Asian Journal of Andrology (2022) 24, 274–286
www.asiaandro.com; www.ajandrology.com

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REVIEW

Male Infertility

Endocrine aberrations of human nonobstructive azoospermia

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Nonobstructive azoospermia (NOA) refers to the failure of spermatogenesis, which affects approximately 1% of the male population and contributes to 10% of male infertility. NOA has an underlying basis of endocrine imbalances since proper human spermatogenesis relies on complex regulation and cooperation of multiple hormones. A better understanding of subtle hormonal disturbances in NOA would help design and improve hormone therapies with reduced risk in human fertility clinics. The purpose of this review is to summarize the research on the endocrinological aspects of NOA, especially the hormones involved in hypothalamic–pituitary–testis axis (HPTA), including gonadotropin-releasing hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, testosterone, estradiol, sex hormone binding globulin, inhibin B, anti-Müllerian hormone, and leptin. For the NOA men associated with primary testicular failure, the quality of currently available evidence has not been sufficient enough to recommend any general hormone optimization therapy. Some other NOA patients, especially those with hypogonadotropic hypogonadism, could be treated with hormonal replacement. Although these approaches have succeeded in resuming the fertility in many NOA patients, the prudent strategies should be applied in individuals according to specific NOA etiology by balancing fertility benefits and potential risks. This review also discusses how NOA can be induced by immunization against hormones.

Asian Journal of Andrology (2022) **24**, 274–286; doi: 10.4103/aja202181; published online: 14 January 2022

Keywords: endocrine; follicle-stimulating hormone; gonadotropin-releasing hormone; inhibin B; nonobstructive azoospermia; prolactin; sperm retrieval; testosterone

INTRODUCTION

With the progress of human reproductive function evaluation, the responsibility of male factor has been rising to contribute to infertility in recent years. Azoospermia refers to the condition of a man whose semen contains no sperm. It has been the most challenging topic associated with infertility treatment.[1] Azoospermia could be posttesticular, which is caused by genital tract obstruction. Therefore, this condition is called obstructive azoospermia (OA). In contrast, nonobstructive azoospermia (NOA) could be either pretesticular, caused by impaired hormone production for spermatogenesis, or primary testicular failure caused by any abnormalities in the function or structure of the testicles. Besides, a genetic classification was also suggested.[2] Approximately, NOA affects 1% of the general population and 10%–20% of infertile men worldwide.[3–5] Testicular biopsy is the only definitive diagnostic method to distinguish OA and NOA.

There are numerous causes of NOA, either congenital or acquired. Acquired causes include radiation and toxins, medications, hormone imbalances, varicocele, and lifestyle. Accumulating studies have developed a number of genetic factors contributing to NOA, including Y chromosome deletion, azoospermia factor (AZF) subdivision, gene mutations of *BRD7*, *CFTR*, *BRCA2*, *MLH*, and *TEX*, Klinefelter syndrome (KS), Kallmann syndrome, and Prader–Willi syndrome.[6–10] Recently, by single-cell RNA sequencing, testicular cell expression patterns and network analysis of genes were developed to identify a number of genes related to the pathogenesis of azoospermia.[11] A retrospective analysis reviewed 1583 azoospermic patients and found 21% genetic causes (14% KS, 1% other chromosomal aberrations, 2% Y-chromosomal microdeletions, 1% hypogonadotropic hypogonadism [HH], and 3% congenital bilateral absence of the vas deferens), 31% current or former maldescended testes, varicocele, and urogenital infections, 15% malignancies, 11% obstructions, 7% endocrine or other chronic diseases, and 12% idiopathic azoospermia.[12] Another retrospective study about etiology of azoospermia in patients in an American military population between 2004 and 2012 reviewed 139 outpatient records with azoospermia and found that NOA was diagnosed in 71% men, including 34% identified with Sertoli cell only syndrome (SCOS), and other etiologies included an idiopathic cause in 26% cases, 9% KS, 9% maturation arrest (MA), 5% Y chromosome microdeletion, 4% cryptorchidism, 4% trauma, and 4% exogenous testosterone supplementation.[13] These studies indicate the big variety of NOA and origins. Obesity indicated by body mass index (BMI) could be associated with the incidence of NOA.[14]

It is well known that hypothalamic–pituitary–gonadal axis (HPGA) plays a vital role in human spermatogenesis. Briefly, the hypothalamus begins secreting high pulses of gonadotropin-releasing hormone (GnRH) in GnRH-expressing neurons at the onset of puberty. In response, the anterior portion of the pituitary gland releases follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH enters the testes, stimulating the SCs, which help nourish the sperm cells that the testes produce, to begin facilitating

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Received: 07 June 2021; Accepted: 17 October 2021

spermatogenesis. LH also enters the testes, stimulating Leydig cells, to make and release testosterone (TT) into the testes and the blood. TT stimulates spermatogenesis. Any disorder of HPGA could result in abnormal spermatogenesis and even azoospermia. Understanding the endocrine basis of NOA helps develop the potential therapies for the patients. Clinically, hormone analysis is critical to confirm, evaluate, and manage NOA. It was reported that incidence of hypogonadism increased from 12%, 20%, 30% to 50% approximately in men's 50s, 60s, 70s to 80s, respectively.[15] Some forms of NOA have become amenable to medical treatment after identifying their hormone profile. In recent years, testicular sperm extraction (TESE) and microdissection TESE (microTESE) have been widely used at fertility clinics for sperm retrieval. To predict the sperm retrieval outcome (SRO) and minimize unnecessary invasive biopsy, serum hormonal and/or seminal markers have been developed.[16–22] Alternative managements for NOA include using antioxidants or bioactive compounds to scavenge and halt the production of ROS or neutralize their actions. They include vitamins A, C, and B_{12}, tocopherol, beta-carotene, coenzyme Q, glutathione, carnitine, arginine, selenium, zinc, and plant extracts.[23]

GnRH

GnRH is a decapeptide produced in the hypothalamus and travels to the pituitary where stimulates the production of FSH and LH, which act on the testes to begin spermatogenesis and to develop secondary sex characteristics in the male. Therefore, GnRH is the central neuroendocrine regulator of reproductive function. GnRH is responsible for a series of human spermatogenesis, including spermatogonia proliferation, spermatogenesis progression, and germ cell apoptosis.

Human GnRH has two isoforms, GnRH1 and GnRH2, and both N-terminal and C-terminal are conserved. GnRH1 is the primary isoform with amino acid sequence of pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, or QHWSYGLRPG.[24,25] It was reported that pulsatile release of LH and FSH can be dissociated, so there could be a separate FSH-releasing hormone (FSHRH) and LH-releasing hormone (LHRH), which is commonly called as GnRH, and the physical hypothalamic areas controlling LH and FSH are also separable.[26,27] It is estimated that humans have 1000 to 1500 GnRH neurons. The co-location of GnRH neurons with other central regulators allows the GnRH network to be influenced by a range of neuroendocrine and metabolic inputs.[28] In addition, GnRH is present in human semen, and human spermatogenic cells and mature spermatozoa express the GnRH receptor, which indicates that there may be GnRH autocrine and paracrine roles in the testis that are involved in regulating the production and maturation of spermatozoa.[29]

Since GnRH stimulates the production of FSH and LH, a disorder of GnRH biosynthesis or secretion may disturb gonadotropin profiles and then cause NOA. GnRH receptor (GnRHR) mediates the action of GnRH to stimulate the secretion of LH and FSH by association with G-proteins that activate a phosphatidylinositol-calcium second messenger system. Therefore, GnRHR disorder may cause similar consequence. The two broad categories responsible for NOA are hypogonadotropic hypogonadism (HH) and hypergonadotropic hypogonadism or eugonadism.[30] HH accounts for approximately 1%–2% of cases of male factor infertility and 45% of NOA patients.[31] HH may be a consequence of congenital such as Kallmann syndrome (hypothalamic GnRH deficiency) or acquired factors that affect the hypothalamus and/or the pituitary gland. Mutations of *KAL1*, *FGFR1*, and *GNRHR* (autosomal recessive) are considered to be associated with congenital HH, but the etiology remains unknown in approximately 70% of patients.[32] In contrast, HH is less common but may also jeopardize spermatogenesis by inhibiting the hypothalamic GnRH secretion and inhibiting the binding of LH to the Leydig cells in the testis. Most of hypergonadotropic hypogonadism are congenital due to genetic abnormalities but could be acquired such as varicocele and orchitis.

