermatogenesis is preserved, and therefore, sperm are easily retrieved from the testis or epididymis [8][33]. The retrieved sperm have to be used for ICSI, which results in adequate success rates [8][33]. The female partners should be screened for clinically relevant CFTR mutations. If the partner carries a CFTR mutation, the couple has up to a 50% risk of having a child with cystic fibrosis or CBAVD, depending on the parents' type of mutation [11][30]. Preimplantation genetic testing may be offered for embryo sex selection or to identify non-affected embryos.

Given the solid genetic background of NOA, additional genetic analysis beyond karyotyping and screening for Y-chromosome microdeletions has been investigated. Gene panels using whole-exome sequencing have been proposed as a way to detect genetic variants possibly explaining NOA [56][58]. At present, however, these advanced genetic assessments are not entirely validated and therefore not yet suitable for inclusion in the routine investigation.

2.6. Imaging Studies

Imaging studies may add information to help determine the type and cause of azoospermia.

Scrotal ultrasound (US) is useful to detect signs of testicular dysgenesis (e.g., microlithiasis, heterogeneous testis architecture) which are often related to NOA-STF [5]. As a general rule, men with suspected NOA-STF should undergo scrotal ultrasonography because these patients have an increased chance of testicular cancer [30]. A scrotal scan may also help to determine testis volume, epididymis characteristics, and presence of a varicocele if a physical examination is inconclusive (e.g., large hydrocele, inguinal testis, obesity) [11][21]. Additionally, indirect signs of obstruction might be seen during a scrotal US examination, including a dilated rete testis, enlarged epididymis, or absent/partially absent epididymis

in patients with CBAVD [59]. Scrotal color Doppler US findings obtained from healthy fertile men provide reference ranges for clinicians [59][60]. For example, the lowest reference limit for testes volume (measured according to the ellipsoid formula) was about 12 mL, and thresholds for epididymis heal, tail, and vas deferens were 12, 6, and 4.5 mm [59].

Transrectal ultrasound (TRUS) is indicated in azoospermic patients with hypospermia (ejaculate volume < 1.5 mL) and seminal acidic pH if an obstruction is suspected [34]. Using TRUS, seminal vesicle abnormalities and prostatic cysts may be detected [34][59]. These lesions can obstruct the ejaculatory ducts and result in azoospermia [61]. Moreover, the presence of seminal vesical cysts should alert the clinician for possible concomitant genitourinary anomalies, including renal agenesis, dysgenesis, and autosomal dominant polycystic kidney disease [62][63]. Treatment to relieve the obstruction can be offered for these patients [8]. Besides, TRUS can help confirm CBAVD as the seminal vesicles of these patients are either absent or hypoplasic [11][28].

Magnetic resonance may also be used, and it is helpful to assess the distal parts of the seminal tract, the presence of prolactinomas, and an intra-abdominal location of an undescended testis [11]. Lastly, renal imaging studies should be performed in men with anatomical vas deferens abnormalities and no evidence of CFTR mutations. The unilateral absence of the vas deferens is usually associated with an ipsilateral absence of the kidney. Moreover, renal abnormalities (e.g., pelvic kidney) may be found in patients with bilateral absence of vas deferens without CFTR mutations [64].

