# Central Hypogonadism in the Male: Physiopathology, Diagnosis, and Treatment

10

Julia Rohayem and Eberhard Nieschlag

## Contents



**Abstract**

The hypothalamic-pituitary-gonadal (HPG) axis coordinates two functions essential for male reproductive capacity: synthesis and secretion of sex steroid hormones, primarily testosterone, and production of spermatozoa. Our understanding of the physiologic principles of male reproductive health have substantially increased in recent years, enabling physicians not only to identify genetic causes of hypogonadism among a wide spectrum of possible disorders, but also to establish therapeutic strategies. Men with hypothalamic or pituitary disorders

J. Rohayem (✉) · E. Nieschlag
Center of Reproductive Medicine and Andrology, University of Münster, Münster, Germany
e-mail: Julia.Rohayem@ukmuenster.de; Eberhard.Nieschlag@ukmuenster.de



F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_10

nowadays have an excellent prognosis for fertility restoration and for paternity by natural intercourse or using assisted reproductive techniques.

**Keywords**
Central hypogonadism · Secondary hypogonadism · Tertiary hypogonadism · Functional hypogonadism · GnRH · LH · FSH · GnRH replacement · hCG/rFSH replacement · Gonadotropin replacement

## Physiology of the Hypothalamo-Pituitary-Gonadal (HPG) Axis

The male gonads have two major functions: the synthesis of androgens and the production of male gametes. Testicular function is regulated by the hypothalamo-pituitary-gonadal axis: gonadotropin-releasing hormone (GnRH), a decapeptide produced in hypothalamic GnRH-neurons, is released into the portal blood via discrete pulses and acts on specific receptors on gonadotropin-secreting cells in the anterior pituitary. The glycoprotein hormones LH and FSH are secreted into the bloodstream upon GnRH stimulation, then binding to their target cells in the male gonads. While LH binds to the LHCG receptor of Leydig cells, thereby stimulating testosterone synthesis and secretion, FSH acts on its receptor in Sertoli cells, thereby inducing spermatid maturation (spermiogenesis) during spermatogenesis. For induction and maintenance of quantitatively and qualitatively normal spermatogenesis, both gonadotropins are required (Nieschlag et al. 1999b).

Negative feedback on GnRH neurons is provided by gonadal sex steroids (testosterone, its bioactive form, dihydrotestosterone, and its derivative, estradiol) and the Sertoli cell product inhibin B. The negative feedback of androgens and of other regulatory signals (such as stress or starvation) to the hypothalamus is conveyed by KNDy cells in the hypothalamic arcuated nucleus, via the neuropeptides kisspeptin, dynorphin, and neurokinin (Lehman et al. 2010). In addition, a dual inhibitory effect at the hypothalamic and pituitary level is exerted by estrogens that are generated in adipose tissues through the aromatization of androgens (Hayes et al. 2000; Rochira et al. 2006; Pitteloud et al. 2008) (Fig. 1).

## Classification, Pathophysiology of Hypogonadism in the Male

The term **hypogonadism** is used to describe testicular dysfunction in men. Due to the dual function of the gonads, testicular disturbances may induce both testosterone deficiency and impaired fertility. While disturbed endocrine gonadal dysfunction disrupts spermatogenesis, infertility itself does not impair testosterone production.

If the functional disturbance affects the testes, this is indicated by a compensatory rise in serum gonadotropin levels. This condition is referred to as **primary or hypergonadotropic hypogonadism**. If **central** structures, i.e., either **the hypothalamus or the pituitary** function are affected, resulting in a decrease of serum

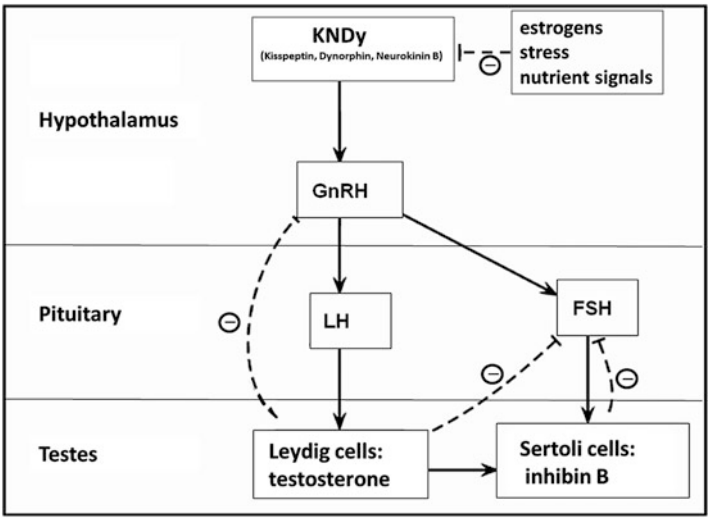


**Fig. 1** Regulation of the hypothalamo-pituitary-gonadal (HPG) axis

luteinizing hormone (LH) and follicle-stimulation hormone (FSH) levels, this is called **hypogonadotropic hypogonadism.** While the term **secondary hypogonadism** should be reserved for conditions with exclusive impairment of pituitary function, the term **tertiary hypogonadism** applies to conditions with an underlying hypothalamic disturbance, that is, with defective GnRH secretion, with the consequence of inadequate pituitary stimulation to secrete gonadotropins.

**Late-onset-hypogonadism** refers to conditions where both testicular and central functional defects are involved**.**

Symptoms of hypogonadism may also appear if LH, FSH, and testosterone serum levels are in the normal range. This is the case if the effect of gonadal sex steroids is hampered at the level of androgen receptors on target organs (**androgen insensitivity**).

This chapter deals with **central, i.e., secondary and tertiary hypogonadism**. Central hypogonadism can be either congenital or acquired.

## Tertiary/Hypothalamic Hypogonadism

### Congenital Tertiary/Hypothalamic Hypogonadism

#### Isolated Congenital HH (CHH) and Kallmann Syndrome

Conditions with congenital hypothalamic hypogonadism (CHH) are characterized by absent or inadequate pulsatile secretion of GnRH by hypothalamic neurons (Belchetz et al. 1978), i.e., by an impaired function of the "GnRH pulse generator." The term CHH includes two major conditions: Kallmann syndrome and isolated congenital hypogonadotropic hypogonadism.

While isolated CHH is defined by HH with normosmia, patients with Kallmann syndrome have an associated inability to smell. Their anosmia is a consequence of a defective development of the olfactory bulbs (Takeda et al. 1992). Kallmann patients are not always aware of their inability to perceive aromatic substances (such as soap or coffee) due to their uncompromised perception of substances that stimulate the trigeminus nerve (such as ammonia or vinegar). Therefore, it may be necessary to perform semiquantitative olfactometry to reliably identify impaired olfaction (Dunkel and Quinton 2014).

Both Kallmann syndrome and CHH may be associated with other phenotypic disorders such as dental and digital anomalies, cleft lips and palate, iris coloboma, and kidney agenesis. Patients may also exhibit neurologic features such as synkinesis (mirror movements) and cerebral ataxia.

CHH remains undiagnosed in most cases of HH until late childhood. The clinical suspicion of CHH is usually raised in boys with absent pubertal development after age 14.

As HPG axis dysfunction is already present during fetal development, maldescended testes and/or micropenis (with a stretched penile length below 2.5 cm) in the male newborn may offer a valuable clue to underlying CHH. Detection of an absent physiologic gonadotropin surge during the first 6 months of life (the so-called minipuberty) represents another opportunity for early diagnosis of the condition (Grinspon and Rey 2010).

If not recognized upon these features during infancy, CHH becomes obvious at an age when normal puberty is expected: as a consequence of insufficient increases of LH and FSH, serum levels of testosterone remain low, i.e., in the prepubertal range. Depending on the severity of hormonal deficiencies, pubertal development may either be delayed, arrested, or totally absent. The definition of "pubertal delay" is based upon pubertal onset at an age that is 2–2.5 SD later than the population mean which is accepted as 14 years in Caucasian boys. In cases with absent puberty, testicular volumes remain prepubertal (i.e., $< 4$ ml each side) after age 14. In boys with spontaneous pubertal onset with early arrest, testicular volumes increase slightly, but do not reach normal adult sizes (i.e., over 10–12 ml each side) and sperm do not appear in seminal fluids. Failure of penile enlargement, absence of pubertal body hair, absence of beard growth and of voice mutation, and nocturnal ejaculations are additional typical clinical features of pubertal failure (Table 1).

Of note, pubic and axillary hair do develop at the expected age, as they result from maturation of the adrenal glands (adrenarche) with the secretion of adrenal androgens, a process that is usually uncompromised in HH. However, psychosexual maturation (with awakening of libido), being dependent on a normal pubertal increase in androgen serum levels, does not occur.

Late epiphyseal closure in the long bones of adolescents affected by CHH is a consequence of low estrogen levels that result from low rates of testosterone aromatization in adipose tissues. Late maturation and fusion of the growth plates give rise to eunuchoid body proportions with long legs and arms (with an arm span longer than body height).

**Table 1** Symptoms of hypogonadism due to testosterone deficiency relative to age at manifestation

| Affected organ/ function | Onset of testosterone deficiency | |
|---|---|---|
| | Before completed puberty | After completed puberty |
| **Hair** | Straight frontal hairline, no androgenic hair loss/no baldness | Previous androgenic hair loss possible, arrested loss |
| **Beard growth** | No beard growth | Beard growth diminishing |
| **Body hair** | Horizontal pubic hairline | Male body hair decreasing |
| **Larynx** | No voice mutation | Accomplished voice mutation (unchanged) |
| **Skin** | Prepubertal sebum production, lack of acne, pallor | Diminished sebum production, wrinkling, hot flashes |
| **Bones** | Eunuchoid body proportions | Osteopenia ➔ osteoporosis |
| **Bone marrow** | Mild anaemia | Mild anaemia |
| **Skeletal muscles** | Underdeveloped | Hypotrophic |
| **Prostate** | Prepubertal size | Volume reduction |
| **Penis** | Prepubertal size | Adult size (unchanged) |
| **Testes** | Prepubertal volumes, maldescended testes possible | Decrease in volume and consistency |
| **Spermatogenesis** | Not initiated | Arrested: secondary oligo−/ azoospermia |
| **Ejaculate** | No emissions | Decrease in volume |
| **Libido** | Prepubertal | Decreased /lost |
| **Potency** | Spontaneous erections possible, Not sexually stimulated | Reduced erectile capacity |

The genetic basis of congenital tertiary/hypogonadotropic hypogonadism could be elucidated in less than half of the patients over the past decades.

CHH and Kallmann syndrome may occur sporadically or clustered in families. Inheritance is either autosomal-dominant, autosomal-recessive, or X-linked (Costa-Barbosa et al. 2013) and may be either monogenic or digenic (Pitteloud et al. 2007; Quaynor et al. 2011). More than 25 gene loci have been identified to date (Dwyer et al. 2015). Recently, targeted multiplex next-generation sequencing has become available, allowing for simultaneous mutation analysis of all genes known to be associated with CHH or Kallmann syndrome (Table 2).

Mutations in *KAL1* are the most common genetic cause of **anosmic CHH = Kallmann syndrome** with an X-linked (recessive) inheritance. *KAL1* is located on Xp22.3. It codes for anosmin, a protein responsible for the migration of GnRH neurons to the hypothalamus and the concomitant migration of olfactory axons to the olfactory epithelium during embryonic development. Further mutations known to cause Kallmann syndrome genes concern *SEMA3A, SOX10, FEZF1, HESX, ILI7RD*, and *NLF*.