Based on hormone analysis and diagnosis of NOA noted above, GnRH administration has become a potential therapy for NOA patients. GnRH treatment was reported as an effective therapy for HH.[33] In an earlier study, 100 μg GnRH was administered intravenously to 8 azoospermic men, and the results showed that the serum LH levels peaked at 30 min after GnRH injection (76.1 mIU ml^{-1}), and the azoospermic men had higher serum FSH levels from 8.8 mIU ml^{-1} to 33.9 mIU ml^{-1},[34] which indicated the potential of GnRH therapy. Blumenfeld *et al.*[35] demonstrated that two HH azoospermic men administrated with GnRH resumed functional spermatogenesis. Specifically, a portable GnRH pump connecting to each patient administered 5–20 μg of GnRH intravenously every 89 min. Spermatogenesis was first detected after 42 and 78 days, respectively, and 4.5 months from the start of treatment, their wives became pregnant.[35] Further, they investigated GnRH antibody formation after such injection to five HH men and found that anti-GnRH antibodies were detected in one patient and that intravenous pulsatile GnRH treatment was superior to subcutaneous GnRH treatment and to human menopausal gonadotropin (hMG)/human chorionic gonadotropin (hCG) treatment.[36]

On the other hand, NOA can be accidentally or purposely induced from normal spermatogenesis. Drug use for leisure or disease treatment, chemotherapy, and radiotherapy may impair spermatogenesis and even cause temporary, long-term, or permanent azoospermia in male patients.[37,38] Azoospermia may also be induced, reversibly or irreversibly, for the purpose of contraception or as a result of transgender. A study in monkeys showed that GnRH antagonist (GnRHα) treatment induced azoospermia after 9-week continuous infusion via osmotic mini-pumps, and the endocrine parameters returned to normal within 2 weeks of termination of treatment. Seminiferous tubule function was restored 14 to 18 weeks after treatment, as indicated by normal ejaculate parameters.[39] This study indicated the potential of GnRH antagonists in contraception. Indeed, it was reported that GnRHα predictably and reversibly induced azoospermia in human beings.[40–42] Interestingly, a study used GnRHα to reset gonadotropin profile in 35 NOA men with negative SRO after TESE, and the method improved the spermatogenesis through restoring the sensitivity of SCs and Leydig cells.[43] Immunization against GnRH can also arrest spermatogenesis and induce azoospermia in some animal models[44,45] but not in others.[46]

In summary, GnRH is a key neuroendocrine regulator of spermatogenesis reproductive function. A disorder of GnRH biosynthesis or secretion may cause NOA, so as GnRHR. GnRH can be used to treat NOA patients with HH, and NOA can also be induced by GnRHα treatment.

FSH AND LH

As two pituitary gonadotropins, FSH and LH are the key endocrine stimuli for, and play pivotal roles in, human spermatogenesis. Specifically, the testicular target cells of FSH are the SCs present in the seminiferous tubules, and FSH improves the proliferation, maturation, and function of SCs, which produce regulatory molecules and nutrients needed for spermatogenesis.[47] Mutation of gene encoding FSH beta-ligand may cause azoospermia. The testicular target cells of

LH are Leydig cells in the interstitial space, and LH stimulates Leydig cell to produce TT. Serum FSH and LH levels in normozoospermic men range 2–7 IU l^{-1} and 1.8–8.6 IU l^{-1}, respectively. As men age, both increased.[48] Similarly, another study found in 37 healthy men at the age of 50–104 years reported that serum FSH and LH levels were stable up to 70 years, increased up to 75–85 years, and thereafter gradually decreased.[49]

An elevated serum FSH level (>7.6 IU l^{-1}) and a normal or low serum TT concentration in a patient with bilateral testicular atrophy imply primary testicular failure.[50] As noted above, HH in men with gonadotropin deficiency or dysfunction could be a result of disease or damage to HPGA and a well-known cause of azoospermia. A study examined serum FSH and LH levels of 239 patients with idiopathic NOA at ages of 20s, 30s, and >40s; both serum FSH (23.3, 27.9, and 28.7 IU l^{-1}, respectively) and LH levels (5.8, 6.9, and 7.2 IU l^{-1}, respectively) tended to increase with aging NOA patients.[51] Another study with 52 azoospermic patients at the age range of 29–36 years (mean: 31 years) found that human serum FSH and LH levels were 9.0 and 5.6 IU l^{-1}, respectively.[52] A study found that serum FSH and LH levels of 84 NOA men were 4.2 IU l^{-1} and 5.4 IU l^{-1}, respectively.[53] Ortac *et al.*[54] analyzed serum FSH and LH levels of 112 azoospermic patients with idiopathic HH with an average age of 28 years and found that the baseline FSH and LH levels were 0.6 IU l^{-1} and 0.5 IU l^{-1}, respectively. The value gaps could be due to the difference of measuring protocols and these studies did not compare the values to normozoospermic men.

Many studies have directly compared the serum FSH and LH levels between NOA patients and OA or fertile men. Schoor *et al.*[55] found that the serum FSH levels in NOA patients (16.9 mIU l^{-1}, n = 54) were significantly higher than those in OA patients (5.3 mIU l^{-1}, n = 97). Combined with testis size (long axis <4.6 cm), serum FSH can be used to distinguish NOA men from OA patients. In contrast, serum LH levels (7.1 *vs* 4.1 mIU l^{-1}) proved a weaker predictor (**Table 1**). Another study reported the similar results of serum FSH in NOA patients (20.1 IU l^{-1}, n = 40) and OA patients (8.5 IU l^{-1}, n = 80).[56] Liu *et al.*[57] analyzed serum FSH and LH levels between NOA patients (n = 286) and age-matched fertile men (n = 100) and found that NOA patients had dramatically higher levels of FSH (16.7 *vs* 6.0 IU l^{-1}) and LH (8.5 *vs* 4.7 IU l^{-1}) than fertile men. Among NOA patients, SCOS men (n = 199) had higher serum FSH levels (19.2 IU l^{-1}) than those with MA (11.9 IU l^{-1}, n = 48) and hypospermatogenesis (HS) men (9.3 IU l^{-1}), while serum LH levels showed a bit different pattern (8.9, 9.2, and 5.4 IU l^{-1}, respectively). Interestingly, HS patients caused significantly higher sperm retrieval rate (SRR; 89.7%) than that of SCOS (18.1%) and MA men (20.8%).[57] Huang *et al.*[20] compared serum FSH and LH levels between OA (n = 51) and NOA patients (n = 156) and found that NOA patients had elevated levels of both FSH (25.4 *vs* 5.6 IU l^{-1}) and LH (11.6 *vs* 3.7 IU l^{-1}).

A number of studies have demonstrated that SRO-negative patients have higher serum FSH levels than SRO-positive ones in NOA men, and a FSH cutoff is even provided as a noninvasive predictor before biopsy. For instance, Chen *et al.*[16] found that SRO-negative patients (28.0 IU l^{-1}, n = 108) had dramatically higher FSH levels than SRO-positive counterparts (7.9 IU l^{-1}, n = 98), and the FSH cutoff value was 19.4 IU l^{-1}. Zhu *et al.*[58] analyzed serum FSH and LH levels of 403 NOA patients at the age of 29–30 years and found that 213 haploid gamete retrieval-positive patients had lower serum FSH level (7.1 IU l^{-1}) than 190 negative patients (18.0 IU l^{-1}), while LH levels were 7.4 IU l^{-1} and 13.6 IU l^{-1}, respectively. This finding was supported by another recent study with 72 NOA patients.[59] Jahromi *et al.*[22] reviewed serum FSH level of 171 NOA patients and found that serum FSH levels were dramatically higher with microTESE-negative patients than positive ones (29.0 *vs* 9.7 IU l^{-1}) while LH levels were close, and the cutoff value for FSH was 14.6 IU l^{-1} (**Table 1**).

However, there were some reports showing different results from noted above. A retrospective study recruited 145 NOA men and found no difference in either serum FSH or LH levels between SRO-positive and -negative patients (FSH: 18.2 *vs* 20.6 IU l^{-1}; LH: 9.0 *vs* 10.2 IU l^{-1}).[60] Ramasamy *et al.*[61] classified 792 NOA men into four groups based on serum FSH levels: <15, 15–30, 31–45, and >45 IU ml^{-1}, and the total SRR was 60% while SRR of NOA patients with FSH <15 IU ml^{-1} (51%) was lower than that of men with FSH values of 15–30, 31–45, and >45 IU ml^{-1} (60%, 67%, and 60%, respectively). Besides the SRR difference, Ramasamy *et al.*[61] also reported different serum FSH patterns in SCOS, MA, and HS patients from Liu *et al.*[57] It should be noted that Ramasamy *et al.*[61] got much lower serum FSH values than most other researchers with the reference range at 1.3–19.4 IU ml^{-1}, which could be related to the measuring method. Zhang *et al.*[21] retrospectively studied 241 consecutive men with NOA, and found that the total SRR of all patients was 20.0%, and men with severely increased FSH level (≥24.8 IU l^{-1}) had higher SRR (32.6%) than those with <24.8 IU l^{-1} FSH (15.8%), while the patients with an LH level of 8.6–17.2 IU l^{-1} had low SSR. HS patients had significantly higher SRR (71.4%) than MA (36.4%) and SCOS (8.1%). The detailed but different settings in different studies might contribute to the discrepancy. Further, it is indicative of the variety of NOA etiology and the variations among different types of NOA.

It seems that serum FSH and FSH levels are irrelevant to chromosomal alterations,[57] obesity,[62] or smoking habit[52] in NOA patients. It is surprising that the major chromosomal alterations of NOA did not happen to affect the endocrine-related genes. For instance, microdeletion of the Y chromosome region containing the AZF did not cause FSH or LH secretion difference in any type of NOA cohorts although it is considered to be the most common genetic cause of male infertility.