References

1. Aziz, N. The importance of semen analysis in the context of azoospermia. Clinics 2013, 6 (Suppl. 1), 35–38.
2. Cocuzza, M.; Alvarenga, C.; Pagani, R. The epidemiology and etiology of azoospermia. Clinics 2013, 68 (Suppl. 1), 15–26.
3. Tüttelmann, F.; Werny, F.; Cooper, T.G.; Kliesch, S.; Simoni, M.; Nieschlag, E. Clinical experience with azoospermia: Aetiology and chances for spermatozoa detection upon biopsy. Int. J. Androl. 2011, 34, 291–298.
4. Olesen, I.A.; Andersson, A.M.; Aksglaede, L.; Skakkebaek, N.E.; Rajpert-de Meyts, E.; Joergensen, N.; Juul, A. Clinical, genetic, biochemical, and testicular biopsy findings among 1213 men evaluated for infertility. Fertil. Steril. 2017, 107, 74–82.e7.
5. Esteves, S.C. Clinical management of infertile men with nonobstructive azoospermia. Asian J. Androl. 2015, 17, 459–470.
6. Fraietta, R.; Zylberstejn, D.S.; Esteves, S.C. Hypogonadotropic hypogonadism revisited. Clinics 2013, 68 (Suppl. 1), 81–88.
7. Miyaoka, R.; Esteves, S.C. Predictive factors for sperm retrieval and sperm injection outcomes in obstructive azoospermia: Do etiology, retrieval techniques and gamete source play a role? Clinics 2013, 68 (Suppl. 1), 111–119.
8. Esteves, S.C.; Lee, W.; Benjamin, D.J.; Seol, B.; Verza, S., Jr.; Agarwal, A. Reproductive potential of men with obstructive azoospermia undergoing percutaneous sperm retrieval and intracytoplasmic sperm injection according to the cause of obstruction. J. Urol. 2013, 189, 232–237.
9. Esteves, S.C.; Miyaoka, R.; Agarwal, A. Surgical treatment of male infertility in the era of intracytoplasmic sperm injection—New insights. Clinics 2011, 66, 1463–1478.
10. Miyaoka, R.; Orosz, J.E.; Achermann, A.P.; Esteves, S.C. Methods of surgical sperm extraction and implications for assisted reproductive technology success. Panminerva Med. 2019, 61, 164–177.
11. Esteves, S.C.; Miyaoka, R.; Agarwal, A. An update on the clinical assessment of the infertile male. Clinics 2011, 66, 691–700, Erratum in Clinics 2012, 67, 203.
12. Hamada, A.J.; Esteves, S.C.; Agarwal, A. A comprehensive review of genetics and genetic testing in azoospermia. Clinics 2013, 68 (Suppl. 1), 39–60.
13. Thakker, S.; Persily, J.; Najari, B.B. Kallman syndrome and central non-obstructive azoospermia. Best Pract. Res. Clin. Endocrinol. Metab. 2020, 34, 101475.
14. Patel, A.S.; Leong, J.Y.; Ramos, L.; Ramasamy, R. Testosterone Is a Contraceptive and Should Not Be Used in Men Who Desire Fertility. World J. Men’s Health 2019, 37, 45–54.
15. Boeri, L.; Capogrosso, P.; Ventimiglia, E.; Cazzaniga, W.; Pozzi, E.; Belladelli, F.; Pederzoli, F.; Alfano, M.; Abbate, C.; Montanari, E.; et al. Testicular volume in infertile versus fertile white-European men: A case-control investigation in the real-life setting. Asian J. Androl. 2021.