Genes currently recognized to be involved in **both Kallmann syndrome and normosmic CHH** include *KAL2 (=FGFR1); FGF8; KAL3 (=PROK2); KAL4 (=PROKR2), HS6ST, AXL,* and *WDR11*. Heterozygous *FGFR1* mutations with an

**Table 2** Genes potentially mutated in patients with central hypogonadism

| ***Congenital hypothalamic disorder/ congenital tertiary HH*** | ***Possibly affected genes*** |
|---|---|
| **(anosmic) Kallmann syndrome** | *KAL1, SEMA3A, SOX10, FEZF1, HESX1, IL17RD, NELF* |
| **Kallmann syndrome and CHH** | *KAL2 = FGFR1; FGF8;*<br>*KAL3 = PROK2; KAL4 = PROKR2,*<br>*HS6ST, AXL, WDR11* |
| **(normosmic) CHH** | *KISS1, KISS1R = GRP54,*<br>*TAC3, TAC3R,*<br>*GNRH1,*<br>*LEP, LEPR* |
| ***Syndromic tertiary CHH*** | |
| **CHARGE syndrome** | *CHD7* |
| **Bardet-Biedl syndrome** | *BBS 1–19* |
| **Prader Willi syndrome (combined tertiary and primary HH)** | Lack of gene expression from the paternal chromosome 15q-11-q13 |
| **Gordon-Holmes syndrome**<br>**Boucher-Neuhauser syndrome** | *RNF216, OTUD4, stub, PNPLA6*<br>*PNPLA6* |
| **Combined congenital tertiary, secondary and primary HH** | |
| **CHH with associated adrenal insufficiency** | *DAX1* |
| **Congenital pituitary disorder/ Congenital secondary HH** | |
| **Congenital isolated LH deficiency (Pasqualini syndrome** | *LHß* |
| **Congenital isolated FSH deficiency** | *FSHß* |
| **Congenital combined LH and FSH-deficiency** | *GnRHR* |
| **CHH with associated multiple pituitary deficiencies** | *PROP1, PIT1 (POU1F1), LHX3, LHX4, GLI2, FGF8, KAL4 = PROKR2, HESX1* |

autosomal dominant mode of inheritance account for about 15% of (familial or sporadic) CHH or Kallmann syndrome (Dodé et al. 2003). *FGFR1,* as well as its ligand, the fibroblast growth factor 8 (*FGF8*) is involved in organogenesis (Falardeau et al. 2008); therefore patients with *FGFR1* or *FGF8* mutations may have digital bone anomalies, such as polydactyly and camptodactyly in association with CHH (Costa-Barbosa et al. 2013). *PROK2(KAL3)* codes for prokineticin 2, a chemoattractant for neural precursor cells. The gene coding for its receptor, *PROKR2*, is also referred to as *KAL4* (Dodé et al. 2006).

Mutations of the following genes have been described **exclusively in normosmic patients with CHH**: *KISS1, KISS1R (=GRP54), TAC3, TAC3R, GNRH1, GNRHR, LEP* (Strobel et al. 1998), and *LEPR* (Clement et al. 1998).

GPR54 and its ligands, the Kisspeptins, play an essential role in GnRH secretion (De Roux et al. 2003; Seminara et al. 2003). Likewise, the neuropeptide neurokinin B

has been recognized as a critical central regulator of GnRH secretion, since mutations in its coding gene *TAC3* or its receptor *TACR* cause CHH (Topaloglu et al. 2009).

An obvious candidate gene for CHH, the *GNRH1* gene, has been identified as a rare cause of normosmic isolated GnRH deficiency in humans, with an autosomal recessive trait. The *GNRH1* gene encodes the pre-prohormone of GnRH (Bouligand et al. 2009; Chan et al. 2009). Mutations may result in both mild and severe forms of GnRH deficiency.

(For *GnRHR* gene mutations see chapter "Congenital Combined LH and FSH-Deficiency")

In patients with normosmic congenital hypogonadotropic hypogonadism and with extreme obesity, inactivating mutations in *LEP* or the gene coding for its receptor *LEPR* have been detected (Clément et al. 1998; Strobel et al. 1998; Fischer-Posovszky et al. 2010). Leptin is a fat-derived hormone that regulates food intake, energy expenditure, and hypothalamic reproductive function. Treatment with recombinant leptin has been shown to restore LH pulses and normalize testosterone levels, besides normalizing weight in morbidly obese homozygous leptin-deficient men (Licinio et al. 2004).

### Tertiary HH Due to Syndromic Disorders

Patients with the **CHARGE syndrome** (**c**oloboma, **h**eart failure, **a**tresia choanae, **r**etarded growth and development, **g**enital and **e**ar malformations) also suffer from hypogonadotropic hypogonadism. The syndrome may be caused by an autosomal-dominant mutation of the *CHD7* gene (Janssen et al. 2012).

Patients with a **Prader-Labhart-Willi syndrome** are affected by a combination of tertiary and primary hypogonadotropic hypogonadism (Eiholzer et al. 2006). The syndrome is caused by a reduced expression of genes inherited from the paternal chromosome 15q-11-q13 (Butler et al. 2015) (Table 2). Newborns and infants often come to clinical attention because of a generalized muscular hypotonia. Delayed motor development, moderate mental retardation, and early obesity are characteristic features. If not treated with growth hormone, male patients hardly reach an adult height of 160 cm. Male genitalia remains hypoplastic at a pubertal age, and pubertal development does not progress spontaneously with resulting infertility.

Patients affected by the **Bardet-Biedl syndrome** may have hypogonadotropic hypogonadism. This autosomal-recessive multisystemic syndrome is characterized by obesity, mental retardation, kidney malformations, polydactylia, retinal degeneration, and hypogonadism. Nineteen gene mutations on various chromosomes BBS1–19 have been described to date (Khan et al. 2016).

**Gordon-Holmes syndrome** refers to a condition with hypogonadotropic hypogonadism combined with cerebellar ataxia. The syndrome is thought to be associated with mutations in genes involved in regulation of autophagy of the cells' own cytosolic components (or protein aggregates), a cellular process concerned with cellular homeostasis. So far, mutations in the following genes have been described: *RNF216, OTUD4, STUB1,* and *PNPLA6* (Alqwaifly and Bohlega 2016).

**Boucher-Neuhauser syndrome** is a neurodegenerative disorder, characterized by an association of progressive cerebellar degeneration (with early onset ataxia),

hypogonadotropic hypogonadism and chorioretinal dystrophy. It has recently been linked to autosomal-recessive mutations in the *PNPLA6* gene (Tarnutzer et al. 2015). PNPLA6 codes for a precursor for the synthesis of acetylcholine.

### Tertiary HH Combined with Adrenal Insufficiency: X-Linked Adrenal Hypoplasia

Congenita (AHC)

If central hypogonadotropic hypogonadism is **associated with adrenal insufficiency**, DAX1 mutations may be the cause (Habiby et al. 1996; Achermann et al. 2001; Jadhav et al. 2011). The rare hypogonadotropic condition named X-linked adrenal hypoplasia congenita (AHC) results from a combined and variable deficiency of hypothalamic GnRH secretion and/or impaired pituitary responsiveness to GnRH. The dosage sensitive sex-reversal-1 (DAX-1) gene is located on the short arm of the X-chromosome in the region Xp21.3–21.2 (Table 2).

The majority of affected newborn patients are diagnosed within the first 2 months because of a life-threatening adrenal insufficiency crisis. Some patients have residual cortisol or mineralocorticoid production (Mantovani et al. 2002), giving rise to hypogonadotropic hypogonadism without or with subclinical adrenal insufficiency. Replacement of pulsatile GnRH does not induce adequate LH- and FSH stimulation and treatment with hCG and FSH may not sufficiently stimulate testosterone production and spermatogenesis, revealing a further testicular defect, in addition to the secondary and tertiary defect of the HPG axis. In line with this clinical observation, animal models indicate that *DAX1* plays a critical role in testis development and function. DAX1 functions as a transcriptional repressor, particularly of pathways regulated by other nuclear receptors, such as steroidogenic factor 1 (SF1). It also acts as a negative coregulator of the estrogen receptor (ER, *NR3A1–2*), the liver receptor homologue-1 (LRH-1, *NR5A2*), the androgen receptor (AR, *NR3C4*), and the progesterone receptor (PR, *NR3C3*), each by distinct repression mechanisms. However, how disruption of DAX1 leads to adrenal, hypothalamic, and pituitary developmental defects similar to SF1 disruption remains to be clarified. The treatment of choice of this complex HPG axis impairment, in addition to gluco- and mineralocorticoid replacement, is therefore testosterone. Most patients are primarily azoospermic and testicular histology reveals a Sertoli-cell-only syndrome. However, in patients with residual hormonal activity, isolated foci with spermatogenesis may be found. In these cases, single sperm may be extracted from biopsied tissue and intraplasmatic sperm injection (ICSI) may result in pregnancy and live birth (Frapsauce et al. 2011).

## Acquired Tertiary/Hypothalamic Hypogonadism

### Functional Hypogonadotropic Hypogonadism

In this potentially reversible condition, hypothalamic GnRH pulsatility is downregulated. The phenomenon can be observed in men with severe malnutrition, such as in restrictive eating disorders (anorexia nervosa or starvation due to poverty or malignant diseases) (Wabitsch et al. 2001, with malabsorption (in inflammatory

bowel diseases, celiac disease, or cystic fibrosis), or in patients with chronic diseases. Functional HH may also be caused by excessive obesity with insulin resistance or type 2 diabetes (Dhindsa et al. 2010). Downregulation of GnRH pulse generator activity is also observed in athletes performing extreme exercise or overtraining, resulting in decrease of testosterone and sperm production (Nieschlag and Vorona 2015; Tenforde et al. 2016). Rarely is it due to serious stress, including severe social deprivation or depression. The indication for central hormone replacement has to be based upon the chances for resolution of the underlying condition with causal treatment.

### Drug-Induced Hypogonadotropic Hypogonadism

Prolonged abuse of anabolic androgenic steroids (AAS) (including testosterone preparations) suppresses the HPG axis (Nieschlag and Vorona 2015). As shown in clinical trials of testosterone-based hormonal male contraception, suppression is reversible if androgen application is ceased (Nieschlag 2010); however, reactivation of the GnRH pulse generator may require 3–24 months (Liu et al. 2006). In these men, cessation of exogenous hormone administration or abuse is indicated, rather than initiation of gonadotropin replacement to overcome the AAS-induced hypogonadism (Nieschlag and Vorona 2015).

Inhibition of GnRH secretion occurs with the use of morphine, heroin, and methadone, all activating inhibitory neurons to the hypothalamus (Daniell 2002; Rajagopal et al. 2004). Iatrogenic HPG axis downregulation also occurs as a result of GnRH agonist treatment (for precocious puberty in boys and during antiandrogenic treatment of metastatic prostate cancer in adults). Therefore, inquiries on the recent intake of medications and on drug abuse are essential to recognize potentially reversible causes of HH.

### Congenital Adrenal Hyperplasia (CAH) with Hypogonadotropic Hypogonadism

Undiagnosed or untreated classic congenital adrenal hypoplasia (CAH) due to 21-hydroxylase deficiency results in ACTH hypersecretion, giving rise to adrenal androgen hypersecretion. Adrenal androgens or their metabolites (e.g., estrone, originating from the aromatization of androstenedione) may suppress the hypothalamic GnRH-pulse generator and thus the secretion of pituitary LH and FSH. As a consequence, spermatogenesis is inhibited, resulting in oligozoospermia or azoospermia. However, adrenally derived testosterone in the blood steam is indistinguishable from testicularly derived testosterone, and near normal serum levels prevent symptoms of androgen deficiency. Therefore, the diagnosis has to be made upon measurement of 17-hydroxyprogesterone levels (in serum or saliva).