As noted above, HH patients are not only typically azoospermic but also one of the few treatable forms of male infertility since secondary hypogonadism usually results from a lack of appropriate stimulation by gonadotropins. These NOA men could benefit from specific hormonal therapy aiming to replicate the natural endocrine control of spermatogenesis. A remarkable recovery of spermatogenic function has been found with exogenously administered gonadotropins or GnRH or aromatase inhibitors.[30,63] Bakircioglu *et al.*[64] tried hormonal therapy with 25 azoospermic men diagnosed with HH, and these patients were treated with hCG for 1 month plus recombinant FSH for the following month. Spermatozoa were detected in the ejaculate in all patients after 10 months on an average. It was reported that previously virilized men with adult-onset HH and normal testicular volume responded well to hCG monotherapy, but this approach was rarely successful with congenital HH men such as KS, for whom combined gonadotropin therapy of hCG and FSH could maximize fertility potential. Key baseline predictors of successful spermatogenesis induction include prior spontaneous testicular development (*i.e.*, testicular volume >4 ml) without history of cryptorchidism.[65] A study analyzed the efficacy of serum FSH and LH replacement therapy with 112 azoospermic patients diagnosed with idiopathic HH patients at the average age of 28 years. Intramuscular hCG treatment started with a dose of 1500 IU (twice per week) and adjusted according to testosterone levels and testicular development over 6 months. If the patient remained azoospermic at the end of 6 months, FSH treatment (75–150 IU, twice per week) was added. Target FSH levels of 4–6 IU were achieved

Table 1: Serum and seminal plasma noninvasive predictors for human azoospermia or for sperm retrieval outcome

Sample	*Marker*	*Distinguishment*	*Cutoff*	*Sensitivity*	*Specificity*	*AUC*	*n*	*Age (year)*	*Reference*
Serum	FSH	OA from NOA[a]	≤7.6 IU l^{-1}	0.77	0.93	0.87	153	21–64	55
Serum	FSH	NOA from OA	>10.4 IU l^{-1}	0.82	0.8	0.84	120	26–58	56
Serum	FSH	NOA from OA	>9.2 IU l^{-1}[b]	0.99	0.82	0.96	207	Unspecified	20
Serum	LH	OA from NOA	<4.5 IU l^{-1}	0.76	0.8	0.79	153	21–64	55
Serum	PRL	OA from NOA	<7.7 ng ml^{-1}	0.68	0.66	0.71	153	21–64	55
Serum	Total TT	NOA from OA	<275.0 ng ml^{-1}	NA	NA	0.36	120	26–58	56
Serum	FSH	NOA sperm retrieval	<17.0 IU l^{-1}	0.90	0.43	0.52	17	32.3 (29–36)	110
Serum	FSH	NOA sperm retrieval	<13.7 IU l^{-1}	0.86	0.87	0.94	206	30	16
Serum	FSH	NOA sperm retrieval	<19.4 IU l^{-1}	0.70	1.0	NA	206	30	16
Serum	FSH	NOA sperm retrieval	<14.3 mIU l^{-1}[c]	0.71	0.68	0.62	280[d]	30.4–34.8	17
Serum	FSH	NOA sperm retrieval	≤9.0 IU l^{-1}	0.81	0.90	0.87	403	26–32	58
Serum	FSH	NOA sperm retrieval	<24.8 IU l^{-1}	0.67	0.15	NA	154	31.1 (22–46)	21
Serum	FSH	NOA sperm retrieval	<14.6 IU l^{-1}	0.84	0.80	0.88	171	34.3±8.6	22
Serum	LH	NOA sperm retrieval[e]	≤7.8 IU l^{-1}	0.87	0.60	0.79	403	26–32	58
Serum	LH	NOA sperm retrieval	<8.6 IU l^{-1}	0.80	0.20	NA	154	31.1 (22–46)	21
Serum	tTT	NOA sperm retrieval	>391.3 ng ml^{-1}	0.68	0.65	0.71	403	26–32	58
Serum	TT	NOA sperm retrieval	9.9 nmol l^{-1}	0.82	0.22	NA	154	31.1 (22–46)	21
Serum	INHB	NOA sperm retrieval	>40.0 pg ml^{-1}	0.90	1.00	0.98	17	32.3 (29–36)	110
Serum	INHB	NOA sperm retrieval	>35.0 pg ml^{-1}	0.76	0.81	NS	228	34.5–35.8	116
Serum	INHB	NOA sperm retrieval	>68.5 pg ml^{-1}	0.78	0.92	0.9	403	26–32	58
Serum	AMH	NOA sperm retrieval	<4.62 ng ml^{-1}	1	0.82	0.93	47	32–42	19
Serum	AMH/tTT	NOA sperm retrieval	<1.02	1	0.82	0.95	47	32–42	19
Seminal plasma	Leptin	NOA sperm retrieval	<2.9 ng ml^{-1}	0.43	0.75	0.59	280[d]	30–35	17

[a]Combined with testicular long axis >4.6 cm; [b]combined with right testis size <15 ml; [c]FSH unit as per citation; [d]134 patients in training set and 146 in testing set by artificial neural networks; [e]testicular haploid gametes including spermatids and testicular spermatozoa used for sperm retrieval. AMH: anti-Müllerian hormone; AUC: area under the curve; E_2: estradiol; FSH: follicle-stimulating hormone; INHB: inhibin B; LH: luteinizing hormone; NA: not available; NOA: nonobstructive azoospermia; NS: not specified; OA: obstructive azoospermia; PRL: prolactin; TT: testosterone; NA: not applicable; tTT: total testosterone

by FSH dose adjustments. After the treatment, sperm retrieval was carried out. Out of 112 idiopathic HH patients, 96 (85.7%) patients had sperms present in ejaculate samples. No differences in the serum baseline FSH, TT, or LH levels were found between sperm retrieval-positive or -negative patients. The authors concluded that hormone replacement approach provided a successful treatment modality in regard to successful spermatogenesis and achievement of pregnancy for azoospermic patients with HH.[54] In contrast, primary hypogonadism is testicular to spermatogenic failure and less treatable. ART with adjunctive endocrine manipulation remains the best therapeutic option. However, careful clinical evaluation may identify a significant minority for whom natural fertility can be restored by addressing gonadotropin deficiency or other endocrinopathies[66] (**Figure 1**).

Immunization against gonadotropins could be feasible to induce temporary azoospermia for contraception. Active immunization against FSH in rhesus monkeys caused occasional azoospermia but is not an effective method for male fertility control.[67] It is necessary to carefully evaluate the bioefficacy of antibodies raised to recombinant ovine FSHbeta or FSH receptor protein fragments. The advantage of the FSH/FSH receptor over the LHRH/LH-based vaccine lies in the fact that the former does not require an exogenous testosterone supplement to maintain accessory gland function, libido, *etc*. The LHRH/LH-based vaccine results in azoospermia, while the FSH vaccine causes the production of low numbers of poor-quality spermatozoa which are incapable of impregnating cycling females.[68]

In summary, FSH and LH play pivotal roles in spermatogenesis. NOA patients have higher serum FSH levels than fertile men, and among NOA patients, SRO-negative patients have higher FSH levels than SRO-positive men. Thus, serum FSH levels could be used as a noninvasive predictor for SRO in NOA patients although the cutoff values may vary. NOA patients with HH can be treated with gonadotropin replacement therapy, while testicular NOA is less treatable. Immunization against gonadotropins could purposely induce azoospermia.

PROLACTIN

Prolactin is produced in the anterior pituitary. Elevated level of serum prolactin inhibits the pulsatile release of GnRH from the anterior pituitary and suppresses both FSH and LH.[69] Normal range of serum PRL in adult men is less than 15 ng ml^{-1}. An early study demonstrated that seminal PRL was two to three times higher than serum PRL levels in fertile and infertile men, and PRL concentration of the fraction 2 of the split ejaculate was higher than that of the fraction 1 and of the serum.[70] Hyperprolactinemia, a form of HH caused by excessive prolactin secretion, influences steroidogenesis and spermatogenesis by acting on prolactin receptors present in SCs and Leydig cells in the testes. Hyperprolactinemia is common, but often missed, cause of male infertility as, unlike in women, clinical diagnosis of prolactinoma is late in men.[71] An early study measured plasma PRL concentration in 208 male partners of infertile marriages and found one case of marked hyperprolactinemia in 82 oligozoospermic men (1.2%).[72] Condition of hyperprolactinemia could be treated with dopamine agonists, which leaded to significant improvements in both semen parameters and hormone levels.[69]

It has been found that azoospermic patients have higher serum level of PRL. For instance, one study reported significantly higher serum PRL levels in azoospermic patients (44.2 ng ml^{-1}, n = 5) than those in normozoospermic men (15.9 ng ml^{-1}, n = 20) while oligozoospermic and asthenozoospermic patients had the values in between.[73] Another study with large sample size showed that serum PRL levels in azoospermic patients (10.9 ng ml^{-1}, n = 28) were