16. Schoor, R.A.; Elhanbly, S.; Niederberger, C.S.; Ross, L.S. The role of testicular biopsy in the modern management of male infertility. J Urol. 2002, 167, 197–200.
17. Majzoub, A.; Arafa, M.; Khalafalla, K.; AlSaid, S.; Burjaq, H.; Albader, M.; Al-Marzooqi, T.; Esteves, S.C.; Elbardisi, H. Predictive model to estimate the chances of successful sperm retrieval by testicular sperm aspiration in patients with nonobstructive azoospermia. Fertil. Steril. 2021, 115, 373–381.
18. Hung, A.J.; King, P.; Schlegel, P.N. Uniform testicular maturation arrest: A unique subset of men with nonobstructive azoospermia. J. Urol. 2007, 178, 608–612, discussion 612.
19. Jungwirth, A.; Giwercman, A.; Tournaye, H.; Diemer, T.; Kopa, Z.; Dohle, G.; Krausz, C. European Association of Urology Working Group on Male Infertility. European Association of Urology guidelines on Male Infertility: The 2012 update. Eur. Urol. 2012, 62, 324–332.
20. Mieusset, R.; Bieth, E.; Daudin, M.; Isus, F.; Delaunay, B.; Bujan, L.; Monteil, L.; Fauquet, I.; Huyghe, E.; Hamdi, S.M. Male partners of infertile couples with congenital unilateral absence of the vas deferens are mainly non-azoospermic. Andrology 2020, 8, 645–653.
21. Cocuzza, M.S.; Tiseo, B.C.; Srougi, V.; Wood, G.J.A.; Cardoso, J.P.G.F.; Esteves, S.C.; Srougi, M. Diagnostic accuracy of physical examination compared with color Doppler ultrasound in the determination of varicocele diagnosis and grading: Impact of urologists' experience. Andrology 2020, 8, 1160–1166.
22. Miyaoka, R.; Esteves, S.C. A critical appraisal on the role of varicocele in male infertility. Adv. Urol. 2012, 2012, 597495.
23. Lira Neto, F.T.; Roque, M.; Esteves, S.C. Effect of varicocelectomy on sperm deoxyribonucleic acid fragmentation rates in infertile men with clinical varicocele: A systematic review and meta-analysis. Fertil. Steril. 2021.
24. Esteves, S.C.; Miyaoka, R.; Roque, M.; Agarwal, A. Outcome of varicocele repair in men with nonobstructive azoospermia: Systematic review and meta-analysis. Asian J. Androl. 2016, 18, 246–253.
25. Esteves, S.C. Pro: Should Varicocele Be Repaired in Azoospermic Infertile Men? In Varicocele and Male Infertility, 1st ed.; Esteves, S., Cho, C.L., Majzoub, A., Agarwal, A., Eds.; Springer: Cham, Switzerland, 2019.
26. Dabaja, A.A.; Goldstein, M. When is a varicocele repair indicated: The dilemma of hypogonadism and erectile dysfunction? Asian J. Androl. 2016, 18, 213–216.
27. Hamada, A.; Esteves, S.C.; Agarwal, A. Varicocele Classification. In Varicocele and Male Infertility: Current Concepts, Controversies and Consensus; Hamada, A., Esteves, S.C., Agarwal, A., Eds.; SpringerBriefs in Reproductive Biology, Springer International Publishing: Cham, Switzerland; Heidelberg, Germany; New York, NY, USA; London, UK, 2016; pp. 37–43.
28. Esteves, S.C.; Roque, M.; Bedoschi, G.; Haahr, T.; Humaidan, P. Intracytoplasmic sperm injection for male infertility and consequences for offspring. Nat. Rev. Urol. 2018, 15, 535–562.
29. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed.; WHO Press: Geneva, Switzerland, 2010.
30. Salonia, A.; Bettocchi, C.; Carvalho, J.; Corona, G.; Jones, T.H.; Kadioğlu, A.; Martinez-Salamanca, J.I.; Minhas, S.; Serefoğlu, E.C.; Verze, P. European Association of Urology Guidelines on Sexual and Reproductive Health; European Association of Urology, 2020. Available online: https://uroweb.org/wp-content/uploads/EAU-Guidelines-on-Sexual-and-Reproductive-Health-2020.pdf (accessed on 15 May 2021).
31. Esteves, S.C. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. Int. Braz. J. Urol. 2014, 40, 443–453.
32. Schlegel, P.N.; Sigman, M.; Collura, B.; De Jonge, C.J.; Eisenberg, M.L.; Lamb, D.J.; Mulhall, J.P.; Niederberger, C.; Sandlow, J.I.; Sokol, R.Z.; et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. Fertil. Steril. 2021, 115, 54–61.
33. Esteves, S.C. Percutaneous epididymal sperm aspiration as a method for sperm retrieval in men with obstructive azoospermia seeking fertility: Operative and laboratory aspects. Int. Braz. J. Urol. 2015, 41, 817–818.
34. Achermann, A.P.P.; Esteves, S.C. Diagnosis and management of infertility due to ejaculatory duct obstruction: Summary evidence. Int. Braz. J. Urol. 2021, 47, 868–881.
35. Shiraishi, K.; Ohmi, C.; Shimabukuro, T.; Matsuyama, H. Human chorionic gonadotrophin treatment prior to microdissection testicular sperm extraction in non-obstructive azoospermia. Hum. Reprod. 2012, 27, 331–339.
36. Oduwole, O.O.; Peltoketo, H.; Huhtaniemi, I.T. Role of Follicle-Stimulating Hormone in Spermatogenesis. Front. Endocrinol. 2018, 9, 763.
37. Ishikawa, T.; Fujioka, H.; Fujisawa, M. Clinical and hormonal findings in testicular maturation arrest. BJU Int. 2004, 94, 1314–1316.

38. Martin-du-Pan, R.C.; Bischof, P. Increased follicle stimulating hormone in infertile men. Is increased plasma FSH always due to damaged germinal epithelium? Hum. Reprod. 1995, 10, 1940–1945.

39. Bergmann, M.; Behre, H.M.; Nieschlag, E. Serum FSH and testicular morphology in male infertility. Clin. Endocrinol. 1994, 40, 133–136.

40. Arshad, M.A.; Majzoub, A.; Esteves, S.C. Predictors of surgical sperm retrieval in non-obstructive azoospermia: Summary of current literature. Int. Urol. Nephrol. 2020, 52, 2015–2038.

41. Esteves, S.C. Microdissection testicular sperm extraction (micro-TESE) as a sperm acquisition method for men with nonobstructive azoospermia seeking fertility: Operative and laboratory aspects. Int. Braz. J. Urol. 2013, 39, 440–441.

42. Esteves, S.C.; Agarwal, A. Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: Case series and systematic review. Clinics 2013, 68 (Suppl. 1), 141–150.

43. Esteves, S.C.; Ramasamy, R.; Colpi, G.M.; Carvalho, J.F.; Schlegel, P.N. Sperm retrieval rates by micro-TESE versus conventional TESE in men with non-obstructive azoospermia-the assumption of independence in effect sizes might lead to misleading conclusions. Hum. Reprod. Update 2020, 26, 603–605.

44. Behre, H.M. Clinical Use of FSH in Male Infertility. Front. Endocrinol. 2019, 10, 322.

45. Adamopoulos, D.A.; Koukkou, E.G. 'Value of FSH and inhibin-B measurements in the diagnosis of azoospermia'—A clinician's overview. Int. J. Androl. 2010, 33, e109–e113.