In males with salt-wasting CAH and tertiary hypogonadism, the cause of infertility is nonadherence or insufficient adherence to treatment. In milder/simple virilizing forms of CAH, the diagnosis of CAH may not have been made unless infertility, combined with suppressed LH and FSH levels, but low-normal serum testosterone levels, elevated 17-OHP serum levels, and hyperplasia of "testicular adrenal rests" in the testes (TART) are detected. The latter may be mistaken for

malign testicular tumors. However, as TART develop bilaterally, due to hyperplasia of adrenal cells that physiologically surround the rete testis, these tumors are always benign and do not require resection.

Adequate replacement of glucocorticoids in CAH men with hypogonadotropic hypogonadism may restore GnRH pulsatility and consequently resume gonadotropin suppression via normalization of ACTH-drive on the adrenal glands. In rare cases, LH and FSH suppression is prolonged. In men wishing paternity and without recovery of gonadotropin secretion on corticoid- (and mineralocorticoid) replacement, gonadotropin substitution with hCG and rFSH is a therapeutic option to induce spermatogenesis (Rohayem et al. 2014). Surgical resection of TART has been shown to be unsuccessful for improving semen quality (Claahsen -van der Grinten et al. 2007).

## Secondary/Pituitary Hypogonadism

### Congenital Secondary/Pituitary Hypogonadism

Isolated deficiencies of LH or FSH, each resulting from mutations of the corresponding genes, are very rare causes of defective pubertal maturation. *LHB* and *FSHB* are the genes coding for the unique ß-subunits that confer specificity of action of the heterodimeric glycoprotein-hormones LH and FSH on their corresponding gonadotropin receptors, in contrast to the common alpha-subunit of these glycoprotein hormones (Table 2).

#### Congenital Isolated LH Deficiency (Pasqualini Syndrome)

In this condition, **homozygous or compound heterozygous mutations of the LHß gene** cause the absence or functional deficiency of LH (Lofrano-Porto et al. 2007). Typically, affected male patients have absent puberty, small testes, a prepubertal penis size with LH serum levels below the detection limit, accompanied by prepubertally low serum testosterone levels, but normal or elevated FSH levels and normal inhibin B levels. Spermatogenesis may be reduced or arrested as Leydig cells are not developed (Matthews et al. 1993; Phillip et al. 1998; Lofrano-Porto et al. 2007; Valdes-Socin et al. 2014). Treatment with hCG causes the normalization of testosterone serum levels and spermatogenesis. Testosterone replacement may result in a slight increase of testicular volumes (to 6–7 ml) in the presence of high FSH-levels (Lofrano-Porto et al. 2007).

#### Congenital Isolated FSH Deficiency

Patients with **isolated FSH deficiency** have a normal pubertal development and are normally virilized men, but their testes remain small/of prepubescent size and their ejaculates are azoospermic. Their FSH serum levels are undetectable, while LH levels are normal or increased.

The lack of FSH is caused by inactivating **homozygous mutations in the FSH-β gene**. Treatment with rFSH over 6 months may increase testicular volumes (Simşek

et al. 2016). However, it is not clear from the literature whether spermatogenesis can be fully developed to produce sperm sufficient for paternity (Siegel et al. 2013).

### Congenital Combined LH and FSH-Deficiency Due to GnRHR Mutations

Homozygous *GnRHR* mutations have been found to cause pituitary GnRH insensitivity by impairing GnRH receptor function in pituitary gonadotropin-secreting cells, with ensuing deficiencies in LH/FSH (De Roux et al. 1997; Layman et al. 1998; Beneduzzi et al. 2014). Depending on the degree of functional receptor impairment, patients may experience absence of pubertal development or pubertal arrest.

### Congenital Pituitary Defects Causing Multiple Pituitary Hormone Deficiencies (MPHD)

If multiple pituitary hormonal deficiencies are associated with gonadotropin deficiency, mutations of *PROP1, PIT1 (=POU1F1), LHX3* (Netchine et al. 2000)*, LHX4, GLI2, FGF8, KAL4 (=PROKR2)*, and *HESX1* may be involved.

The *PROP-1* gene encodes the transcription factor PROP-1 (acronym for "prophet of Pit") that is required for the expression of *PIT1*. Pit1-dependent cell lines in the anterior pituitary include somatotrophs, lactotrophs and thyrotrophs and variably gonadotrophs and corticotrophs (Wu et al. 1998).

## Acquired Secondary/Pituitary Hypogonadism

Acquired forms of HH are mostly due to pituitary tumors (craniopharyngeoma, pituitary adenoma, glioma) or surgery in the sella region. Pituitary failure with multiple pituitary hormone deficiencies may also result from cranial radiotherapy. Likewise, inflammatory processes (hypophysitis), iron overload (due to hemochromatosis or repetitive erythrocyte transfusions), granulomatosis, and vascular disorders can lead to pituitary insufficiency (Behre et al. 2010). Trauma from accidents, sports (e.g., boxing, football, soccer), or military activities are often overlooked as cause of hypopituitarism and especially when secondary hypogonadism occurs as an isolated posttraumatic sequela (Karaca et al. 2016; Kelly et al. 2014).

Endocrine deficiencies are to be expected if more than 75% of the anterior pituitary tissue is destroyed. Partial forms of HH with residual testosterone secretion and less severe symptoms of androgen deficiency and residual spermatogenesis may result from all above-mentioned conditions (Spratt et al. 1987; Waldstreicher et al. 1996).

In addition to endocrine deficiencies, pituitary tumors may cause headache and visual field defects due to their location and size.

Prolactin-secreting pituitary micro- or macroadenomas cause hypogonadism by a dual mechanism: prolactin itself induces a suppression of pituitary gonadotropin secretion; in addition, the adenoma displaces and compresses normal pituitary tissue, thereby impairing hormone secretion.

Hyperprolactinemia may also be caused pharmacologically by various prescribed drugs (e.g., H2-blockers, metoclopramide, imipramine, alpha-methyldopa, neuroleptic agents) Pharmacological hyperprolactinemia is best treated by eliminating the

respective drug. Surgical or radiologic removal of a prolactin-secreting macroadenoma has become necessary only in rare cases, as adenoma-caused hyperprolactinemia is primarily treated by dopamine agonists such as bromocriptine, cabergoline, quinagolide, or metergoline. These drugs are effective in suppressing hyperprolactinemia and adenoma growth so that additional testosterone or gonadotropin treatment is rarely required. Rarely, dopamine agonist treatment of prolactinomas may lead to hypersexuality. This condition, named "Dopa-testotoxicosis" is assumed to be induced by synergy between reward pathway stimulation and restoration of the eugonadal state after prolonged hypogonadism (De Sousa et al. 2017).

## Diagnosis of Central Hypogonadism in the Male

During adolescence, hypogonadotropic hypogonadism is suspected in boys with delayed, absent, or partial and then arrested puberty and prepubescent gonadotropin serum levels. In adulthood, suspicion is raised by symptoms of testosterone deficiency (Table 1) and/or infertility, combined with low LH and FSH levels.

Suspicion of permanent HH has to be based upon meticulous pretreatment diagnostic work-up.

### Diagnostic Work-Up

Decreased energy levels, fatigue and loss of libido are the first objective **signs of testosterone deficiency**. Depressive mood, lack of concentration, sleep disturbance, and erectile dysfunction are additional typical complaints. However, the patient may not spontaneously report erectile dysfunction due to reduction of his sexual desire. Therefore, the question regarding sexuality has to be explored explicitly. A standardized hypogonadism symptom questionnaire may help reveal this and other sensitive anamnestic items (Gelhorn et al. 2016). Further symptoms of androgen deficiency include the arrest of spermatogenesis, reduction of seminal fluid, ejaculatory frequency, and sperm count in semen. In the long term, reduction of body hair and beard growth, muscle and bone mineral mass loss (osteoporosis), and anemia will develop.

**Family history** should assess the parents' age at entering puberty and age at maternal menarche and the presence of signs of hypogonadism or infertility in relatives.

**Previous medical history** assesses the presence of undescended testes at birth (with age at possible orchidopexy) and previous diseases, including malignancies with chemo- and/or radiotherapy, inflammatory diseases (meningitis, orchitis), and testicular trauma or surgery. Inquiries on the ability to smell and on specific congenital features (that may be associated with congenital HH or Kallmann syndrome), including renal agenesis, dental and digital anomalies, cleft lip and palate, coloboma, synkinesia/mirror movements, and ataxia are similarly important.

**Physical examination** includes auxological measurements and pubertal Tanner staging with measurement of testicular volumes using a **Prader orchiometer**.

**Scrotal ultrasound** provides information on testicular and epididymal morphology, in addition to the (more accurate) assessment of testicular volumes.

While **hormone investigations** of the HPG axis focus on LH, FSH, and testosterone levels, measurement of TSH, T3, fT4, IGF1, IGFBP3, prolactin, and cortisol enables investigations of associated pituitary and adrenal hormone deficiencies.

**Other laboratory analyses and function tests** with the measurement of inhibin B levels and GnRH agonist (buserelin) testing may help differentiate HH from CDGP.

**Further diagnostic work-up** includes imaging procedures, such as **magnetic resonance imaging** (**MRI)** of the hypothalamo-pituitary region to rule out intracranial malformations, neoplasms, or infiltrating diseases. **Perimetry of visual fields** is helpful for detecting scotomas, specifically bitemporal hemianopsia in case of compression of the chiasma opticum by neoplasms in the sella region. Determination of bone age according to Greulich and Pyle (1959) by a **left-hand carpo-radiogram** is used to estimate residual longitudinal growth potential in adolescents with absent or arrested puberty. **Semiquantitative olfactometry** by University of Pennsylvania Smell Identification Test (UPSIT) (Doty et al. 1995) or Sniffin'sticks (Burghart Messtechnik GmbH, Wedel, Germany) (Hummel et al. 2007) can be performed to reliably distinguish between normal, partially, or totally defective olfaction.

**Semen analysis** is relevant to evaluate exocrine testicular function, especially if paternity is desired and should be performed according to the "WHO Laboratory Manual for Semen Analysis" (2010) under quality control.

**Karyotyping (+ fluorescence in situ hybridization (FISH))** may be undertaken to rule out associated chromosomal anomalies. **Sequencing of candidate genes or performance of targeted multiplex NGS** in subjects with CHH with the description of mutations or polymorphisms is indicated for investigations on the genetic origin of HH. Results are also helpful for genetic counseling of a couple affected by male CHH prior to hormone replacement for spermatogenic induction.

## Differential Diagnosis of Tertiary Hypogonadism: Constitutional Delay of Growth and Puberty (CDGP)

In boys presenting for delayed puberty or growth retardation, the most important differential diagnosis to congenital normosmic HH is the normal variant of a constitutional (self-limited) delay of growth and puberty (CDGP). CDGP is by far the most common cause of delayed puberty (pubertas tarda): up to 65% of boys with pubertal delay have CDGP (Sedlmeyer and Palmert 2002).

Once testosterone or gonadotropin replacement has been started, this measure will suppress the endogenous GnRH pulse generator activity and gonadotropin secretion, thereby impeding proper diagnosis. Therefore, it is imperative to previously establish the differential diagnosis between self-limited/potentially reversible hypogonadotropic states and permanent disorders.