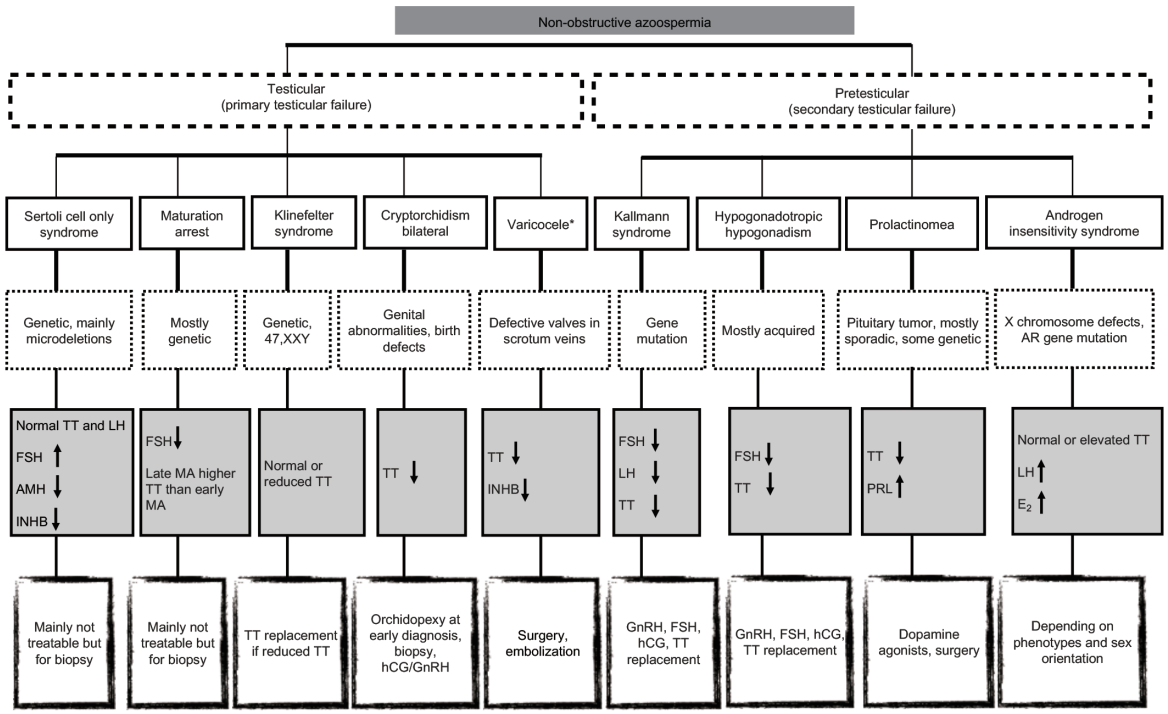

Figure 1: Endocrine aberrations of main conditions of nonobstruction azoospermia. *Most varicocele cases have low sperm production and decreased sperm quality, but not azoospermia. AMH: anti-Müllerian hormone; E_2: estradiol; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; hCG: human chorionic gonadotropin; INHB: inhibin B; LH: luteinizing hormone; MA: maturation arrest; PRL: prolactin; TT: testosterone.

significantly higher than those in healthy men (7.3 ng ml^{-1}, n = 46).[74] Schoor *et al.*[55] found that the serum PRL levels in NOA patients (10.3 mIU l^{-1}, n = 54) were higher than those in OA patients (6.9 mIU l^{-1}, n = 97) but proved not a strong marker to distinguish NOA from OA (**Table 1**). Ellithy *et al.*[75] reported much higher serum PRL levels in NOA patients (n = 40) than in normozoospermic men (6.1 ng ml^{-1}, n = 6). Other reports demonstrated similar results.[70,76] One study measured the PRL concentrations in seminal plasma in azoospermic (n = 11), oligozoospermic (n = 20), and normozoospermic men (n = 20) and found no difference.[76] These results suggest the strong but negative correlation between serum PRL levels and semen quality. However, a study further measured serum PRL levels in NOA patients with SRO-negative or -positive outcome and found that there was no difference in serum PRL levels between SRO-positive NOA patients (14.1 ng ml^{-1}, n = 52) and -negative NOA patients (12.5 ng ml^{-1}, n = 82).[17] Ellithy *et al.*[75] got similar results with SRO-positive (25.9 ng ml^{-1}, n = 11) and -negative NOA patients (17.3 ng ml^{-1}, n = 29) with no statistical difference. One more recent study demonstrated that serum anti-Müllerian hormone (AMH) levels, or AMH/total TT ratio, achieved independent predictor status for SRO in NOA patients, but not serum FSH, LH, PRL, thyrotropin hormone (TSH), sex hormone binding globulin (SHBG), INHB, or estradiol (E_2) was tested in the study. The cutoff values were <4.6 ng ml^{-1} for serum AMH and <1.0 for AMH/tTT[19] (**Table 1**).

One study monitored serum PRL levels of 204 men attending an infertility clinic for 1 year and found that PRL levels ranged from 1.4 to 24.7 ng ml^{-1} with a median of 5 ng ml^{-1}, there was no significant correlation of serum PRL concentration with the results of semen analysis, and the functional sperm capacity was even better in the groups of patients with serum PRL above the median level. The authors suggested that, instead of routine screening of asymptomatic men, PRL should be preferentially determined in patients with clinical symptoms of hyperprolactinemia to exclude pituitary adenoma.[77] These results may not be controversial to those noted above in that only one patient out of 204 in this study had serum PRL levels greater than 20 ng ml^{-1}.

The condition of reduced serum PRL level, known as hypoprolactinemia, is rare and could be due to autoimmune disease or hypopituitarism from genetic abnormality. Guidelines for diagnosing hypoprolactinemia are defined as serum PRL levels below 5 ng ml^{-1} in men. Patients with reduced PRL showed a higher risk of metabolic syndrome, erectile dysfunction, and premature ejaculation.[78]

Administration with exogenous PRL seems more efficient to adjust the endocrine and then spermatogenesis. It was reported that PRL administration, as a reversible contraceptive method, is efficient and safe and has the potential to be developed as a male contraceptive based on the experiments in 14 male mongrel dogs.[79,80] Sperm count decreased to azoospermia in 3 months after PRL injection with decrease of sperm motility and increase of abnormal forms. Testicular biopsy showed degenerated seminiferous tubules. Reproductive hormones, renal function, and serum sodium and potassium revealed insignificant change. Dog mating during the period of PRL administration induced no pregnancy. After 3 months of drug withdrawal, the sperm count normalized and dog mating produced pregnancy; offsprings showed no anomalies.[79]

In summary, elevated levels of serum PRL have a detrimental effect on spermatogenesis, even causing azoospermia, through inhibition of the pulsatile release of gonadotropins from the anterior pituitary gland. Elevated serum PRL levels are correlated to men's infertility.

ANDROGENS AND ESTROGENS

Androgens are a group of hormones that play a role in male traits and reproductive activity. The principle androgens are TT and

androstenedione. Although androstenedione has weak androgenic activity in its own right, it is mainly an intermediate in the biosynthesis of TT and estrone from dehydroepiandrosterone as a precursor.[81] In men, TT is the primary sex hormone and anabolic steroid, playing a variety of roles in the development of male reproductive tissues, promoting secondary sexual characteristics. In the testis, TT is the primary sex hormone that regulates spermatogenesis. Specifically, TT is mainly produced by Leydig cells in the interstitial space of the testis in response to stimulation with LH and acts as a paracrine factor that diffuses into the seminiferous tubules. The androgen receptor that is located on SCs plays a major role in spermatogenesis. Androgen receptor variants and abnormal expression were found associated with NOA.[82,83] Measurement of total TT could be sufficient for diagnosis when combined with measurements of serum LH and FSH. The normal range of total serum TT in adult men (≥19 years old) is 240–950 ng ml^{-1}. However, TT is present in plasma as free (unbound testosterone), albumin-bound, and SHBG-bound, and testing total TT might be not sensitive enough,[84] which is to be addressed later.

As men age, serum TT levels decline gradually as serum FSH and LH increase. Morley *et al.*[48] tracked 77 healthy men with the age at entry ranging from 61 to 87 years and examined the longitudinal alterations of TT and found that total serum TT level decreased gradually from 633 ng ml^{-1} to 464 ng ml^{-1}. Total TT declined with the age by 1%–2% per year approximately.[48,85–87]

In the absence of testosterone or the androgen receptor, spermatogenesis does not proceed beyond the meiosis stage. Compared with fertile men, NOA patients have declined or comparable serum TT levels. A study reported that serum TT levels in NOA patients (298.8 ng ml^{-1}, n = 40) were higher than those in OA patients (405.5 ng ml^{-1}, n = 80).[56] Liu *et al.*[57] found that NOA patients (n = 286) had dramatically lower TT level than fertile men (n = 100, 409 *vs* 548 ng fl^{-1}). Similarly, Huang *et al.*[20] demonstrated that NOA patients had much lower serum TT levels (n = 156) than OA men (n = 51), so as serum E_2 levels (19.2 *vs* 26.3 pg ml^{-1}), as shown in **Table 1**. Another study also showed that serum TT level of azoospermic patients (n = 112) with idiopathic HH was lower than the normal reference. Further, azoospermic patients with cryptorchidism had significantly lower serum total TT level than those with descended testes, while no difference was found in either FSH or LH levels, indicating the importance of proper testis temperature condition and TT concentration in spermatogenesis.[54] NOA patients with HH can be treated with gonadotropins to stimulate spermatogenesis, which is called hormonal replacement therapy. Using anti-estrogens and aromatase inhibitors in the meantime may improve the effectiveness of gonadotropin therapies by optimizing the intratesticular TT levels.[30,63] Ortac *et al.*[54] found that after FSH and LH replacement therapy, 85.7% of NOA patients with HH (n = 112) had sperm detected in ejaculates, and TT levels dramatically increased with FSH increased from basal 0.6 IU l^{-1} to 3.3 IU l^{-1}. However, an earlier study demonstrated that the azoospermic patients with SCOS (n = 15) had lower levels of serum estrone, estradiol, progesterone, 17alpha-hydroxyprogesterone, and dihydrotestosterone but higher levels of serum estriol and TT (5.7 *vs* 10.0 ng ml^{-1}) than fertile men (n = 20).[88]

