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Review Article

An update on non-obstructive azoospermia; a narrative review

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Abstract:

Azoospermia can stem from either an obstructive issue or a non-obstructive problem originating in the testes. Distinguishing between these two root causes relies on clinical evaluation of testis size and consistency, hormone testing of FSH levels, and genetic analysis looking at chromosomes, Y chromosome microdeletions, and genes involved in hypogonadotropic hypogonadism.

NOA encompasses both primary testicular failure where sperm production is impaired, as well as secondary failure driven by hypothalamic or pituitary dysfunction leading to inadequate gonadotropin levels. The treatment approach for NOA is still largely empirical, lacking definitive evidence-based guidelines.

However, for cases of hypogonadotropic hypogonadism specifically, gonadotropin replacement with hCG and recombinant FSH is the primary established treatment aimed at improving semen quality and increasing chances of conception. GnRH therapy can be added for men who don't respond adequately to gonadotropins alone.

While high-level clinical data is scarce, there are some indications that combining aromatase inhibitors with gonadotropin therapy may enhance outcomes for men requiring surgical sperm retrieval procedures. Overall, this review summarizes the current understanding of the causes, treatments, and clinical management of non-obstructive azoospermia.

Keywords: non-obstructive azoospermia; treatment; fertility

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Introduction:

Azoospermia is diagnosed when at least two ejaculate samples, including the centrifuged portion, completely lack spermatozoa, confirming impaired sperm production or obstruction. The term derives from Greek meaning "without animal sperm/seed". Diagnosis requires confirming absence of sperm across multiple semen analyses of both liquid and centrifuged samples. (1)