Hormone profiles in CDGP are infantile and therefore indistinguishable from hypogonadotropic hypogonadism. A family history of late pubertal development can be found in 50–75% of subjects with CDGP (Wehkalampi et al. 2008; Sedlmeyer and Palmert 2002). Other indicators of CDGP are short stature for chronologic age, in concert with delayed bone age. This is due to a transitory partial growth hormone deficiency which requires no treatment.

A variety of physiological and stimulation tests have been proposed to differentiate between CHH and CDGP (Harrington and Palmert 2012), such as gonadotropin response to GnRH, testosterone response to hCG (Bang et al. 2017), assessment of LH pulsatility by frequent sampling, first morning-voided urinary LH and FSH, and prolactin response to various provocations.

Low-serum levels of the Sertoli cell marker inhibin B may be the best indicator of HH in prepubertal boys. Inhibin B levels below cut-off values of 35 pg/mL (Coutant et al. 2010) or 28.5 pg/mL (Rohayem et al. 2015) are indicative of HH. In addition, pituitary functional testing with the GnRH agonist buserelin, resulting in stimulated LH levels above 4 U/L after 4 h may be helpful to identify boys with CDGP among those with pubertal delay (Wilson et al. 2006). However, due to overlap in the results of all tests between the two entities, the diagnostic process remains challenging.

While reassurance and a "wait-and-see" approach may suit many boys with CDGP, in case of remaining diagnostic uncertainty, "priming" with testosterone may be performed, using three (to six)-monthly 50–100 mg testosterone enanthate i.m. injections every 4 weeks. A rise in LH serum levels on consequent reassessment of HPG axis function after additional 3 months without treatment, together with testicular growth above 4 ml each side would indicate pubertal activation of the GnRH pulse generator, thus CDGP, with no need for further hormone substitution. The above-mentioned procedure can be repeated if necessary (Soliman and De Sanctis 2012).

## Hormonal Treatment of Central Hypogonadism in the Male

In all HH men, symptoms of androgen deficiency are the most prevalent complaints. Consequently, substitution with testosterone appears to be the first choice of treatment. Indeed, due to the rapid onset of testosterone action, this treatment is satisfying for the patient as well as for the attending physician. However, besides androgen deficiency, infertility is or will be a problem for many afflicted men. Therefore, induction of spermatogenesis should be prospectively discussed and possibly initiated even in patients without immediate wish for paternity. Hormone replacement strategies in adults with hypogonadotropic hypogonadism differ from those used in adolescents concerning initial dosages.

Generally, adults have already attained normal adult stature, thus eliminating the therapeutic conflicts between maximizing final height (according to genetic target height) and promptness of pubertal induction.

In boys with HH, however, one major therapeutic goal is to induce the pubertal growth spurt. Of note, structural changes of the brain during **adolescence** are

dynamic and protracted, occurring over the course of a decade or more and encompass not only reproductive maturation, but also cognitive, emotional, and social maturation. These behavioral maturational processes may and may not be influenced by gonadal steroid hormones (Sisk and Zehr 2005).

Independent of age, hormonal substitution aims at inducing and maintaining secondary sexual characteristics (penile growth, beard and body hair growth, voice mutation, and muscle and bone mass acquisition) and psychosexual maturation/activation (awakening of libido with sexually stimulated erections and regular ejaculations), thereby improving self-esteem and well-being.

If puberty is induced at an adult age, after psychosocial adaption to the undervirilized/juvenile appearance has occurred, psychological changes affecting partnership may ensue. It remains to be seen whether late hormone substitution in adulthood reverses all effects of low androgen levels during adolescence, as differences in spatial ability and of some reproductive behavioral aspects of psychosocial development have been reported in small patient cohorts (Hier and Crowley 1982; Gooren 1988).

The therapeutic goal of normalizing serum testosterone levels according to age references can be achieved by replacement of testosterone, GnRH, or gonadotropins. In the following, the indications for these therapies are described.

## Pulsatile GnRH Substitution

In patients with HH due to hypothalamic GnRH deficiency, but normal pituitary function, GnRH is the most physiological but cumbersome treatment: As continuous application of GnRH results in downregulation of pituitary GnRH receptors, GnRH has to be substituted in a pulsatile fashion. This is performed by a portable minipump which has to be worn for at least one to 2 years and sometimes longer to stimulate gonadotropin secretion in the pituitary and consequently testosterone secretion and spermatogenesis in the testes. Only few patients are motivated to be compliant with this treatment modality for longer periods (Delemarre-van de Waal 2004). A recent study from China demonstrated that those patients who adhere to GnRH replacement may be rewarded by faster testicular growth and appearance of sperm (Mao et al. 2016) compared to gonadotropin replacement regimens, in contrast to earlier observations with no differences in outcome (Schopohl 1993; Büchter et al. 1998).

The needle of the catheter linked to the portable GnRH mini pump is placed into the patient's abdominal subcutaneous tissue and has to be changed every 2 days. The pump is programmed to deliver GnRH boluses every 120 minutes, as this frequency was shown to be most effective for gonadotropin stimulation. After a starting dose of 4 μg per pulse, increases of 2 μg may be performed every 4 weeks, aiming at testosterone levels in the normal adult range after 3–12 months (depending on the maturation progress of the testes). Response to treatment is monitored by the assessment of testicular growth, testosterone serum levels, and appearance of sperm in the ejaculate. The doses necessary to induce spermatogenesis vary between

HH patients, ranging 5–20 μg GnRH per bolus or 25–600 ng/kg GnRH per bolus. One to 3 years of treatment may be required to induce spermatogenesis sufficient to induce a pregnancy. Patients with previously undescended testes may require considerably longer treatment for induction of spermatogenesis than patients with eutopic testes (Büchter et al. 1998).

It has recently been shown that infusion of kisspeptin-54 is efficient in restoring GnRH and LH-pulsatility in patients with a mutation in *TAC3* or *TAC3R* with a loss of signaling by neurokinin B (Young et al. 2013). These data have had implications for the development of kisspeptin 54 as an efficacious trigger of oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS) during in vitro fertilization (IVF) therapy (Abbara et al. 2015). Currently kisspeptin is undergoing testing for diagnosis of hypogonadotropic hypogonadism (Chan et al. 2014).

## Gonadotropin Substitution

### General Considerations

While GnRH treatment is reserved for patients with hypogonadotropic hypogonadism due to lack of GnRH synthesis or action, but with preserved capacity for gonadotropin secretion, gonadotropin substitution can be applied to all hypogonadotropic patients (Boehm et al. 2015) (Table 3). This applies to those men, who, besides virilization, aim for fertility and/or testicular growth. The subcutaneous administration of human chorionic gonadotropin (hCG) serves as a substitute for LH and stimulates Leydig cells to secrete testosterone (Büchter et al. 1998; Liu et al. 1988). This effect of hCG is enhanced in combination with FSH.

Follicle-stimulating hormone (FSH) is required for spermatid maturation (spermiogenesis) during initiation, and for maintenance of quantitatively normal spermatogenesis at puberty and thereafter (Matsumoto et al. 1986, 2009).

While available hCG preparations are derived from the urine of pregnant women, FSH preparations are either urinary-derived from postmenopausal women (hMG)

**Table 3** Therapeutic options licensed for puberty induction and fertility treatment in male hypogonadotropic hypogonadism

| *Drug* | *Trade name* | *Application* | *Dose* |
|---|---|---|---|
| **Human chorionic gonadotropin (hCG)** | Brevactid®[a]<br>Pregnyl®[b]<br>Novarel®[b] | s.c. or i.m. | 1000–2500 IU s.c. twice weekly<br>(on Mondays and Fridays) |
| **Recombinant FSH (rFSH)** | Gonal F®[a],<br>Puregon®[a]<br>Follistim®[b] | s.c. or i.m. | 75–150 IU s.c.<br>three times weekly<br>(on Mondays, Wednesdays, Fridays) |
| **Pulsatile GnRH** | LutrePulse®[a] | Minipump s.c. | 4–20 μg per pulse<br>every 120 min |

[a]Trade names of drugs marketed in Europe
[b]Trade names of drugs marketed in the USA

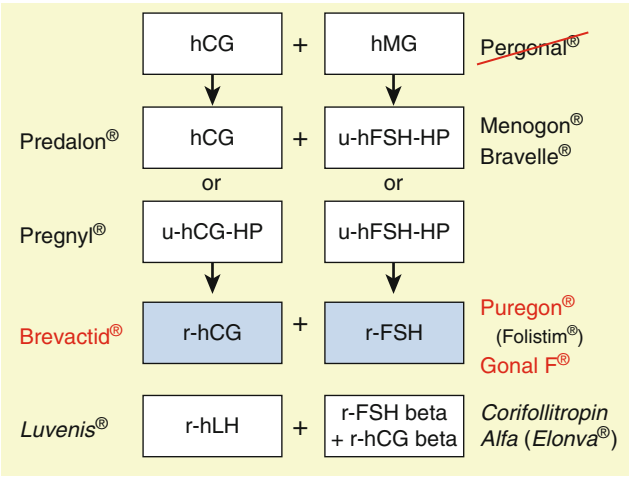


**Fig. 2** Historical development of gonadotropin treatment for secondary or tertiary hypogonadism. The blue fields signify the preparations in use, the lower white fields indicate preparations under development

and purified, or recombinant (*rFSH*) (Kliesch et al. 1995; Bouloux et al. 2003; Warne et al. 2009). The long-acting recombinant FSH-CTP (Corifollitropin alfa/MK 8962) has to be subcutaneously injected only every second week. It has already been licensed for female indications and is at this time (2017) under investigation for use in the treatment of male hypogonadotropic hypogonadism (Nieschlag et al. 2017) (Fig. 2).

Induction of spermatogenesis and achievement of fertility in males can be attained within 6 months to 2–2.5 years, depending on previous testicular maturation before initiation of treatment. Subjects with initially absent puberty require longer replacement to achieve maximum testicular growth and sperm concentrations in their ejaculate than those with pubertal arrest or postpubertal onset of hypogonadotropic hypogonadism. In these patients, final testicular volumes often plateau at a subnormal level (Fig. 3) and maximal seminal sperm counts do not reach normal values. However, quality of spermatozoa seems to be high, as most patients are nevertheless able to impregnate their partner spontaneously (Burris et al. 1988; Pitteloud et al. 2002; Liu et al. 2002; Rohayem et al. 2016) (Fig. 4).

Once a gonadotropin treatment cycle has been completed, this reduces time to reinduction of fertility to 6–10 months by repeating this treatment in later years (Liu et al. 1988; Büchter et al. 1998). Spermatogenesis can be maintained at a lower level with hCG alone for some time (Depenbusch et al. 2002).

Maldescended testes, although a negative predictor of GnRH and gonadotropin response, do not preclude chances of attaining fertility, but require longer stimulation (Büchter et al. 1998).