46. Krausz, C.; Riera-Escamilla, A. Genetics of male infertility. Nat. Rev. Urol. 2018, 15, 369–384.

47. Kohn, T.P.; Kohn, J.R.; Owen, R.C.; Coward, R.M. The Prevalence of Y-chromosome Microdeletions in Oligozoospermic Men: A Systematic Review and Meta-analysis of European and North American Studies. Eur. Urol. 2019, 76, 626–636.

48. Peña, V.N.; Kohn, T.P.; Herati, A.S. Genetic mutations contributing to non-obstructive azoospermia. Best Pract. Res. Clin. Endocrinol. Metab. 2020, 34, 101479.

49. Liu, J.L.; Peña, V.; Fletcher, S.A.; Kohn, T.P. Genetic testing in male infertility—Reassessing screening thresholds. Curr. Opin. Urol. 2020, 30, 317–323.

50. Corona, G.; Minhas, S.; Giwercman, A.; Bettocchi, C.; Dinkelman-Smit, M.; Dohle, G.; Fusco, F.; Kadioglou, A.; Kliesch, S.; Kopa, Z.; et al. Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: A systematic review and meta-analysis. Hum. Reprod. Update 2019, 25, 733–757.

51. Krausz, C.; Hoefsloot, L.; Simoni, M.; Tüttelmann, F. European Academy of Andrology; European Molecular Genetics Quality Network. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: State-of-the-art 2013. Andrology 2014, 2, 5–19.

52. Kleiman, S.E.; Yogev, L.; Lehavi, O.; Hauser, R.; Botchan, A.; Paz, G.; Yavetz, H.; Gamzu, R. The likelihood of finding mature sperm cells in men with AZFb or AZFb-c deletions: Six new cases and a review of the literature (1994–2010). Fertil. Steril. 2011, 95, 2005–2012.e4.

53. Stouffs, K.; Vloeberghs, V.; Gheldof, A.; Tournaye, H.; Seneca, S. Are AZFb deletions always incompatible with sperm production? Andrology 2017, 5, 691–694.

54. Simoni, M.; Tuüttelmann, F.; Gromoll, J.; Nieschlag, E. Clinical consequences of microdeletions of the Y chromosome: The extended Muünster experience. Reprod. Biomed. Online 2008, 16, 289–303.

55. Bieniek, J.M.; Lapin, C.D.; Jarvi, K.A. Genetics of CFTR and male infertility. Transl. Androl. Urol. 2021, 10, 1391–1400.

56. Cioppi, F.; Rosta, V.; Krausz, C. Genetics of Azoospermia. Int. J. Mol. Sci. 2021, 22, 3264.

57. Dequeker, E.; Stuhrmann, M.; Morris, M.A.; Casals, T.; Castellani, C.; Claustres, M.; Cuppens, H.; des Georges, M.; Ferec, C.; Macek, M.; et al. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders--updated European recommendations. Eur. J. Hum. Genet. 2009, 17, 51–65.

58. Fakhro, K.A.; Elbardisi, H.; Arafa, M.; Robay, A.; Rodriguez-Flores, J.L.; Al-Shakaki, A.; Syed, N.; Mezey, J.G.; Abi Khalil, C.; Malek, J.A.; et al. Point-of-care whole-exome sequencing of idiopathic male infertility. Genet. Med. 2018, 20, 1365–1373.

59. Lotti, F.; Frizza, F.; Balercia, G.; Barbonetti, A.; Behre, H.M.; Calogero, A.E.; Cremers, J.F.; Francavilla, F.; Isidori, A.M.; Kliesch, S.; et al. The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: Scrotal ultrasound reference ranges and associations with clinical, seminal, and biochemical characteristics. Andrology 2021, 9, 559–576.

60. Lotti, F.; Maggi, M. Ultrasound of the male genital tract in relation to male reproductive health. Hum. Reprod. Update 2015, 21, 56–83.

61. Netto, N.R., Jr.; Esteves, S.C.; Neves, P.A. Transurethral resection of partially obstructed ejaculatory ducts: Seminal parameters and pregnancy outcomes according to the etiology of obstruction. J. Urol. 1998, 159, 2048–2053.
62. Kim, B.; Kawashima, A.; Ryu, J.A.; Takahashi, N.; Hartman, R.P.; King, B.F., Jr. Imaging of the seminal vesicle and vas deferens. Radiographics 2009, 29, 1105–1121.
63. Danaci, M.; Akpolat, T.; Başternir, M.; Sarikaya, S.; Akan, H.; Selçuk, M.B.; Cengiz, K. The prevalence of seminal vesicle cysts in autosomal dominant polycystic kidney disease. Nephrol. Dial. Transplant. 1998, 13, 2825–2828.
64. Kolettis, P.N.; Sandlow, J.I. Clinical and genetic features of patients with congenital unilateral absence of the vas deferens. Urology 2002, 60, 1073–1076.