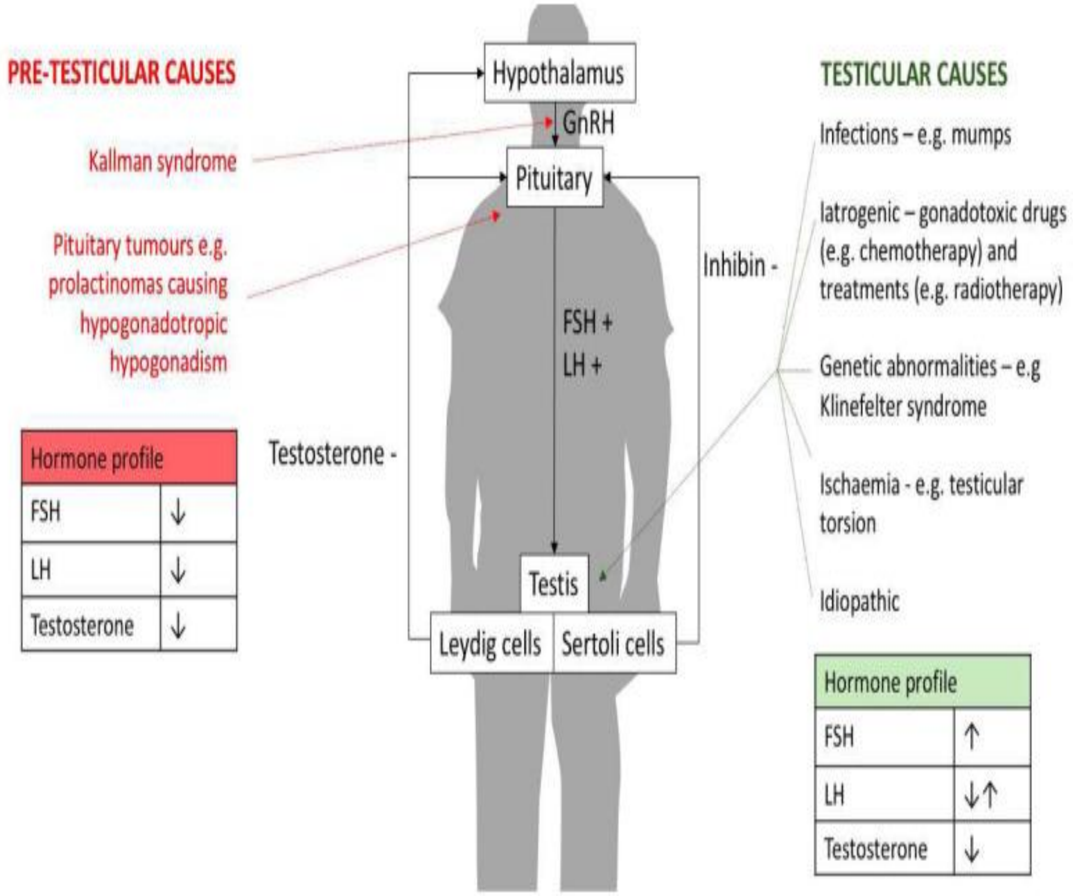
Epidemiology

Infertility affects approximately 15% of reproductive couples. (2) Male factors contribute to 50% of these cases. Azoospermia, defined as the complete absence of spermatozoa in the ejaculate, manifests in 10-15% of infertile mal corresponding to a prevalence of nearly 1% in the general male population **[3]**. The United States has an estimated 600,000 azoospermic males of reproductive age at any given time, with non-obstructive azoospermia

(NOA) being the predominant etiology. [3] In the United Kingdom, one in seven heterosexual couples experiences infertility, and male factor infertility is the primary causative factor, accounting for 30% of cases .[4] Clinically, azoospermia can be classified as obstructive (post-testicular) or non-obstructive (pre-testicular or testicular). Obstructive azoospermia (OA) affects 15-20% of azoospermmeandless prevalent than NOA .[5]

Functional (non-obstructive) azoospermia: NOA is typically considered an incurable condition, affecting up to 10% of infertile males. It results from defective spermatogenesis. The diagnosis is established by the absence of normal spermatogenesis on testicular histopathology or an elevated serum follicle-stimulating hormone (FSH) level, indicating primary testicular failure. .[6] Causes of NOA are shown in Figure .[1]

Figure (1): Non-obstructive azoospermia causes and male reproductive hormone profiles .[7]



A-Hypogonadotropic hypogonadism:

Hypogonadotropic hypogonadism (HH) is characterized by low serum testosterone levels resulting from diminished secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland. HH can have idiopathic, acquired, or congenital etiologies.[6]

I. Congenital Hypogonadotropic Hypogonadism

Congenital hypogonadotropic hypogonadism (CHH) is an uncommon hereditary disorder caused by gonadotropin-releasing hormone (GnRH) deficiency. It manifests as infertility and absent or delayed onset of puberty. [8] Syndromic forms of congenital HH include Prader-Willi syndrome, Kallmann syndrome, and Laurence-Moon syndrome [6] **Table (1)**.

Table (1): Congenital Hypogonadotropic Hypogonadism syndromes.

Kallmann syndrome	Prader-Willi syndrome	Laurence-Moon syndrome
Congenital form of hypogonadotropic hypogonadism causing hypo/anosmia, failure of GnRH neurons to migrate from olfactory mucosa to hypothalamus, low sex steroids due to GnRH deficiency, Lack of sexual maturation and secondary sexual characteristics. Symptoms: lack of testicular growth, infantile voice, absent pubic hair, may present with Micropenis, cryptorchidism and associated with low FSH, LH, and testosterone levels [14; 8).	Prader-Willi syndrome (PWS) is a rare, complex genetic disorder that impacts multiple neurological, endocrine, and metabolic systems, leading to impaired behavior and cognition. Most cases are sporadic, but familial PWS can occur due to a paternal microdeletion in the imprinted genomic region inherited from the mother .[11; 12] Clinical manifestations in early infancy include severe hypotonia, poor appetite, and feeding difficulties. In childhood, hyperphagia and progressive morbid obesity develop unless food intake is strictly controlled. Delayed attainment of motor and language developmental milestones is observed. Varying degrees of cognitive impairment are present. Hypogonadism, characterized by genital hypoplasia, incomplete pubertal development, and infertility in the majority, affects both sexes. Short stature is common if not treated with growth hormone therapy. Behavioral issues such as temper outbursts, stubbornness, manipulative conduct, and obsessive-compulsive traits are typical. Additional symptoms include scoliosis, kyphosis, osteoporosis, hypopigmentation, viscous and thick saliva, heightened pain sensitivity, decreased vomiting, and temperature dysregulation. Characteristic facial features, including strabismus, are frequently present .[13]	Rare ciliopathic, autosomal recessive disorder. Primarily affects offspring of consanguineous couples. Initial symptom: poor night vision in first decade of life .[9] Diagnostic criteria: 4 primary features or 3 majors plus 2 additional features. Secondary features: speech difficulties, polyuria, ataxia, diabetes, developmental delay, cardiac hypertrophy, brachydactyly, hepatic fibrosis, spasticity, hearing loss. Primary features: cone-rod dystrophy, polydactyly, obesity, learning disabilities, renal anomalies, associated with short stature, crowded teeth, hypermobile joints and early osteoarthritis. [10]