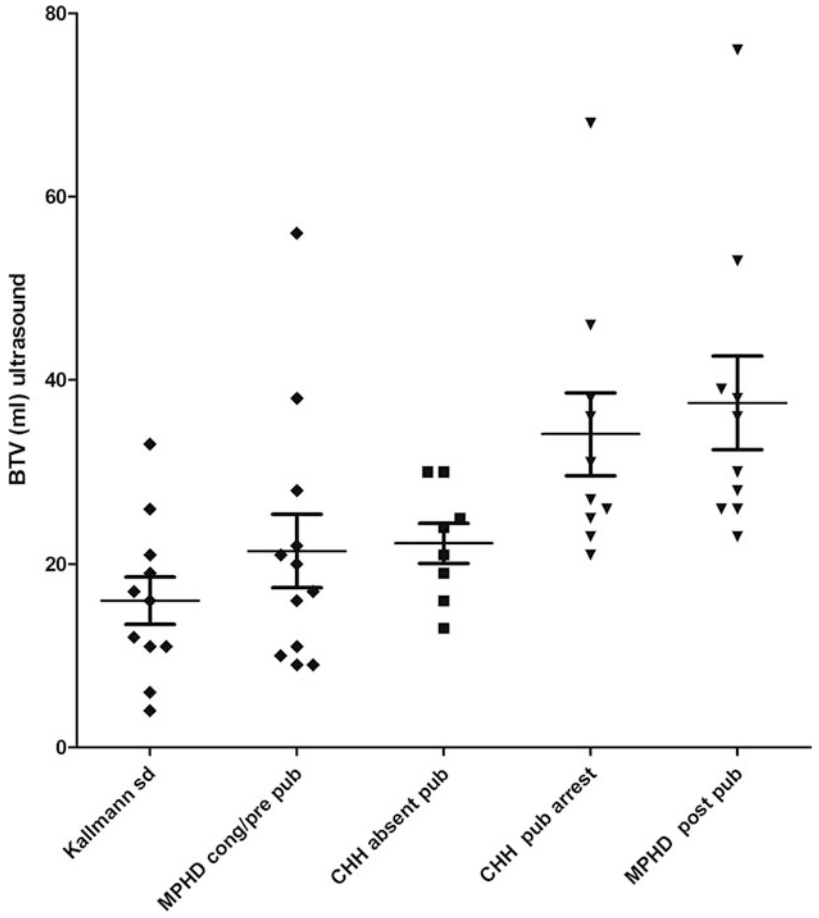


**Fig. 3** Final bitesticular volumes (BTV) in 38 adult patients with hypogonadotropic hypogonadism under hCG/rFSH treatment correlate with onset of disease (Rohayem et al. 2016). Kallmann sd: Kallmann syndrome; MPHD cong/pr pub: congenital or pre pubertally acquired multiple pituitary hormone deficiencies; CHH absent pub: congenital hypogonadotropic hypogonadism with absent puberty; CHH pub arrest: congenital hypogonadotropic hypogonadism with spontaneously initiated puberty, with consequent pubertal arrest; MPHD post pub: postpubertally acquired multiple pituitary hormone deficiencies (by surgery or tumour in the sella region)

## Hormone Replacement in Prepubertal Onset HH

A treatment regimen beginning with testosterone for induction of puberty stimulates normal linear growth, pubertal virilization, and psychosexual maturation in congenital HH. This is achieved by increasing doses of testosterone enanthate i.m., starting with 50 mg every 4 weeks. The dose is increased to 125 mg after 6–12 months and further increased to the full replacement dose for adult men: 250 mg after 1.5–2 years. If the adolescent feels that the effect of testosterone vanishes before the next injection is due, the interval of shots can be reduced to 3 weeks.

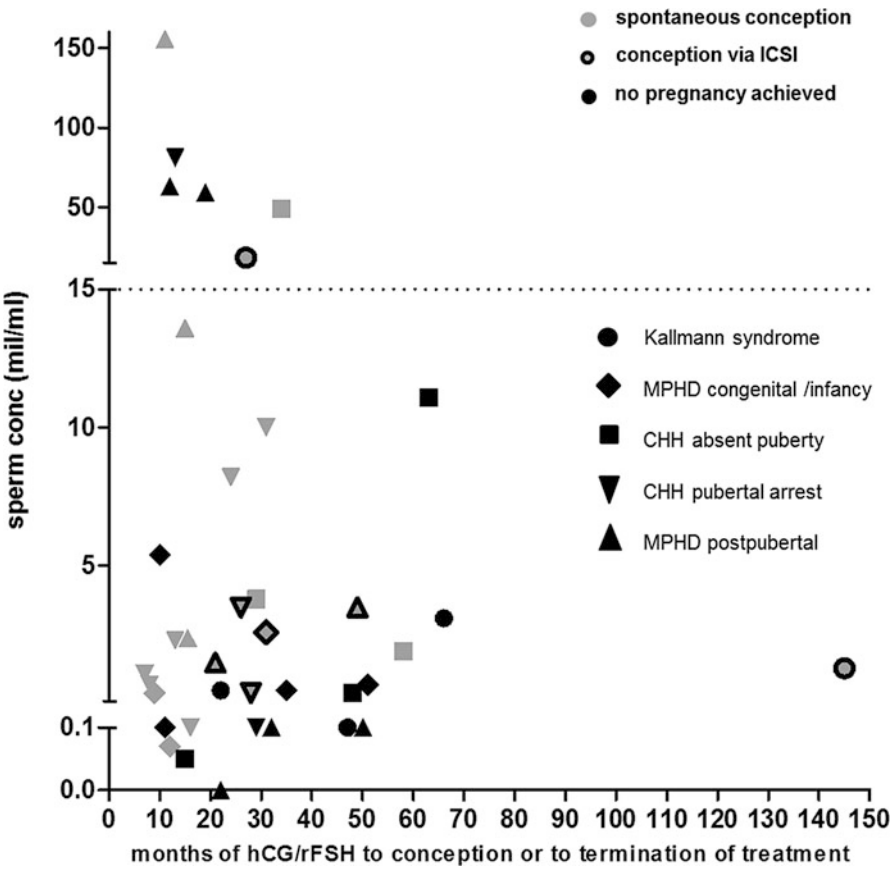


**Fig. 4** Sperm concentrations at conception or termination of hCG/rFSH from 38 adult men with hypogonadotropic hypogonadism of different ethiology (Rohayem et al. 2016)

However, this traditional approach neglects testicular growth and the acquisition of fertility as components of normal puberty. The testes remain immature and small (< 4 ml each side), i.e., in a prepubertal state, and spermatogenesis is not initiated. Meanwhile, there is sufficient evidence that complete male puberty, comprising pubertal virilization in concert with testicular growth and initiation of spermatogenesis can be successfully achieved during adolescence by replacing gonadotropins (hCG/FSH) (Liu et al. 1988; Schopohl 1993; Barrio et al. 1999; Sinisi et al. 2008; Zacharin et al. 2012; Rohayem et al. 2017).

Gonadotropin treatment may be suggested for pubertal induction even if fertility is a matter for the future, and even though rFSH is costly, as normalization of testicular size and initiation of spermatogenesis may positively affect body image, thus providing self-assurance and confidence for the future in teenage boys with HH (Shiraishi et al. 2014, Rohayem et al. 2017). In addition, spermatogenesis, once driven to full maturation, can be restimulated much faster when fertility is desired

later in life (Büchter et al. 1998; Liu et al. 2002). Subsequent treatment with testosterone does not seem to jeopardize the outcome of later stimulation therapy (Rohayem et al. 2017). This is important for a few prepubertal boys who are unwilling to commit to five s.c. injections per week for 2–3 years. However, some of these young patients may be more agreeable to gonadotropin stimulation once pubertal induction with testosterone is accomplished (Rogol 2005).

In adolescent patients with open epiphyses (i.e., with a bone age <16 years), hCG treatment ought to be performed in small increments over the first year (until serum testosterone levels are in the normal adult range) to induce pubertal virilization. Thereby a pubertal growth spurt and attainment of final height in the range of midparental expectations can be achieved. Adverse pubertal effects including premature epiphyseal fusion, severe acne, or gynecomastia can successfully be avoided by this regimen. Subsequent combined hCG/rFSH replacement over 2–3 years is required to accomplish testicular growth and fully activate the individual's spermatogenic potential. Over 70% of adolescents will thereby reach normal adult testicular sizes and in more than 90% sperm will develop in semen (Zacharin et al. 2012; Rohayem et al. 2017).

In view of the challenging differential diagnosis of constitutional delay of puberty (CDGP), this regimen bears an important further advantage: low initial hCG doses have only weak suppressive properties at the hypothalamic or pituitary level, therefore activation of the pubertal GnRH pulse generator in cases of unrecognized CDGP is possible. Patients wrongly diagnosed with HH may thus be recognized if special attention to a potential rise in LH levels is given during the first phase of gonadotropin replacement.

In patients with pubertal failure but a late diagnosis of CHH in adulthood, or those with previous testosterone treatment, final height may already have been attained. In these adult subjects, higher initial hCG replacement doses and faster dose escalations can be applied than in HH boys with open epiphyses/immature growth plates.

Success rates concerning spermatogenic induction in adults are slightly below those of adolescents, ranging from 65% to 90% (Büchter et al. 1998; Burgués and Calderon 1997; EMHSG 1998; Bouloux et al. 2002).

### Treatment Protocol for Testosterone-Naïve Prepubertal Adolescents

A starting dose of (250-) 500 IU hCG, injected subcutaneously on Mondays and Fridays, with incremental increases of 250–500 IU hCG per injection every 6 months to a maximum of 3 × 2500 IU hCG s.c./week is recommended. The aim is to achieve pubertal levels of serum testosterone (≥1.5 ng/ml, [5.2 nmol/l]) after around 6 months, and levels in the midnormal adult range (testosterone >3.5 ng/ml, [12 nmol/l]) by 1 year. rFSH (follitropin alpha) 3 × (75-)150 IUs.c/week (injected Mondays, Wednesdays, and Fridays) is added when pubertal serum testosterone levels (>5.2 nmol/l) are reached. Subsequent rFSH dosage modifications above 150 IU per injection are not recommended as they are not beneficial for enhancement of spermatogenesis.

### Treatment Protocol for Testosterone-Virilized Adolescents with Prepubertal Onset HH

These patients can be treated like post- pubertal adult patients (see section "Gonadotropin Replacement in Postpubertal Onset/Adult HH").

### Gonadotropin Replacement in Postpubertal Onset/Adult HH

In patients with postpubertally acquired HH, testicular maturation has already been completed, as indicated by normal adult testicular size. However, if gonadotropin stimulation has been lacking for a prolonged period, paused spermatogenesis may have led to a (slight) reduction in testicular volume. Since gonadotropins are costly, this therapeutic investment is justified only if paternity is desired. In all other cases, testosterone is the preferred modality for replacement. When stimulation therapy for fertility induction is initiated in patients with previously accomplished testicular maturation, Leydig cell response (as indicated by a rise in serum testosterone levels to the adult range) to hCG occurs within 3 months. In rare cases, this is sufficient for initiation of spermatogenesis (Finkel et al. 1985). However, prolonged treatment with hCG alone will suppress endogenous residual FSH secretion. Therefore the addition of FSH is required.

**Protocol**: A full hCG starting dose of 1500 IU s.c. is applied twice weekly. The HCG dose should be reduced in cases of erythrocytosis, gynecomastia, or excessive acne. If testosterone levels remain below the normal adult range (<12 nmol/l) after 6–9 months, the hCG dose can be increased by increments of 500–1000 IU per injection every (3)–6 months to a maximum of 3 × 2500 IU s.c./week.

A full dose of rFSH (follitropin alpha) 3 × 150 IU is additionally injected thrice weekly, without subsequent dose modifications.

Reawakening of spermatogenesis occurs (with first spermatozoa appearing in semen) after 3–13 months of combined hCG/FSH replacement (Liu et al. 2009; Rohayem et al. 2016). Stimulation of spermatogenesis will not proceed further when the sperm concentration in the ejaculate reaches a plateau that is indicative of the individual's spermatogenic capacity.