Among NOA patients, it was found that serum TT concentration slightly decreased from the age of 20s (n = 39), 30s (n = 163), and >40s (n = 37).[51] In NOA patients, SCOS (389 ng fl^{-1}), MA (483 ng fl^{-1}), and HS (450 ng fl^{-1}) patients had similar TT levels, no matter patients had any chromosomal alterations, or AZF microdeletion or not.[57] Eken and Gulec[60] examined 145 NOA patients at the age of 33–34 years who underwent microTESE and found no difference of serum total TT levels between SRO-positive and -negative NOA patients (381 *vs* 342 ng dl^{-1}). In NOA patients, serum TT levels were found correlated with BMI and the obese men had much lower serum TT than lean men,[62] but not correlated with smoking.[52] It was newly reported that NOA patients with normal TT levels had a significantly higher chance of successful sperm retrieval compared to those with subnormal T levels.[89]

The androgen receptor (AR) is also essential for spermatogenesis. Mutations in the *AR* gene cause a wide variety of androgen insensitivity syndromes (AIS), including mild, partial, and complete AIS. Even the mild AIS could cause oligozoospermia or even NOA. Typically, patients with AIS have elevated or normal basal serum TT levels associated with high LH, while FSH levels are usually normal.[90]

In contrast, estrogens in the male can be synthesized locally from testosterone, by aromatase enzymes, in many tissues. There are three major endogenous estrogens that have estrogenic hormonal activity: estrone (E_1), E_2, and estriol. E_2, a predominant form of estrogen, is essential for modulating libido, erectile function, and spermatogenesis in men.[91] The normal range of serum E_2 in adult men is 10–40 pg ml^{-1}, while E_1 is 10–50 pg ml^{-1}. The age trends of men's estrogen levels have been reported variously as declining or remaining steady.[92] Estrogen mediates testosterone negative feedback on GnRH expression and secretion. On the other hand, estrogen can also regulate numerous aspects of spermatogenesis, including proliferation, differentiation, survival, and apoptosis of germ cells.

It has been reported that subfertile and azoospermic patients may have abnormal estrogen profile. Hargreave *et al.*[93] found that subfertile patients (n = 451) had lower levels of serum E_2 than fertile men (n = 80) and that there was no relationship to obesity, smoking or age. Salama and Blgozah[94] found a wide spectrum of serum E_2 concentrations in NOA patients (n = 166) and fertile men (n = 40), and serum levels were not correlated with BMI, varicocele prevalence, prolactin, or smoking habits. Shiraishi and colleagues found that the aromatase was mainly expressed in Leydig cells, and the levels of its transcript and protein expression levels were increased in men with NOA (n = 76); there were significant associations between decreased intratesticular TT/increased intratesticular E_2, aromatase expression, and sperm retrieval but no correlation between serum TT/E_2 and intratesticular TT/E_2 levels.[95]

On the one hand, some NOA patients, such as those with KS, can be treated with testosterone therapy.[96] On the other hand, azoospermia could be induced by administration of GnRHα, and the combination of GnRHα and androgens can suppress spermatogenesis to azoospermia, which is effective in nonhuman primates and can be maintained more by androgens alone.[97] Behre *et al.*[42] tested the combined administration of GnRH antagonist cetrorelix and 19-nortestosterone hexyloxyphenylpropionate (19NT-HPP), a long-acting androgen, in a clinical trial with six healthy volunteers. They found that serum concentrations of LH, FSH, and testosterone were effectively suppressed, and within 12 weeks, azoospermia was achieved in all six volunteers. After cessation of cetrorelix injections in week 12, gonadotropins and testosterone increased significantly despite continued 19NT-HPP injections. In parallel, spermatogenesis was re-stimulated in five of six volunteers. The suppression was effective and reversible, but complete azoospermia cannot be maintained by continued injections of the nonaromatizable 19NT-HPP alone.[42] Exogenous estrogen can also be used to induce azoospermia. Exogenous TT therapy, despite its positive effects on sexual function in subfertile men, can negatively affect the hypothalamic–pituitary–testis axis (HPTA) functions and inhibit spermatogenesis, so TT is a contraceptive and should not be used in men who desire fertility.[98] Hormone treatment for transwomen consists of anti-androgens combined with estrogens to achieve feminization,

which can lead to azoospermia.[99] A retrospective cohort study included a total of 260 transwomen aged between 16 and 52 years between 1972 and 2017. Semen quality in transwomen was significantly decreased and 21 transwomen had an azoospermia (8%).[100] Therefore, fertility preservation is highly recommended before gender-affirming hormone therapy in their desire of parenthood.

In summary, spermatogenesis is in part under the control of a balance of androgens and estrogens, with aromatase serving as a modulator. TT plays an important role in spermatogenesis as a primary androgen. NOA patients have reduced level of serum TT, whereas NOA can be reversibly induced by a combination of GnRHα and androgen or by estrogen.

SHBG

SHBG, also known as sex steroid-binding globulin, is a plasma glycoprotein mainly produced by hepatocytes and secreted into the blood. Testes-produced SHBG is called androgen-binding protein. SHBG transports the androgens such as TT and DHT and the estrogens such as E_2 in the blood as biologically inactive forms. Therefore, changes in SHBG levels can affect the free form of hormone that is functional. Approximately, 45% to 65% of testosterone in the blood is tightly bound to SHBG in men, and slightly less than one-third of the protein-bound testosterone is loosely and reversibly bound to albumin, the main protein in the fluid portion of the blood. Only 2% to 3% of TT is free. The free testosterone plus the albumin-bound testosterone is the bioavailable testosterone (BAT), which is the portion of TT that is available to act on target tissues. Direct tests of free TT are expensive and unreliable, but free TT or bioavailable TT can be obtained by calculation.[101] Clinically, SHBG and total TT levels may be tested for men with infertility, decreased sex drive, or erectile dysfunction when total TT results are inconsistent with clinical signs. Estrogen and its related steroids, thyroid hormone, and insulin increase SHBG levels, while SHBG decreases in response to androgens, and in the presence of hypothyroidism and insulin resistance. The human plasma SHBG levels tend to increase with increasing age while total TT levels steadily decrease.[92]

A study measured normal serum SHBG and TT levels in 84 adult healthy men at 40–70 years old and found that total TT was 6.3 ng ml^{-1}, measured free TT was 12.6 pg ml^{-1}, SHBG varied 54.5–91.6 nmol l^{-1} with an average of 72.0 nmol l^{-1}, calculated free TT was 80.0 pg ml^{-1}, and BAT was 1.88 ng ml^{-1}.[102] Ring and colleagues assessed serum SHBG as a predictor of men infertility. In 168 patients, serum levels were TT 338.4 ng ml^{-1}, free TT was 7.8 ng ml^{-1}, BAT was 177 ng ml^{-1}, and SHBG was 27.8 nmol l^{-1}, and they found that serum SHBG levels independently predicted deceased sperm concentrations and motility with a similar magnitude as FSH and that the addition of SHBG to TT facilitated more accurate diagnosis.[103,104] Heracek *et al.*[53] examined intratesticular sex steroids (TT, DHT, androstenedione, E_2, and epitestosterone) and serum hormones (LH, FSH, PRL, SHBG, TT, and E_2) of 84 NOA men and found no correlation of serum SHBG with either intratesticular sex steroids or serum hormones examined. Bolufer *et al.*[105] reported no difference of basal serum SHBG levels in men with normozoospermia (31.1 nmol l^{-1}, n = 10), oligozoospermia (26.3 nmol l^{-1}, n = 29), and azoospermia (25.6 nmol l^{-1}, n = 11). One hundred milligrams of clomiphene per day was administered for 11 consecutive days, and then, the values were 38.4 nmol l^{-1}, 30.7 nmol l^{-1}, and 28.3 nmol l^{-1}, respectively. Zorn *et al.*[106] reported the similar results with much bigger sample size that serum SHBG levels of NOA patients (31.8 nmol l^{-1}, n = 42), OA patients (31.3 nmol l^{-1}, n = 15), oligoasthenoteratozoospermic patients (30.6 nmol l^{-1}, n = 68), and normozoospermic men (32.1 nmol l^{-1}, n = 85) had no significant difference. Among NOA patients, there was no difference of serum INHB levels between SRO-positive (29.0 nmol l^{-1}, n = 23) and -negative NOA patients (28.0 nmol l^{-1}, n = 24).[19] Simoni *et al.*[107] determined serum SHBG levels of 8 men participating in a contraceptive trial with single im injection of 1200 mg of testosterone buciclate after intravenous administration of GnRH (100 μg), and 3 volunteers developed azoospermia in 10 weeks. No difference of serum SHBG levels was found between responders and nonresponders. Collectively, limited research so far has found no difference with serum SHBG levels in azoospermic patients from healthy men, no difference of SHBG in men with TT-induced azoospermia, and no difference between SRO-positive and -negative patients with NOA, either.