II-Acquired Hypogonadotropic Hypogonadism:

Acquired causes of hypogonadotropic hypogonadism (HH) include medications (GnRH analogs, sex steroids), pituitary/brain radiation, hyperprolactinemia, strenuous exercise, pituitary lesions (infiltrative or infectious), traumatic brain injury, and substance abuse (alcohol, illicit drugs) . [15]

Table (2): Genetic causes of Hypergonadotropic Hypogonadism/ Eugonadism

1-Klinefelter's Syndrome	2-XX males	3-Myotonic Dystrophy (DM):
A phenotypical guy with two or more extra X chromosomes will have Klinefelter syndrome (KS). Gynecomastia in males, tiny testes, tall stature and azoospermia were the first to be identified as the clinical presentation of KS, and the genetic cause of extra X chromosomes was discovered in 1959 [22] Excess X chromosomes contribute to testicular hyalinization, fibrosis, and hypofunction, leading to genital diseases primarily characterized by hypogonadism and infertility. Neurocognitive impairments associated with KS began to be understood in the latter half of the twentieth century. Management of KS often involves testosterone replacement therapy, cognitive interventions, and adaptable therapies, yielding favorable outcomes. [23-25] The predominant KS karyotype is 47 XXY, accounting for over 90% of cases, with mosaic karyotypes like 46 XY/47 XXY and diverse aneuploidies like 48 XXXY and 49 XXXXY also reported. Acquisition of the extra X chromosome typically occurs randomly through post-zygotic or meiotic nondisjunction, with the severity of the condition often linked to the amount of additional X chromosomal material present. [23; 26] In KS patients, a low upper/lower segment ratio is common due to their tall stature and elongated limbs. Weight and head circumference typically fall within the 50th percentile, while mean height is typically in the 75th percentile. During childhood, the phallus and testicles may be relatively small, with pubic and phallic hair growth common during adolescence. However, testicular size remains small, usually below 4 mL, and is often firm due to hyalinization and fibrosis. Testosterone levels typically range from low to low-normal, with gynecomastia being a prevalent feature [23; 26]	The XX male syndrome, occurring in approximately 1 in 20,000 live births, results from a crossover event between the X and Y chromosomes outside the pseudo autosomal region during Meiosis I.This crossover leads to the transfer of the Y chromosome's sex-determining region (SRY) to the X chromosome, thereby inducing testicular development instead of ovarian development. Individuals with this syndrome typically exhibit low to normal levels of testosterone, elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and normal external and internal genitalia. Despite their normal appearance, they are infertile due to the absence of the Y chromosome's azoospermia factor (AZF) region, which is crucial for spermatogenesis and renders them incapable of producing sperm .[21]	Myotonic dystrophy, an autosomal dominant multisystem disorder, presents in two main forms: type 1 (DM1), also known as Steinert disease, and type 2, also known as proximal myotonic myopathy. DM1 affects various body systems including the central nervous system, heart, eyes, skeletal/smooth muscles, and endocrine system. [16] The phenotypic manifestations of myotonic dystrophy are categorized into congenital, classic, and mild forms. The predominant symptoms of DM2 include myotonia (90%), muscular dysfunction (82%), along with cataracts (36%), and cardiac conduction abnormalities (19%) [17] Reproductive abnormalities are a common feature across all forms of myotonic dystrophy. A key characteristic of both DM1 and DM2 is progressive testicular atrophy, affecting up to 60%-80% of cases respectively. Histological abnormalities such as seminiferous tubule fibrosis, hyalinization, and atrophy are observed. Approximately 73% of DM1 patients report oligospermia or azoospermia, with small testes being a prominent physical indicator of gonadal dysfunction. Elevated levels of FSH and LH, coupled with decreased testosterone levels, are typically observed in affected individuals .[18-20]