Thereafter, sperm production may be maintained with hCG alone for several months (in patients with residual endogenous gonadotropin secretion) (Johnson 1978; Depenbusch et al. 2002).

### Monitoring Gonadotropin Substitution

Measurement of serum LH, FSH, testosterone levels, and testicular volumes (via Prader orchiometer and/or ultrasound) ought to be performed at 3 to 6 monthly intervals during gonadotropin substitution, along with annual bone age estimation in adolescents, until a bone age of 16 years (and thus near final height) is reached. Special attention should be given to LH levels, as a spontaneous increase into a range above >1 U/L would indicate spontaneous HPG axis activation during gonadotropin substitution, which would question the diagnosis of HH. In such cases of CDGP, total cessation of hormone replacement is indicated.

Regular measurement of serum testosterone levels (e.g., every 3 months) is useful not only to monitor the Leydig cell response, but also, along with FSH levels,

adherence to treatment. Once maturity for semen is attained in previously prepubertal subjects, ejaculates can be collected and analyzed according to WHO guidelines (WHO 2010) after at least 48 h of sexual abstinence. Thereafter, semen analyses may be repeated every 3 months until a plateau of sperm concentrations is documented in at least two follow-up visits or until pregnancy is achieved. Sperm cryopreservation may be offered before gonadotropin replacement is stopped and patients are switched to testosterone replacement. However, as restimulation of testes with gonadotropins by a second treatment cycle is known to be successful in most subjects, even after a longer period of testosterone substitution (Büchter et al. 1998), sperm cryopreservation in adolescent patients is recommended only if primary spermatogenic response to treatment is very poor. In adult men with no wish for immediate fatherhood, cryostorage of semen may be indicated.

### Misdiagnosis of CDGP and CHH Reversal

With respect to the clinical and genetic overlap between CHH patients and those with constitutionally delayed puberty, erroneous diagnosis of CDGP as HH cannot be excluded (Zuh et al. 2015). Therefore, interruption of treatment of HH is discussed with the patient after achievement of the primary treatment goals. Such interruption allows the physician to determine whether a spontaneous reactivation of the HPG axis occurs or whether substitution therapy has to continue life-long. Around 10% of patients with Kallmann syndrome or CHH (with *CHD7, FGFR1, GNRHR, TACR* mutations) bear potential for reversal of GnRH deficiency (Quinton et al. 1999; Raivio et al. 2007; Laitinen et al. 2012; Dwyer et al. 2016). Even with a replacement strategy with testosterone (enanthate or gel), continuous attention should be given to LH levels (the parameter reflecting the patient's endogenous HPG axis activity), in order to recognize potential spontaneous GnRH pulse generator activation. If reversal of HH occurs, it may not be sustained (Tommiska et al. 2013; Sidhoum et al. 2014). This vulnerability and plasticity of the reproductive axis emphasize the need for lifelong attention to symptoms of androgen deficiency and surveillance of HPG axis hormones in patients with spontaneous HH reversal.

## Testosterone Substitution

### Criteria for Testosterone Substitution

All patients with hypogonadism including central hypogonadism will eventually require testosterone replacement as hypogonadism implies testosterone deficiency. However, in patients with central hypogonadism, testosterone substitution should only be initiated once the patient's current or future fertility perspectives have been discussed. If the patient desires paternity in the immediate future, stimulation therapy by GnRH or gonadotropins should be the first choice as it stimulates spermatogenesis *as well as* testosterone production in the Leydig cells (see above).

If testosterone substitution is the treatment of choice, the question arises at which level of testosterone serum concentrations substitution is indicated.

Two factors contribute to controversial opinions and explain why the lower limit of normal and the limits for initiating testosterone substitution vary in various countries (Nieschlag et al. 2004) and in different guidelines (Bhasin et al. 2010; Dohle et al. 2012; Swerdloff and Wang 2012, Flaseriu et al. 2016):

First, serum testosterone undergoes a clear diurnal rhythm with higher levels in the morning and about 25% lower levels in the evening. Therefore, and for the sake of comparability, therapeutic decisions should be based on serum testosterone measurements during the morning hours.

Second, various testosterone symptoms have different threshold levels, loss of libido, and vigor may already occur below 12–15 nmol/L. Below 10 nmol/L depressive moods, sleep disturbances, lack of concentration, and diabetes type 2 are observed, while erectile dysfunction and hot flashes are reported below 8 nmol/L (Zitzmann et al. 2006).

Often perception of the physician rather than the patient's complaints and strain may govern prescribing behavior. Loss of libido is the most frequent and an early symptom demanding treatment and occurring below 12 nmol/L and provides a distinct signal for beginning testosterone substitution. Several large epidemiological studies from the USA (Bhasin et al. 2011), Australia (Sartorius et al. 2012), and Europe (Huhtaniemi et al. 2012) show that 12 nmol/L is the lowest value for serum testosterone of healthy men.

If several measurements provide ambivalent results, free testosterone in serum may aid the therapeutic decision. Free testosterone should be measured based on total testosterone and SHBG by using a generally available formula (http://issam.ch/freetesto.htm). Values below 225 pmol/L are considered subnormal.

Determination of serum testosterone varies depending on laboratory methodology and still provides a problem as wide scatters in external quality control programs show. Meanwhile, mass spectrometry methods (LLC-MS/MS) are the gold standard for measuring testosterone. Some guidelines and journals require measurements by these techniques. However, mass spectrometry may not be available everywhere. It has been shown that immune assays correlate well with results from mass spectrometry in the normal and upper subnormal range (Bhasin et al. 2011; Huhtaniemi et al. 2012; Haring et al. 2013), but are less reliable in in the low hypogonadal range.

## Testosterone Preparations and Modes of Application

Testosterone was synthesized in 1935 and has remained in clinical use since that time (Nieschlag and Nieschlag 2014). Until the 1990s only preparations resulting in mostly unphysiologic serum levels existed. Today intramuscular, subdermal, transdermal, oral, and buccal testosterone preparations are available (Behre and Nieschlag 2012) (Table 4). WHO, NIH, and FDA have jointly formulated general principles of testosterone therapy as "Guidelines for the use of androgens in men" (Nieschlag et al. 1992) which are still valid today. The following are the most important:

- Only preparations of natural testosterone should be used for substitution therapy in hypogonadism since the full spectrum of testosterone action in the body can

**Table 4** Testosterone preparations available for substitution

| | | |
|---|---|---|
| ***Intramuscular testosterone*** | **testosterone enanthate**<br>250 mg every 2–3 weeks<br>**testosterone cypionate**<br>200 mg every 2–3 weeks<br>**testosterone undecanoate**<br>1000 mg, first and after 6,<br>then every 12 weeks:<br>750 mg first and after 4,<br>then every 10 weeks | Testoviron® Depot 250<br>Testosteron Depot®<br>Delatestryl®<br>Nebido®<br>Aveed® (USA) |
| ***Transdermal Testosterone*** | One system daily<br>50–100 mg in 5–10 g gel daily<br>62.5 mg in 5 g gel daily<br>30–120 mg in 1.5–4.5 ml | Testopatch®<br>Androgel® / Testim ®<br>Testotop® / Tostran®<br>Axiron® |
| ***Oral Testosterone*** | Testosterone undecanoate<br>3–4 capsules à 40 mg daily | Andriol® / Testocaps® |
| ***Buccal Testosterone*** | Testosterone<br>2 × 30 mg tablets daily | Striant® |

only be achieved if testosterone is aromatized to estradiol and 5α-reduced to dihydrotestosterone (DHT). As conversion to these metabolites occurs at physiologically well-balanced rates for natural testosterone secreted by the testes, and not with synthetic androgens, testosterone remains the only choice for substitution in central hypogonadism. The well-balanced synergism between testosterone and estradiol is specifically important for sexual function, bone metabolism, and body composition, while synergism with DHT is important for accessory sex organs and action on the skin (hair pattern, sebum production). Nor can the full spectrum of testosterone action be achieved by androgenic anabolic steroids or by selective androgen receptor modulators (SARMs).

- Testosterone substitution should result in circulating serum levels as close to physiology as possible. Testosterone treatment of hypogonadal males should avoid supraphysiologically high testosterone serum levels as well as subnormally low levels.

These demands are not achieved by intramuscular testosterone enanthate or cypionate resulting in a roller coaster pharmacokinetic pattern. Patients dislike the ups and downs of well-being, vigor, sexual activity, and emotional stability. Because these preparations are cheap, they are still in use but have been replaced, wherever affordable, by transdermal gels and intramuscular testosterone undecanoate resulting in physiologic serum testosterone levels.

In the following, testosterone substitution in the adult HH patient is described, for testosterone treatment in the adolescent HH patient see section "Hormone Replacement in Prepubertal Onset HH."

### Oral Testosterone

**Testosterone** applied orally is readily absorbed by the intestine, but is also quickly eliminated by the first-pass effect in the liver. In order to overcome this effect a

modified synthetic testosterone molecule, **17α-alkylated methyltestosterone** was used for some time, but became obsolete because of its liver toxicity. However, esterified **testosterone undecanoate** administered orally, due to its long aliphatic side chain absorbed via the lymph, reaches circulation and target organs before the liver. Capsules of 40 mg (Andriol®) have to be taken 3 or 4 times/day for the treatment of hypogonadism. Pharmacokinetic analysis shows high intra- and interindividual variability in serum testosterone concentrations. Therefore this preparation is best suited as a supplement for low, but still ongoing testosterone production.

### Buccal Testosterone

**Testosterone incorporated into polyethylene matrices** can be **formed into mucoadhesive tablets**. These tablets adhere to the gingiva above the incisors for many hours and slowly release testosterone into the circulation. Twice daily applications result in constant serum levels. However, up to 15% of patients experience irritation, inflammation, or gingivitis, but those who become accustomed to the tablets tolerate them well. Care must be taken that the tablets do not become disattached, e.g., during meals (Dinsmore and Willie 2012).

### Intramuscular Testosterone

Testosterone, when injected, is very quickly degraded and therefore not suitable for substitution, the testosterone molecule has to be esterified to produce longer-acting substances. The most widely used preparations are **testosterone enanthate** and **cypionate,** of which 200–250 mg need to be injected in 2–3 week intervals for substitution purposes. **Testosterone propionate** is a shorter-acting ester. 50–100 mg last for only 2–3 days and are therefore not suited for long-term substitution.

The same *orally* effective testosterone undecanoate can also be used for ***intramuscular*** injection when administered in castor oil (Nieschlag et al. 1999a; Nieschlag and Nieschlag 2014). When injected in doses of 1000 mg in 4 ml castor oil (Nebido®) and then after 6 weeks, followed by further injections every 12 weeks, this preparation results in stable adult serum levels lasting for 10–14 weeks. After the initial loading dose, the patient on average requires injections only every 3 months (Zitzmann and Nieschlag 2007; Behre and Nieschlag 2012). In the USA, this preparation is available in doses of 750 mg in 3 ml castor oil (Aveed®) requiring further injections after 4 and then at 10 week intervals (Wang et al. 2010).

While the injectable preparations are generally considered very safe, pulmonary oil micro embolism (POME) characterized by brief respiratory symptoms (including cough, urge to cough, and dyspnea) immediately after the injection has been observed in rare cases (Mackey et al. 1995). Therefore special care has to be observed when injecting these oily solutions intramuscularly to avoid intravenous injections.

### Transdermal Testosterone

Transdermal testosterone preparations mimic physiological diurnal variations and their kinetic profiles are closest to ideal substitution. They may be used as the first choice and are especially well suited for patients with fluctuating symptoms caused

by other preparations. In addition, upon removal, testosterone is immediately eliminated if any adverse health effects should occur (e. g. polycythemia).