It was known that human *SHBG* gene mutations, especially in rs6259 and rs727428 loci, were associated with male infertility. Cui *et al.*[108] analyzed the correlation between *SHBG* gene polymorphism and infertility in a Chinese population. Their study recruited 183 infertility patients with either azoospermia or severe oligozoospermia and another 183 healthy men. Besides rs6259 and rs727428 locus polymorphisms, genotype frequencies, allele frequency, and haplotype were also analyzed. They found that men infertility was associated with GA genotype and A allele of rs6259 locus, as well as CC genotype of rs727428 locus in *SHBG* gene.

In summary, the fraction of TT bound to SHBG in the serum is proportional to the SHBG level. Hepatocyte-produced SHBG mainly affects spermatogenesis and sexual functions through adjusting free and combined TT ratio in the serum. While evaluating total TT levels, SHBG should be considered for an accurate calculation of free and bioavailable TT under some circumstances.

INHIBIN B

Inhibins, belonging to transforming growth-beta (TGF-beta) superfamily, consist of a dimer of two homologous subunits, an alpha subunit and either a beta A or beta B subunit, to form inhibin A (INHA) and inhibin B (INHB), respectively. INHB is predominantly produced by SCs in the testis and plays an important role in human spermatogenesis, while serum INHA is undetectable. INHB controls FSH secretion via a negative feedback mechanism in that INHB is a strong inhibitor of FSHbeta transcription. In the adult, INHB production depends both, on spermatogenic status, FSH and TT. There is a strong negative correlation between serum INHB and FSH in the adult men, and in infertile patients, INHB decreases as FSH increases.

Azoospermic patients have lower serum INHB levels than normozoospermic men. Pierik *et al.*[109] found that serum INHB levels in idiopathic azoospermic patients (52.0 pg ml^{-1}, n = 15) were significantly lower than those of normozoospermic men (166.1 pg ml^{-1}, n = 49) and severe oligozoospermic patients (128.4 pg ml^{-1}, n = 58). Serum INHB levels were closely but negatively correlated with the serum FSH levels, suggesting an endocrine marker of human spermatogenesis in subfertile men. Ballesca *et al.*[110] reported similar results with NOA patients (57.0 pg ml^{-1}, n = 17), OA patients (118.0 pg ml^{-1}, n = 22), and fertile men (117.6 pg ml^{-1}, n = 29). Furthermore, among NOA patients, INHB levels were higher in SPO-positive (78.3 pg ml^{-1}, n = 10) than negative patients (26.7 pg ml^{-1}, n = 7), but no difference with serum FSH. Therefore, serum INHB levels can be used as a strong noninvasive predictor of SPO with cutoff of >40 pg ml^{-1} in addition to serum FSH measurement before undergoing sperm retrieval. Brugo-Olmedo *et al.*[111] found that NOA patients had significantly lower levels of serum INHB (13.3 pg ml^{-1}, n = 6) than normozoospermic (231.2 pg ml^{-1}, n = 10) and OA (156.8 pg ml^{-1}, n = 15) patients.

NOA patients with positive SRO had higher serum INHB levels than negative patients (89.3 pg ml^{-1}, n = 30 *vs* 19.2 pg ml^{-1}, n = 42). They concluded that serum INHB level seemed to be more accurate than serum FSH level in predicting SRO in NOA patients. Zorn *et al.*[106] also found that serum INHB levels of NOA patients (73.0 ng ml^{-1}, n = 42) were dramatically lower than those of OA patients (194.3 ng ml^{-1}, n = 15), oligoasthenoteratozoospermic patients (167.0 ng ml^{-1}, n = 68), and normozoospermic men (192.3 ng ml^{-1}, n = 85). Muttukrishna *et al.*[112] also reported that serum INHB levels were significantly lower in NOA patients (10 pg ml^{-1}, n = 17) than those in fertile men (154 pg ml^{-1}, n = 40) while OA men in between (124 pg ml^{-1}, n = 14). Duvilla *et al.*[113] examined the seminal INHB concentrations of azoospermic (59.6 pg ml^{-1}, n = 67), oligozoospermic (417.5 pg ml^{-1}, n = 28), and normozoospermic (714.4 pg ml^{-1}, n = 47) men and found that seminal INHB was a poor predictors of SRO due to huge variation. Mitchell *et al.*[114] demonstrated that SRO-positive NOA patients had higher levels of serum INHB (97.8 pg ml^{-1}, n = 60) than negative NOA patients (36.3 pg ml^{-1}, n = 79), whereas no statistical difference was found between SRO-positive (25.9 pg ml^{-1}, n = 60) and -negative (227.4 pg ml^{-1}, n = 79) NOA patients due to the big variation.

Moradi *et al.*[115] investigated if the measurement of serum INHB helped for SRO with 41 azoospermic patients and then concluded that serum INHB should be measured when serum FSH was less than twice the normal, while testicular biopsy should be cancelled without testing serum INHB when serum FSH was less than 100 pg ml^{-1}. Another study had similar results with 228 idiopathic NOA patients while the threshold of 35.0 pg ml^{-1} serum INHB was suggested.[116] Zhu *et al.*[58] also reported similar finding with larger sample size that the NOA patients who generated testicular haploid gamete had higher serum INHB level (112.5 pg ml^{-1}, n = 213) than those who did not (29.0 pg ml^{-1}, n = 190), and they concluded that serum INHB as an effective marker for spermatogenesis was a significant predictor of SPO in NOA patients with cutoff 68.5 pg ml^{-1}, even superior to elevated serum FSH (**Table 1**). Barbotin *et al.*[117] found that serum INHB might be a predictor of SRO for NOA patients with cryptorchidism (n = 225), although there was serum INHB difference neither between bilateral (64.4%) and unilateral cryptorchidism patients (35.6%) nor between SRO-positive and -negative men.

Pierik *et al.*[109] also found that among NOA patients, KS patients (47,XXY) had even lower levels of serum INHB (7.3 pg ml^{-1}, n = 4), indicating the variation depending on the etiology. Indeed, it was reported that among NOA patients, serum INHB had significant correlation inversely with testicular fibrosis and SCOS and directly with incomplete spermatocytic MA and OA, and they suggested that at clinics, if FSH is less than twice the normal, INHB should be measured, and if its level is less than 100 pg ml^{-1}, testicular biopsy can be cancelled.[115] Another retrospective study investigated the relationship of genetic causes and INHB in NOA with 322 NOA patients and found that, compared to normal karyotype 46,XY (84.0 pg ml^{-1}, n = 210), the level of INHB in chromosomal abnormality from lowest to highest was 46,XX (or 45,X; 3.7 pg ml^{-1}, n = 8), 47,XXY (10.5 pg ml^{-1}, n = 34), mosaics (33.2 pg ml^{-1}, n = 12), polymorphisms (68.6 pg ml^{-1}, n = 27), inversion (111.1 pg ml^{-1}, n = 10), and translocation (158.0 pg ml^{-1}, n = 8).[118] Another study found that most NOA patients with SCOS (48/76) had undetectable serum INHB level.[117] It seems that among NOA patients, the serum INHB levels are widely dispersed depending on the origin of NOA.

Interestingly, Demyashkin[119] examined INHB expression by immunohistochemistry and found that INHB expression index in the SCs was higher in SCs of azoospermic patients (98.0%, n = 82) than in healthy men (65.9%, n = 10). INHB expression index was low in the cytoplasm of spermatogonia in healthy men (4.0%) but high SCOS NOA patients with focal spermatogenesis (45.0%). Among NOA patients, severe lesion to the seminiferous tubules had the lowest level of INHB expression in the SCs.[119] These results indicate the variation of INHB expression in SCs cells from etiology of NOA, just as serum INHB.

Foresta *et al.*[120] investigated the relationship between testicular damage and serum INHB levels in 30 normozoospermic men and 89 azoospermic patients, including SCOS, severe hypospermatogenesis, spermatogonial and/or spermatocytic arrest, spermatidic arrest, and OA. They found that serum INHB as a diagnostic parameter in azoospermic patients was not specific and it did not discriminate OA from spermatidic arrest, and serum INHB levels were normal in some SCOS patients.

However, one study analyzed the electronic database available by 2010 and found that serum INHB cannot serve as a marker of SRO alone.[121] Meachem *et al.*[122] reviewed the research and concluded that, although INHB is a valuable index of spermatogenesis and can be used as a strong noninvasive endocrine marker to predict the outcome of SRO, the combination of both serum INHB and FSH was more sensitive and specific for spermatogenesis. Alfano *et al.*[19] found that there was even no difference of serum INHB levels in SRO-positive (32.7 pg ml^{-1}, n = 23) and -negative (37.7 pg ml^{-1}, n = 24) NOA patients.