4-Inherited disorders of LH and FSH	5-LH and FSH resistance
Mutations in the gonadotropin α-chain are unknown in humans. Mutations in the LH and FSH chains have been reported. In diseases caused by these mutations, low levels of testosterone are commonly accompanied by high levels of one or both gonadotropins, which may be a sign of hypergonadotropic hypogonadism. The afflicted man exhibited slowed spermatogenesis, low testosterone, and delayed puberty; one LH mutation has been found. This patient has a missense mutation in the LH beta gene. Although the hormone was no longer able to bind to its receptor, the mutation nonetheless permitted hormone production and immunoreactivity. [20] The patient's LH level was therefore elevated but his FSH level was normal, according to radioimmunoassay results. The patient's infertility remained despite the HCG therapy's effects on the patient's testosterone levels, testicular size, virilization, and sperm count. At age 44, all of his gonadotropin levels were elevated .[20]	The LH and FSH receptors belong to the seven transmembrane domains G protein-coupled family of receptors. The failure of the cyclic AMP-regulated receptor activation cascade or the receptor's inability to bind ligands is two potential effects of mutations in the LH and FSH receptors (cAMP). LH resistance, which is caused by LH receptor-inactivating mutations, is a relatively unusual form of hypergonadotropic hypogonadism. .[20] The syndrome's most severe symptoms include male pseudo-hermaphroditism, feminine or ambiguous genitalia, low testosterone levels, high LH levels, the absence of male secondary sexual characteristics, and a lack of response to HCG or LH challenge. [20]

Developmental causes:

Cryptorchidism, the congenital absence of one or both testes from the scrotum, is the most common male genital anomaly. It affects approximately 3% of full-term and 30% of preterm male neonates. Testicular descent typically occurs by the 7th month of gestation, with spontaneous descent occurring in around 80% of cryptorchid cases by 3 months after birth. The actual incidence is about 1%, as natural descent is improbable after 6 months, necessitating surgical intervention .[27; 28]

Cryptorchidism is the leading cause of non-obstructive azoospermia (NOA). Unilateral cryptorchidism results in azoospermia in 13% of cases, while untreated bilateral cryptorchidism leads to azoospermia in 89% of patients. [29; 30]

III- Acquired causes:

Chemotherapy:

The testes are more susceptible than ovaries to radiation or chemotherapy-induced damage. Leydig cells are less affected than the germinal epithelium. Patients may exhibit azoospermia, elevated LH and FSH, and testicular injury on pathology, with normal testosterone levels. Testicular damage is often dose-dependent. [20; 31]

Radiotherapy:

The testis is highly radiosensitive, and even low doses can significantly impact its function, either through direct radiation or scattered radiation during treatment of nearby tissues. Younger testicular cells are more vulnerable. Spermatogenesis recovery depends on the condition of type A spermatogonia and the absorbed radiation dose. Leydig cells are more resistant than the germinal epithelium, but high radiation doses can still cause damage.[32; 33]

Infections:

Viral orchitis is typically caused by lymphocytic choriomeningitis virus, echovirus, mumps, and group B arbovirus. [34] Mumps orchitis incidence has decreased with vaccination. Over 50% of post-pubertal mumps cases risk infertility due to associated orchitis .[35] Acute orchitis causes testicular inflammation, pain, swelling, and potential atrophy or recovery. Leprosy can also lead to orchitis and gonadal insufficiency.[20; 33; 36]

Trauma/Torsion:

Blunt scrotal trauma can cause testicular rupture, intratesticular hematoma, tissue damage, and anti-sperm antibody production due to blood-germinal epithelial barrier breach. Testicular torsion affects 1 in 4000 males under 25 and can lead to ischemic necrosis, long-term damage, testicular atrophy if not treated within 6 hours .[37; 38] Additionally, following this might be the development of sperm antibodies that harm the testes. [38]

Varicocele:

A varicocele is an enlargement of the testicular pampiniform or cremasteric plexus, affecting 4-14% of NOA men .[39; 40] It impairs both steroidogenic and spermatogenic testicular functions. Left varicocele increases testicular temperature, negatively affecting Leydig cell secretory function and potentially causing low peripheral testosterone levels in some men. [41-43] .[44] Androgens play a crucial role in regulating Sertoli cell secretory activity and completing spermiogenesis. Varicocele may also lead to testicular hypoxia due to impaired venous drainage and directly impact Sertoli cell function and structure. [45-49]

Diagnosis:

History and clinical examination:

The diagnosis of azoospermia is supported when two separate semen analyses, including examination of the centrifuged sediment, demonstrate a complete absence of spermatozoa. The primary objective in evaluating azoospermic patients is differentiating obstructive versus non-obstructive etiologies. A core concept of obstructive azoospermia (OA) is preservation of natural testicular processes like spermatogenesis and testosterone production, a detailed medical history and physical examination provide information on the state of testicular function. The patient interview should explore the reproductive history, risk factors for obstruction, and any female partner concerns. Further analysis of symptoms related to the hypothalamic-pituitary-testicular (HPT) axis status is important, as hypogonadism is often incompatible with OA. [50]

The history should evaluate prior sexually transmitted infections, tuberculosis exposure, and conditions that may indicate an underlying ciliary disorder or cystic fibrosis. A history of chemotherapy, undescended testicles, or medications that can impact spermatogenesis may suggest non-obstructive etiology. [51; 52]

A physical examination aids in identifying obstructive etiology. Normal testicular volume (>15 ml) is expected in OA patients. Scrotal and inguinal regions should be examined for surgical scars, with palpation of spermatic cord and epididymis during genital examination. Congenital absence of vasa deferentia, seen in 2% of infertile males, may indicate anatomical differences. Missing excurrent duct segments warrant further CFTR gene testing. Digital rectal examination detects midline cysts or SV fullness, linked to EDO. [53; 54]

Tanner phases assess secondary sexual traits' development. Poor pubic hair or genital development signals hypogonadism. Varicocele, a common disorder, may be revealed during examination. Examination should be done lying down and standing, visually inspecting and palpating the scrotum. Varicocele may lead to spermatogenesis dysfunction or azoospermia, hence its importance in NOA diagnosis [55). (56-58].

Laboratory and genetic testing:

Azoospermia diagnosis entails ejaculate analysis, with at least 2 samples for precision. Semen centrifugation should be done confirms sperm absence. Microscopic inspection may reveal sperm in initially labeled NOA patients (Schlegel, 2004; Ron-El et al., 1997).

Hormonal analysis aids NOA diagnosis; high gonadotropin levels suggest primary testicular failure. Intramuscular HCG administration helps distinguish anorchia from cryptorchidism, in cryptorchid men, there should then be a spike in plasma testosterone. There won't be an increase in testosterone in anorchid guys [59; 60).

Additionally, anti-Mullerian factor hormone (AMH) in anorchid men is undetectable during infancy. Therefore, the existence of testicular tissue in prepubertal men is indicated by measurable levels of AMH [61]. Radiographic **Assessment:**

Diagnosing NOA involves assessing testicular volume using ultrasonography or an orchidometer. Reduced testicular size indicates spermatogenesis failure, often below 15 cc with a flat epididymis. Ultrasonography aids in volume assessment and understanding testicular pathophysiology .[62; 39] .[63]

Testicular microlithiasis, indicative of spermatogenesis failure, may occur in those with testicular dysgenesis syndrome (TDS). Testicular microlithiasis isn't directly linked to testicular cancer, contrary to previous speculation. Follow-up ultrasound is advised for identified risk factors, including testicular atrophy and history of germ cell tumors. Suspicion of testicular cancer warrants further tests like tumor markers, MRIs, or orchidectomies .[64; 58]

In azoospermic men with palpable vasa differentia, normal-sized testes, normal FSH levels, and negative anti-sperm antibody test, open testicular biopsy may distinguish OA from NOA. Biopsy solutions like collidine-buffered glutaraldehyde or Bouin are preferred over formaldehyde to preserve testicular architecture. [65]

While testicular sperm may have higher implantation rates, TESE might not always be necessary for ICSI. Typically, biopsy isn't required due to accurate diagnosis through total testicular volume, LH, and FSH measurement. [59; 66].

Additional investigations for NOA patients: Additional tests like Karyotyping and genetic testing need to be done when NOA is diagnosed [67].

Additional investigations for NOA include karyotyping and genetic testing. AZF sub-region analysis on the Y chromosome helps manage NOA, with Yq microdeletions found in about 8% of Western NOA patients. A new genetic tool aids Y-chromosome deletion evaluation in Japanese patients [68; 69].

Treatment:

With the advancement of assisted reproductive technologies, infertile couples now have several ways to grow their family biologically.