**Scrotal patches** consisting of a thin film soaked with testosterone were the earliest transdermal preparations for clinical use (Behre et al. 1999). Later, non-scrotal transdermal systems were developed but had the disadvantage of skin irritation occurring in a high percentage of patients.

These preparations were soon superceded by **testosterone gels** needing to be applied to sufficiently large skin areas in order to allow enough testosterone to be resorbed. Physiological levels result when these gels, applied in the morning to the upper arms, shoulders and abdomen, are left to dry for 5–10 minutes. Some manufactures suggest covering the skin area with underwear and others recommend washing the skin after evaporation of the alcohol in order to avoid unintended transfer to children or women (DeRonde 2009; Nelson et al. 2013). Long-term use showed good results (Wang et al. 2004; McNicholas and Ong 2006; Behre et al. 2012; Kühnert et al. 2005), if the appropriate dose of the gel is administered; serum levels, although variable, remain within the normal range (Swerdloff et al. 2015).

### Subdermal Testosterone Implants

Testosterone pellet implants were among the first modalities applied for testosterone replacement therapy; however, as a tunneling technique is required for implantation in the abdominal wall by a trocar, extrusion of the pellet and infection of the implantation site may occur. Three to six implants are inserted, resulting in initially supraphysiological levels and then slowly declining to the normal range for up to 4–6 months. In a cross-over study of pellets versus injectable testosterone undecanoate most patients preferred injections, mainly because of the simpler delivery (Fennell et al. 2010). Such implants have lost popularity and are only used in a few places (Kelleher et al.1999).

## Monitoring of Testosterone Substitution

Once testosterone substitution has been initiated, the patient should be carefully monitored at regular intervals. As a general rule, the patient should be seen after 3, 6, and 12 months and then at least annually. Intervals may be more frequent if certain symptoms prevail or comorbidities occur requiring immediate attention. Often, the patients feel badly instructed about the purpose and schedule of the treatment regimen, and then do not spontaneously contact their physicians (Dwyer et al. 2016). Mechanisms should be installed by the attending physician to remind patients of necessary control visits and to encourage additional visits if required.

Inquiries on present complaints should include information on mental and physical activity, libido and sexual activity, and satisfaction.

Physical examination should assess body weight, waist circumference and fat distribution, beard growth, and hair pattern as well as possible development of breast tissue.

Evaluation of size and surface of the prostate (by digital rectal examination preferably supplemented by transrectal ultrasonography) should be performed annually. Laboratory work-up includes the measurement of red blood cell counts and

hematocrit to detect and avoid polycythemia and its sequelae, e.g., apoplexy and thromboembolism.

Bone density should be measured prior to the commencement of testosterone substitution and then at regular intervals of about 2 years.

Taking into consideration the pharmacokinetics of the respective testosterone preparation, serum testosterone should always be measured before the next dose is applied and visits should be scheduled accordingly to allow for dosage adjustments.

Of note: On replacement with testosterone, the prostate of the hypogonadal patient will grow to its normal size. PSA will also increase, but should not exceed the normal range. At present there is no compelling evidence that testosterone treatment increases the incidence of prostate carcinoma (Debruyne et al. 2017).

## References

Abbara A, Jayasena CN, Christopoulos G, Narayanaswamy S, Izzi-Engbeaya C, Nijher GM, Comninos AN, Peters D, Buckley A, Ratnasabapathy R, Prague JK, Salim R, Lavery SA, Bloom SR, Szigeti M, Ashby DA, Trew GH, Dhillo WS. Efficacy of kisspeptin-54 to trigger oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS) during in vitro fertilization (IVF) therapy. J Clin Endocrinol Metab. 2015;100:3322–31.

Achermann JC, Meeks JJ, Jameson JL. Phenotypic spectrum of mutations in DAX-1 and SF-1. Mol Cell Endocrinol. 2001;185:17–25.

Alqwaifly M, Bohlega S. Ataxia and hypogonadotropic hypogonadism with intrafamilial variability caused by RNF216 mutation. Neurol Int. 2016;8:6444.

Bang AK, Nordkap L, Almstrup K, Priskorn L, Petersen JH, Rajpert-De Meyts E, Andersson AM, Juul A, Jørgensen N. Dynamic GnRH and hCG testing: establishment of new diagnostic reference levels. Eur J Endocrinol. 2017;176:379–91.

Barrio R, De Luis D, Alonso M, Lamas A, Moreno JC. Induction of puberty with human chorionic gonadotropin and follicle-stimulating hormone in adolescent males with hypogonadotropic hypogonadism. Fertil Steril. 1999;71:244–8.

Behre HM, Nieschlag E. Testosterone preparations for clinical use in males. In: Nieschlag E, Behre HM, Nieschlag S, editors. Testosterone: action, deficiency, substitution. 4th ed. Cambridge: Cambridge University Press; 2012. p. 309–35.

Behre HM, Nieschlag E, Partsch CJ, Wieacker P, Simoni M. Diseases of the hypothalamus and the pituitary gland. In: Nieschlag E, Behre HM, editors. Andrology: male reproductive health and dysfunction. 3rd ed. Berlin: Springer; 2010. p. 169–92.

Behre HM, von Eckardstein S, Kliesch S, Nieschlag E. Long-term substitution therapy of hypogonadal men with transscrotal testosterone over 7–10 years. Clin Endocr. 1999;50:629–35.

Behre HM, Tammela TL, Arver S, Tolrá JR, Bonifacio V, Lamche M, Kelly J, Hiemeyer F, European Testogel® Study Team, Giltay EJ, Gooren LJ. A randomized, double-blind, placebo-controlled trial of testosterone gel on body composition and health-related quality-of-life in men with hypogonadal to low-normal levels of serum testosterone and symptoms of androgen deficiency over 6 months with 12 months open-label follow-up. Aging Male. 2012;15:198–207.

Behre HM, Zitzmann M. Imaging diagnosis. In: Nieschlag E, Behre HM, editors. Andrology: male reproductive health and dysfunction. 3rd ed. Berlin: Springer; 2010. p. 101–7.

Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophyseal responses to continuous and intermittent delivery of hypothalamic gonadotropin releasing hormone. Science. 1978;202:631–3.

Beneduzzi D, Trarbach EB, Min L, Jorge AA, Garmes HM, Renk AC, Fichna M, Fichna P, Arantes KA, Costa EM, Zhang A, Adeola O, Wen J, Carroll RS, Mendonça BB, Kaiser UB,

Latronico AC, Silveira LF. Role of gonadotropin-releasing hormone receptor mutations in patients with a wide spectrum of pubertal delay. Fertil Steril. 2014;102:838–46.

Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM, Task force, Endocrine Society. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010;6:2536–59.

Bhasin S, Pencina M, Jasuja GK, Travison TG, Coviello A, Orwoll E, Wang PY, Nielson C, Wu F, Tajar A, Labrie F, Vesper H, Zhang A, Ulloor J, Singh R, D'Agostino R, Vasan RS. Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham heart study and applied to three geographically distinct cohorts. J Clin Endocrinol Metab. 2011;96:2430–9.

Boehm U, Bouloux PM, Dattani MT, de Roux N, Dodé C, Dunkel L, Dwyer AA, Giacobini P, Hardelin JP, Juul A, Maghnie M, Pitteloud N, Prevot V, Raivio T, Tena-Sempere M, Quinton R, Young J. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism–pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11:547–64.

Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombès M, Millar RP, Guiochon-Mantel A, Young J. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med. 2009;360:2742–8.

Bouloux P, Warne DW, Loumaye E. Efficacy and safety of recombinant human follicle-stimulating hormone in men with isolated hypogonadotropic hypogonadism. Fertil Steril. 2002;77:270–3.

Bouloux PM, Nieschlag E, Burger HG, Skakkebaek NE, FC W, Handelsman DJ, Baker GH, Ochsenkuehn R, Syska A, McLachlan RI, Giwercman A, Conway AJ, Turner L, van Kuijk JH, Voortman G. Induction of spermatogenesis by recombinant follicle-stimulating hormone (puregon) in hypogonadotropic azoospermic men who failed to respond to human chorionic gonadotropin alone. J Androl. 2003;24:604–11.

Büchter D, Behre HM, Kliesch S, Nieschlag E. Pulsatile GnRH or human chorionic gonadotropin/human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. Eur J Endocrinol. 1998;139:298–303.

Burgués S, Calderón MD. Subcutaneous self-administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotrophic hypogonadism. Hum Reprod. 1997;12:980–6.

Burris AS, Rodbard HW, Winters SJ, Sherins RJ. Gonadotropin therapy in men with isolated hypogonadotropic hypogonadism: the response to human chorionic gonadotropin is predicted by initial testicular size. J Endocrinol Metab. 1988;66:1144–51.

Butler MG, Wang K, Marshall JD, Naggert JK, Rethmeyer JA, Gunewardena SS, Manzardo AM. Coding and noncoding expression patterns associated with rare obesity-related disorders: Prader-Willi and Alström syndromes. Adv Genomics Genet. 2015;2015:53–75.

Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, Gaspert A, Lavoie HB, Wu CH, Crowley WF Jr, Amory JK, Pitteloud N, Seminara SB. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A. 2009;106:11703–8.

Chan YM, Lippincott MF, Butler JP, Sidhoum VF, Li CX, Plummer L, Seminara SB. Exogenous kisspeptin administration as a probe of GnRH neuronal function in patients with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2014;99:E2762–71.

Claahsen-van der Grinten HL, Otten BJ, Takahashi S, Meuleman EJ, Hulsbergen-van de Kaa C, Hermus AR. Testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia: evaluation of pituitary-gonadal function before and after successful testis-sparing surgery in eight patients. J Clin Endocrinol Metab. 2007;92:612–5.

Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougnères P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the

human leptin receptor gene causes obesity and pituitary dysfunction. Nature. 1998;392:398–401.

Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, Rouleau S, Lahlou N. Baseline inhibin B and anti-Mullerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. J Clin Endocrinol Metab. 2010;95:5225–32.

Costa-Barbosa FA, Balasubramanian R, Keefe KW, Shaw ND, Al-Tassan N, Plummer L, Dwyer AA, Buck CL, Choi JH, Seminara SB, Quinton R, Monies D, Meyer B, Hall JE, Pitteloud N, Crowley WF Jr. Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. J Clin Endocrinol Metab. 2013;98:E943–53.

Daniell HW. Hypogonadism in men consuming sustained-action oral opioids. The J Pain. 2002;3:377–84.

Debruyne FM, Behre HM, Roehrborn CG, Maggi M, Wu FC, Schröder FH, Jones TH, Porst H, Hackett G, Wheaton OA, Martin-Morales A, Meuleman E, Cunningham GR, Divan HA, Rosen RC, Investigators RHYME. Testosterone treatment is not associated with increased risk of prostate cancer or worsening of lower urinary tract symptoms: prostate health outcomes in the registry of hypogonadism in men. BJU Int. 2017;119:216–24.

Delemarre-van de Waal HA. Application of gonadotropin releasing hormone in hypogonadotropic hypogonadism-diagnostic and therapeutic aspects. Eur J Endocrinol. 2004;151(Suppl 3): U89–94.

Depenbusch M, von Eckardstein S, Simoni M, Nieschlag E. Maintenance of spermatogenesis in hypogonadotropic hypogonadal men with hCG alone. Eur J Endorinol. 2002;147:617–24.

de Ronde W. Hyperandrogenism after transfer of topical testosterone gel: case report and review of published and unpublished studies. Hum Reprod. 2009;24:425–8.