In summary, SCs-produced INHB plays an important role in human spermatogenesis by controlling FSH secretion via a negative feedback mechanism (**Figure 2**). NOA patients have reduced levels of serum INHB. Among NOA patients, serum INHB levels vary to the

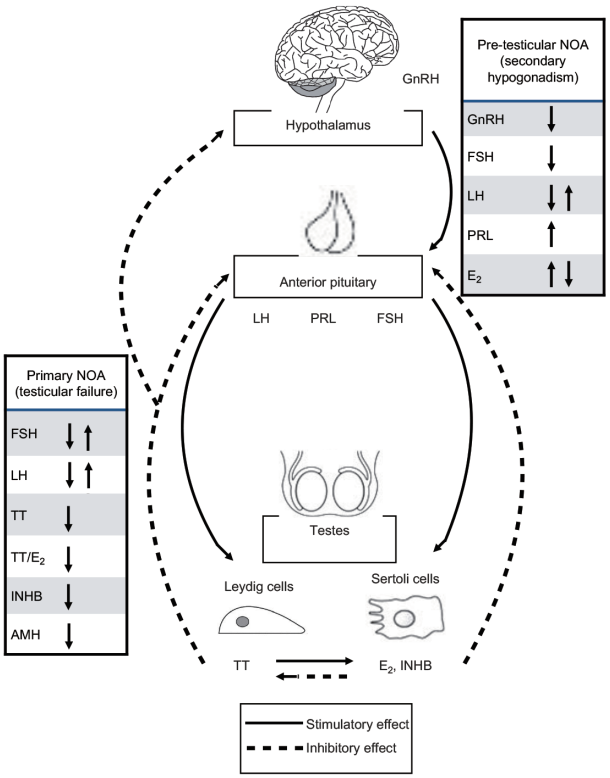

Figure 2: Hypothalamus–pituitary–testis axis and hormonal aberrations in men with NOA. AMH: anti-Müllerian hormone; E_2: estradiol; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; INHB: inhibin B; LH: luteinizing hormone; NOA: nonobstructive azoospermia; PRL: prolactin; TT: testosterone.

etiology of NOA. Serum INHB, not seminal INHB, may be used as an endocrine marker for male infertility and a noninvasive predictor before sperm retrieval, better in combination with serum FSH levels.

AMH

AMH, also known as Müllerian inhibiting substance or factor, is a SCs-secreted glycoprotein dimer structurally related to inhibin and TGF-β. Its well-known function is responsible for Müllerian duct regression at male fetal stage. In male humans, circular AMH increases in the first week after birth, then rises rapidly during the first month, and peaks at about 6 months of age. It then slowly declines during childhood and persists at a very low level in adults. After puberty, AMH is released preferentially by the apical pole of the SC toward the lumen of the seminiferous tubules, resulting in higher concentrations in the seminal plasma than in the serum.[123]

Fenichel *et al.*[124] measured both seminal and serum AMH levels in fertile men and found that the seminal AMH of fertile men ($n = 18$) was 153 pmol l^{-1}, varying from undetectable (<3.5 pmol l^{-1}) to 543 pmol l^{-1}, significantly higher than the serum concentration (10.7 pmol l^{-1}). In contrast, seminal AMH in NOA patients was undetectable in 14 cases out of 23, while in the remaining 9 cases, AMH was much lower (17.0 pmol l^{-1}) than that of healthy men. No correlation could be found between seminal AMH and plasma FSH or TT. Among 14 undetectable cases, 11 were associated with lack of spermatozoa by biopsy. Among 9 detectable cases, 2 lacked of spermatozoa. These results indicate that seminal AMH may represent a noninvasive marker to predict sperm retrieval outcome in NOA men.[123] Another study showed that oligozoospermic men had lower level of seminal AMH (149.3 pmol l^{-1}, $n = 39$) than healthy men (249.0 pmol l^{-1}, $n = 10$), and seminal AMH concentration was correlated with sperm concentration and testicular volume and serum LH, but not serum FSH, TT, or E_2.[125] Muttukrishna *et al.*[112] found that serum AMH levels were lower in NOA men (6.0 ng ml^{-1}, $n = 17$) than fertile men (7.3 ng ml^{-1}, $n = 40$), suggesting that serum AMH could be a marker of NOA patients. Plotton *et al.*[126] measured plasma AMH levels in OA and NOA patients, including genetic, cryptorchidism, cytotoxic, and unexplained NOA subgroups. They found that plasma AMH levels were lower in NOA (28.7 pmol l^{-1}, $n = 36$) relatively to OA (48.6 pmol l^{-1}, $n = 13$), and genetic NOA patients had the lowest plasma AMH concentrations (11.4 pmol l^{-1}, $n = 9$) while the values observed in case of cytotoxic NOA (45.7 pmol l^{-1}, $n = 4$) were as high as the values observed in OA. Plasma AMH levels were correlated to FSH, INHB, bioavailable TT, and testicular volumes. It seems that the decrease in the plasma AMH levels in NOA patients was related to the origin of NOA. A recent study demonstrated that among 47 NOA patients with serum AMH concentration of 3.4 ng ml^{-1}, sperm retrieval-positive patients had lower serum AMH levels (2.0 ng ml^{-1}, $n = 23$) than those negative men (6.0 ng ml^{-1}, $n = 24$). It concluded that serum AMH levels (<4.6 ng ml^{-1}) achieved independent predictor status for sperm retrieval outcome, also AMH/total TT ratio (<1.0), but not serum FSH, LH, prolactin, TSH, SHBG, INHB, or E_2[19] (**Table 1**). Another study reported significantly lower serum AMH levels in azoospermic patients (0.5 ng ml^{-1}, $n = 47$) than normozoospermic men (3.0 ng ml^{-1}, $n = 25$) and oligozoospermic men (2.6 ng ml^{-1}, $n = 28$), so serum AMH was a significant marker for male infertility. Further, serum AMH negatively correlated with FSH in oligozoospermic and azoospermic men and positively with TT in azoospermic patients.[127]

However, some studies showed different results. Isikoglu *et al.*[128] measured semen AMH levels instead of seminal AMH because the presence of seminal proteases could influence AMH levels. They found that the difference between serum AMH levels in normozoospermic men (67.6 pmol l^{-1}, $n = 23$) and NOA patients (82.7 pmol l^{-1}, $n = 24$) did not reach statistical significance, and there was no difference between serum AMH levels in SRO-positive (77.5 pmol l^{-1}, $n = 11$) and -negative (62.0 pmol l^{-1}, $n = 11$) NOA patients. Therefore, serum AMH cannot predict the testicular sperm retrieval outcome in NOA men. Duvilla *et al.*[113] also evaluated the predictive value of seminal AMH on the SRO in NOA patients ($n = 68$) as well as normozoospermic men ($n = 47$) and oligozoospermic patients ($n = 28$), and they found that seminal AMH was a poor predictor of SRO mainly because of widely dispersed seminal AMH levels due to the etiology of NOA. Toulis *et al.*[121] analyzed the data from previous studies about serum AMH (2 studies) and seminal AMH (4 studies) by June 2009 and found that neither serum nor seminal AMH levels served as a marker for sperm retrieval outcome in NOA patients. As noted by the authors, no clear conclusions can be deduced for seminal serum/seminal AMH due to the small number of relative studies. Another systematic review held the similar impression.[129] Among 139 NOA patients, mean seminal AMH was 12.1 pmol l^{-1} and seminal AMH was undetectable in 36% of men. There was no difference between serum AMH in SRO-positive (6.7 pmol l^{-1}, $n = 60$) and -negative (16.1 pmol l^{-1}, $n = 79$) NOA patients. Seminal AMH and serum INHB were positively correlated. Combining serum AMH levels with the serum FSH level did not improve the predictive value for SRO. Seminal AMH was not predictive of testicular sperm retrieval in NOA patients.[114] El-Halawaty *et al.*[130] found no difference in serum AMH levels among NOA patients (4.7 ng ml^{-1}, $n = 41$), oligozoospermic patients (2.5 ng ml^{-1}, $n = 14$), and normozoospermic men (4.9 ng ml^{-1}, $n = 22$). Xu *et al.*[131] tended to idea that serum AMH levels cannot become a strong marker all by itself because of varying levels.

Some studies investigated the pathway(s) how AMH affects the spermatogenesis and found that FSH transcriptionally activated AMH through a nonclassical cyclic adenosine monophosphate (cAMP) pathway by binding to the AMH promoter region located at nuclear factor kappa-B (NF-κB) and transcription factor AP2-binding sites.[132] TT inhibited the transcriptional activation of AMH, hypothetically through transcriptional inhibition of NF-κB. Besides, sex-determining region Y box 9 (Sox9), steroidogenic factor 1 (SF1), and GATA factors were implicated in the transcriptional activation of AMH by regulating the proximal promoter of *AMH*.[131] This understanding of AMH-related NOA is indicative to the potential treatment regimens, such as KS, persistent Müllerian duct syndrome (PMDS), and disorders of sex development (DSD).[131]

In summary, AMH levels reflect the function of SC. Adult men have higher AMH concentrations in the seminal plasma than in the serum. So far, many studies, but not all, have demonstrated that NOA patients have lower seminal AMH levels than fertile men, but the levels vary with the origin of NOA. More studies are needed before AMH can be used as a noninvasive marker for sperm retrieval outcome in NOA patients.

LEPTIN

Leptin, a pleiotropic hormone, is produced by the adipose tissue and acts as a satiety signal to control energy balance. Its levels are correlated with fat mass. Leptin plays an important mediating role in the regulation of neuroendocrine, and thus, leptin can act on all levels of HPG axis and affect the nutritional support of testis and spermatogenesis. For instance, hyperleptinemia has negative effects on male reproductive function.[133,134]

So far, there have been studies investigated serum and seminal leptin levels and its correlations to the results of semen analysis.