Non-obstructive azoospermia:

Non-obstructive azoospermia (NOA) poses significant barriers to fertility, often necessitating advanced assisted reproductive techniques like intracytoplasmic sperm injection (ICSI) with microdissection testicular sperm extraction (micro-TESE). Underlying etiologies contribute to high micro-TESE failure rates, averaging 50-75% despite quotes of up to 75% success. Repeated micro-TESE attempts may be reasonable. Chromosomal abnormalities and sperm DNA damage are prevalent in NOA, with implications for offspring .[70] Iatrogenic causes like exogenous testosterone are often reversible by cessation of therapy and expectant management, with most men recovering baseline sperm counts within 1-2 years .[71-73]

For pre-testicular (e.g. secondary hypogonadism) or hypogonadotropic hypogonadism (HH) cases, gonadotropin regimens combining human chorionic gonadotropin (hCG) and follicle-stimulating hormone (FSH) aim to initiate spermatogenesis. The suggested course of treatment involves injecting HCG (3,000 IU to 10,000 IU) two to three times a week in addition to anastrozole, clomiphene citrate, FSH, or tamoxifen. Up to 6 months may be required, with 75-77% demonstrate return of sperm Gonadotropin releasing hormone (GnRH) infusion pumps show comparable results. Limited evidence supports adjunct estrogen antagonists/aromatase inhibitors. Testosterone is contraindicated.. [74; 73]

Even though it has been shown that up to 11% of azoospermic men who underwent hormone therapy (typically clomiphene) benefit from having sperm in their ejaculate, there is no standardization of this medicine and no high-quality randomized trials. Because of this, many medical professionals including the European Association of Urology (EAU) advise against utilizing hormone therapy in males with primary hypogonadism and NOA in general When possible, microscopic testicular sperm extraction and ICSI are the major treatments for these men. The overall success rate of these expensive procedures in producing a pregnancy is only 25%.

The role of gonadotropins, estrogen receptor modulators, and aromatase inhibitors in males with primary hypogonadism and NOA is even more controversial. These are frequently used to enhance sperm parameters in oligozoospermia men who are infertile, and there is some evidence to support their effectiveness .[76-78] However, their effectiveness in improving sperm retrieval rates through TESE or TESA is somewhat uncertain and has not been definitively proven .[75] Although a progressive strategy starting with clomiphene and rising to HCG has been proposed, the ideal procedure and dose schedule are still to be found .[78] There can be unforeseen complications to the therapy. Nevertheless, despite these disadvantages and the lack of a better course of treatment, hormone stimulation therapy is nevertheless often utilized in clinical settings. [79]

Treatment for HH has a reasonable chance of success. Azoospermic men with HH are given 5 mcg to 20 mcg of GnRH every 2 hours via a pulsatile infusion pump. After 12 to 24 months of treatment, 77% of men who were azoospermic at first were found to have spermatogenesis; the recovery of sperm in the semen was often seen after 6 months of therapy. [80]

The FSH stimulation appears to improve the results prior to GnRH treatment. GnRH is only active in men with normal pituitary function. Gonadotropin therapy with HCG (with or without FSH) is advised in patients with reduced or absent pituitary function. 1000 IU to 3000 IU is the recommended dosage, to be taken two or three times each week. This normally leads to sperm production after three or six months. If that doesn't work, FSH is added at a level of 75 IU to 150 IU twice a week .[81] Overall success rates for spermatogenesis treated medically for HH are about 75%. If medical treatment is not successful, assisted reproductive techniques are recommended .[82; 79]

Men with NOA are three times more likely than infertile men without azoospermia to develop a malignancy in the future, and they are more likely to have pituitary prolactinomas, various neoplasms (such as Sertoli cell, Leydig cell, and germ-cell tumors), and other health-related conditions .[83]

Conclusion:

Testis consistency/volume, laboratory testing (FSH), and genetic testing are used to distinguish patients with NOA (which encompasses primary and secondary testicular failure) from OA. The treatment of NOA is still empirical. For hypogonadotropic hypogonadism, gonadotropin therapy is the only particular indication that consistently improves semen analysis and rates of conception. The typical treatment consists of gonadotropins (hCG and rFSH) combined, with GnRH therapy maintained for non-responders. Although there is a paucity of level I clinical data, drug therapy combining aromatase inhibitors and gonadotropins may be able to improve outcomes for men who need surgical sperm retrieval. Varicocelectomy may be helpful when varicocele is present together with NOA.

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