Farooq *et al.*[135] found that serum leptin level was higher in infertile obese men than in obese fertile men at both young (25–35 years old, 11.8 *vs* 7.0 ng ml^{-1}, n = 16 *vs* 16) and old age (36–60 years old, 10.0 *vs* 8.2 ng ml^{-1}, n = 50 *vs* 44) with a strong positive correlation between BMI and serum leptin levels. Steinman *et al.*[136] found that serum leptin concentration in azoospermic men (n = 36) was 7.6 μg l^{-1}, higher than that of fertile men (5.0 μg l^{-1}, n = 35), while severe oligozoospermic patients had serum leptin levels in between (7.1 μg l^{-1}, n = 17), and leptin levels were not correlated to serum LH or FSH in any patients. Another study also found that NOA patients (1.2 ng ml^{-1}) and high-grade oligozoospermic patients (1.1 ng ml^{-1}) had higher semen leptin levels than those with OA (0.5 ng ml^{-1}) and low-grade oligozoospermic patients (0.5 ng ml^{-1}), comparable with those of normozoospermic men (0.2 ng ml^{-1}).[137] Similarly, Zorn *et al.*[106] found that serum leptin levels of NOA patients (15.3 ng ml^{-1}, n = 42) were higher than normozoospermic men (8.2 ng ml^{-1}, n = 85), while oligoasthenoteratozoospermic patients had the levels in between (12.4 ng ml^{-1}, n = 68). Among NOA patients, serum leptin levels did not differ from SCOS patients (15.0 ng ml^{-1}, n = 15), patients with hypospermatogenesis (17.6 ng ml^{-1}, n = 22), and MA patients (12.6 ng ml^{-1}, n = 15), although there was no correlation between serum leptin and classical sperm characteristics. There was a negative correlation between serum levels of leptin and INHB, total TT, and SHBG.[106]

In contrast, Ma *et al.*[17] demonstrated that seminal leptin, but not serum leptin, could be a good assistant diagnostic marker, secondary to serum FSH, to predict sperm retrieval outcome after TESE. Their results showed that seminal leptin levels were lower with TESE-positive NOA patients (2.5 ng ml^{-1}, n = 52) than TESE negative (3.4 ng ml^{-1}, n = 82) while serum leptin levels had no difference (6.7 *vs* 7.4 ng ml^{-1}), which was similar to the previous report that the serum leptin levels of testicular biopsy-positive NOA patients (16.2 ng ml^{-1}, n = 41) did not differ from negative NOA patients (14.4 ng ml^{-1}, n = 16).[106] Guo *et al.*[138] reported the similar finding that serum leptin level was no difference between asthenozoospermic patients (9.5 ng ml^{-1}, n = 79) and healthy men (9.2 ng ml^{-1}, n = 77), but seminal leptin level was higher with asthenozoospermic patients than normozoospermic men (4.7 *vs* 3.8 ng ml^{-1}).

Ellithy *et al.*[75] measured the serum, testicular, and seminal leptin levels at the same time in NOA, OA, and fertile men, including SRO-positive and -negative NOA patients, and they found that seminal leptin levels were lower with SRO-positive NOA patients (1.1 ng ml^{-1}, n = 11) than negative (1.2 ng ml^{-1}, n = 29) and both much higher than fertile men (0.8 ng ml^{-1}, n = 6), and plus, testicular leptin levels were significantly lower with TESE-positive NOA patients (1.5 ng ml^{-1}, n = 11) than TESE-negative (1.7 ng ml^{-1}, n = 29), while no differences in serum leptin levels (fertile men: 2.8 ng ml^{-1}, OA: 3.9 ng ml^{-1}, NOA-SRO positive: 3.8 ng ml^{-1}, and NOA SRO-negative: 4.6 ng ml^{-1}). There was a positive correlation between seminal and testicular leptin concentrations. They concluded that seminal leptin can be used as an assistant marker to increase the production accuracy for sperm retrieval in combination with FSH in NOA patients.[75] Further, no statistically significant correlations were found between the concentration of the seminal leptin and BMI and concentrations of LH, FSH, PRL, total TT, and serum leptin. No statistically significant correlations were found between the concentration of the testicular leptin and BMI and also concentrations of LH, FSH, PRL, total TT, free TT, and serum leptin.[75]

The molecular mechanisms how leptin affects spermatogenesis remain unknown. Leptin may regulate the functions of Leydig cells[106] and/or SCs.[139] Semen leptin concentrations were directly correlated with serum leptin but inversely correlated with serum TT levels, indicating that leptin was not actively transported but rather leaked through the blood–testis barrier due to dysfunction of testicular epithelia.[137] Martins *et al.*[139] used *in vitro* experimental model of human SCs treated with leptin and found that leptin decreased SCs acetate production and increased glucose transporters, and it seemed that this hormone modulated human SCs acetate production and glycolytic profile, indicating the mechanism of obesity-induced male infertility.

Another study demonstrated that the expression of leptin and its receptor was higher in rat testis with varicocele created by partial ligation of the left renal vein. Leptin expression was inversely associated with the number of sperm in the left epididymis, thickness of the seminiferous epithelium, and the diameter of seminiferous tubules. The expression of leptin receptors in the hypothalamus was also increased. These results suggested that leptin and its receptor may serve significant roles in the pathogenesis of varicocele-induced testicular dysfunction.[140]

In summary, leptin plays an important role in spermatogenesis by mediating of HPGA and the nutritional support of testis. Although most studies have shown that both serum and seminal leptin levels in NOA patients are higher than those in fertile men, rather than serum and testicular leptin concentrations, seminal leptin levels could be an assistant diagnostic marker, secondary to serum FSH, to increase the production accuracy for sperm retrieval in NOA patients.

CONCLUSIONS

This review summarizes the most recent advances in endocrine aberrations associated with human NOA. Hormonal imbalance may either jeopardize proper human spermatogenesis and cause NOA, especially the hormones in the HPTA (**Figure 2**), or happen as a result of NOA. GnRH is the central neuroendocrine regulator of spermatogenesis, and therefore, any disorder of GnRH or GnRHR may cause NOA, and therefore, GnRH and GnRHα can be used to treat NOA patients in the scenarios. FSH and LH play pivotal roles in spermatogenesis, and HH is a common cohort of NOA. Among NOA patients, SRO-positive patients have lower FSH levels than SRO-negative men. Thus, serum FSH levels could be used as a noninvasive marker to predict SRO in NOA patients before testis biopsy. Gonadotropin replacement therapy has been successfully used to treat NOA patients with HH. Elevated levels of serum PRL have a detrimental effect on spermatogenesis, even causing azoospermia, through inhibition of gonadotropin release. A balance of androgens and estrogens is required for spermatogenesis with aromatase serving as a modulator. NOA can be reversibly induced by a combination of GnRHα and androgen or by estrogen. SHBG bound to TT greatly influences the free TT levels in the serum and then affects spermatogenesis. Therefore, measurement of serum SHBG is necessary under some circumstances. NOA patients have reduced levels of serum INHB, and serum INHB levels vary to the etiology of NOA. Serum INHB may be used as an endocrine marker for male infertility and a noninvasive predictor for SRO. AMH levels reflect the function of SC, and NOA patients may have declined seminal AMH levels, but the levels vary with the origin of NOA. Leptin plays roles in spermatogenesis by mediating of HPGA and the nutritional support of testis. Seminal leptin levels could be an assistant diagnostic marker, secondary to serum FSH, to increase the production accuracy for sperm retrieval in NOA patients.

GnRH acts as paracrine and neurotransmitter while other hormones discussed in this review principally act as circulating hormones (endocrine). However, many of them can also serve as paracrine and autocrine, such as TT, E_2, and AMH. Besides the balance of the circulating hormones, the balance of local hormones in the testes

is also critical to maintain normal spermatogenesis. For instance, many men with NOA had an abnormal testosterone to estradiol ratio locally in intratesticular environment.[141,142]

Under some circumstances, NOA could be reversibly or irreversibly induced on purpose of contraception or transgender with hormone treatment. In addition, immunization against many hormones, including GnRH, FSH, and TT, may be able to break the hormonal balance in HPTA, interrupt the spermatogenesis, and then induce NOA.

In general, among a number of hormones, FSH and TT are critical to proper spermatogenesis. Most studies, but not all, so far have demonstrated that serum FSH and INHB may be used as noninvasive endocrine markers for assessing spermatogenesis and for predicting SRO in NOA patients. Some recent studies, for instance, showed that none of serum FSH, LH, TT, PRL or INHB levels tested was a solid predictor for SRO in NOA patients.[143,144] So far, microTESE has been a common management of NOA due to its efficacy and minimal invasiveness of its procedure, and hormone stimulation including gonadotropin replacement therapy before microTESE may increase the success rate to help those NOA patients with HH. NOA men who have an abnormal TT/E_2 ratio could be treated with aromatase inhibitors. However, hormone therapy has yet been well established, and the etiology of azoospermia and potential risks in individuals should be carefully evaluated before starting a time- and cost-consuming treatments in vulnerable patients.[1,145] For the NOA men associated with primary testicular failure, however, the quality of currently available evidence has not been sufficient to recommend hormone optimization therapy.[89,146,147] Hopefully, with the development of novel technologies such as proteomics, genomics, and artificial intelligence systems, processing large collections of data could help formulate algorithms, diagnose the etiology, and then guide the clinic management of designing the treatment of NOA.[148]

COMPETING INTERESTS

The author declares no competing interests.

ACKNOWLEDGMENTS

The author is grateful to the language help from Ms. Anna Engineer (University of Ottawa, Ottawa, ON, Canada).

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