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A. 2003;100:10972–6.

de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med. 1997;337:1597–602.

De Sousa SM, Chapman IM, Falhammar H, Torpy DJ. Dopa-testotoxicosis: disruptive hypersexuality in hypogonadal men with prolactinomas treated with dopamine agonists. Endocrine. 2017;55:618–24.

Dhindsa S, Miller MG, McWhirter CL, Mager DE, Ghanim H, Chaudhuri A, Dandona P. Testosterone concentrations in diabetic and nondiabetic obese men. Diabetes Care. 2010;33:1186–92.

Dinsmore WW, Wyllie MG. The long-term efficacy and safety of a testosterone mucoadhesive buccal tablet in testosterone-deficient men. Br J Urol Int. 2012;110:162–9.

Dodé C, Levilliers J, Dupont JM, De Paepe A, Le Dû N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pêcheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet. 2003;33:463–5.

Dodé C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet. 2006;2:e175.

Dohle GR, Arvers S, Bettocchi C (2012) Guidelines on male hypogonadism. European Association of Urology. www.uroweb.org.

Doty RL, Bromley SM, Stern MB. Olfactory testing as an aid in the diagnosis of Parkinson's disease: development of optimal discrimination criteria. Neurodegeneration. 1995;4:93–7.

Dunkel L, Quinton R. Transition in endocrinology: induction of puberty. Eur J Endocrinol. 2014;170:229–39.

Dwyer AA, Phan-Hug F, Hauschild M, Elowe-Gruau E, Pitteloud N. TRANSITION IN ENDOCRINOLOGY: Hypogonadism in adolescence. Eur J Endocrinol. 2015;173:R15–24.

Dwyer AA, Raivio T, Pitteloud N. MANAGEMENT OF ENDOCRINE DISEASE: Reversible hypogonadotropic hypogonadism. Eur J Endocrinol. 2016;174:R267–74.

Eiholzer U, l'Allemand D, Rousson V, Schlumpf M, Gasser T, Girard J, Grüters A, Simoni M. Hypothalamic and gonadal components of hypogonadism in boys with Prader-Labhart-Willi syndrome. J Clin Endocrinol Metab. 2006;91:892–8.

European Metrodin HP Study Group EMHSG. Efficacy and safety of highly purified urinary follicle-stimulating hormone with human chorionic gonadotropin for treating men with isolated hypogonadotropic hypogonadism. European Metrodin HP Study Group. Fertil Steril. 1998;70:256–62.

Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N. Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest. 2008;118:2822–31.

Fennell C, Sartorius G, Ly LP, Turner L, Liu PY, Conway AJ, Handelsman DJ. Randomized cross-over clinical trial of injectable vs. implantable depot testosterone for maintenance of testosterone replacement therapy in androgen deficient men. Clin Endocrinol. 2010;73:102–9.

Finkel DM, Phillips BA, Snyder PJ. Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. N Engl J Med. 1985;313:651–5.

Fischer-Posovszky P, von Schnurbein J, Moepps B, Lahr G, Strauss G, Barth TF, Kassubek J, Mühleder H, Möller P, Debatin KM, Gierschik P, Wabitsch M. A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness. J Clin Endocrinol Metab. 2010;95:2836–40.

Fleseriu M, Hashim IA, Karavitaki N, Melmed S, Murad MH, Salvatori R, Samuels MH. Hormonal replacement in hypopituitarism in adults: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2016;101:3888–921.

Frapsauce C, Ravel C, Legendre M, Sibony M, Mandelbaum J, Donadille B, Achermann JC, Siffroi JP, Christin-Maitre S. Birth after TESE-ICSI in a man with hypogonadotropic hypogonadism and congenital adrenal hypoplasia linked to a DAX-1 (NR0B1) mutation. Hum Reprod. 2011;26:724–8.

Gelhorn HL, Vernon MK, Stewart KD, Miller MG, Brod M, Althof SE, DeRogatis LR, Dobs A, Seftel AD, Revicki DA. Content validity of the hypogonadism impact of symptoms questionnaire (HIS-Q): a patient-reported outcome measure to evaluate symptoms of hypogonadism. Patient. 2016;9:181–90.

Gooren L. Hypogonadotropic hypogonadal men respond less well to androgen substitution treatment than hypergonadotropic hypogonadal men. Arch Sex Behav. 1988;17:265–70.

Greulich WW, Pyle SI, editors. Radiographic atlas of skeletal development of hand and wrist. 2. ed. California: Stanford University Press; 1959.

Grinspon RP, Rey RA. Anti-mullerian hormone and sertoli cell function in paediatric male hypogonadism. Horm Res Paediatr. 2010;73:81–92.

Habiby RL, Boepple P, Nachtigall L, Sluss PM, Crowley WF Jr, Jameson JL. Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalmic and pituitary defects in gonadotropin production. J Clin Invest. 1996;98:1055–62.

Haring R, Baumeister SE, Nauck M, Völzke H, Keevil BG, Brabant G, Wallaschofski H. Testosterone and cardiometabolic risk in the general population – the impact of measurement method on risk associations: a comparative study between immunoassay and mass spectrometry. Eur J Endocrinol. 2013;169:463–70.

Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. J Clin Endocrinol Metab. 2012;97:3056–67.

Hayes FJ, Seminara SB, Decruz S, Boepple PA, Crowley WF Jr. Aromatase inhibition in the human male reveals a hypothalamic site of estrogen feedback. J Clin Endocrinol Metab. 2000;85:3027–35.

Hier DB, Crowley WF Jr. Spatial ability in androgen-deficient men. N Engl J Med. 1982;306:1202–5.

Huhtaniemi IT, Tajar A, Lee DM, O'Neill TW, Finn JD, Bartfai G, Boonen S, Casanueva FF, Giwercman A, Han TS, Kula K, Labrie F, Lean ME, Pendleton N, Punab M, Silman AJ, Vanderschueren D, Forti G, Wu FC, EMAS Group. Comparison of serum testosterone and estradiol measurements in 3174 European men using platform immunoassay and mass spectrometry; relevance for the diagnostics in aging men. Eur J Endocrinol. 2012;166:983–91.

Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol. 2007;264:237–43.

Jadhav U, Harris RM, Jameson JL. Hypogonadotropic hypogonadism in subjects with DAX1 mutations. Mol Cell Endocrinol. 2011;346:65–73.

Janssen N, Bergman JE, Swertz MA, Tranebjaerg L, Lodahl M, Schoots J, Hofstra RM, van Ravenswaaij-Arts CM, Hoefsloot LH. Mutation update on the CHD7 gene involved in CHARGE syndrome. Hum Mutat. 2012;33:1149–60.

Johnsen SG. Maintenance of spermatogenesis induced by hMG treatment by means of continuous hCG treatment in hypogonadotropic men. Acta Endocrinol. 1978;89:763–9.

Karaca Z, Tanrıverdi F, Ünlühızarcı K, Kelestimur F. GH and pituitary hormone alterations after traumatic brain injury. Prog Mol Biol Transl Sci. 2016;138:167–91.

Kelleher S, Turner L, Howe C, Conway AJ, Handelsman DJ. Extrusion of testosterone pellets: a randomized controlled clinical study. Clin Endocrinol. 1999;51:469–71.

Kelly DF, Chaloner C, Evans D, Mathews A, Cohan P, Wang C, Swerdloff R, Sim MS, Lee J, Wright MJ, Kernan C, Barkhoudarian G, Yuen KC, Guskiewicz K. Prevalence of pituitary hormone dysfunction, metabolic syndrome, and impaired quality of life in retired professional football players: a prospective study. J Neurotrauma. 2014;31:1161–71.

Khan SA, Muhammad N, Khan MA, Kamal A, Rehman ZU, Khan S. Genetics of human Bardet-Biedl syndrome, an update. Clin Genet. 2016;90:3–15.

Kliesch S, Behre HM, Nieschlag E. Recombinant human follicle-stimulating hormone and human chorionic gonadotropin for induction of spermatogenesis in a hypogonadotropic male. Fertil Steril. 1995;63:1326–8.

Kühnert B, Byrne M, Simoni M, Köpcke W, Gerss J, Lemmnitz G, Nieschlag E. Testosterone substitution with a new transdermal, hydroalcoholic gel applied to scrotal or non-scrotal skin: a multicentre trial. Eur J Endocrinol. 2005;153:317–26.

Laitinen EM, Tommiska J, Sane T, Vaaralahti K, Toppari J, Raivio T. Reversible congenital hypogonadotropic hypogonadism in patients with *CHD7, FGFR1* or *GNRHR* mutations. PLoS One. 2012;7:e39450.

Layman LC, Cohen DP, Jin M, Xie J, Li Z, Reindollar RH, Bolbolan S, Bick DP, Sherins RR, Duck LW, Musgrove LC, Sellers JC, Neill JD. Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. Nat Genet. 1998;18:14–5.

Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology. 2010;151:3479–89.

Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, Krauss RM, Veldhuis JD, Wagner AJ, DePaoli AM, McCann SM, Wong ML. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A. 2004;101:4531–6.

Liu L, Banks SM, Barnes KM, Sherins RJ. Two-year comparison of testicular responses to pulsatile gonadotropin-releasing hormone and exogenous gonadotropins from the inception of therapy in men with isolated hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 1988;67:1140–5.

Liu PY, Gebski VJ, Turner L, Conway AJ, Wishart SM, Handelsman DJ. Predicting pregnancy and spermatogenesis by survival analysis during gonadotrophin treatment of gonadotrophin-deficient infertile men. Hum Reprod. 2002;17:625–33.

Liu PY, Swerdloff RS, Christenson PD, Handelsman DJ, Wang C. Hormonal Male Contraception Summit Group. Rate, extent, and modifiers of spermatogenic recovery after hormonal male contraception: an integrated analysis. Lancet. 2006;367:1412–20.

Liu PY, Baker HW, Jayadev V, Zacharin M, Conway AJ, Handelsman DJ. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. J Clin Endocrinol Metab. 2009;94:801–8.

Lofrano-Porto A, Barra GB, Giacomini LA, Nascimento PP, Latronico AC, Casulari LA, da Rocha Neves Fde A. Luteinizing hormone beta mutation and hypogonadism in men and women. N Engl J Med. 2007;357:897–904.

Mackey MA, Conway AJ, Handelsman DJ. Tolerability of intramuscular injections of testosterone ester in oil vehicle. Hum Reprod. 1995;108:862–5.

Mantovani G, Ozisik G, Achermann JC, Romoli R, Borretta G, Persani L, Spada A, Jameson JL, Beck-Peccoz P. Hypogonadotropic hypogonadism as a presenting feature of late-onset X-linked adrenal hypoplasia congenita. J Clin Endocrinol Metab. 2002;87:44–8.

Mao JF, Liu ZX, Nie M, Wang X, HL X, Huang BK, Zheng JJ, Min L, Kaiser UB, Pulsatile WXY. Gonadotropin-releasing hormone therapy is associated with earlier spermatogenesis compared to combined gonadotropin therapy in patients with congenital hypogonadotropic hypogonadism. Asian J Androl. 2016. https://doi.org/10.4103/1008-682X.193568.

Matthews CH, Borgato S, Beck-Peccoz P, Adams M, Tone Y, Gambino G, Casagrande S, Tedeschini G, Benedetti A, Chatterjee VK. Primary amenorrhoea and infertility due to a mutation in the beta-subunit of follicle-stimulating hormone. Nat Genet. 1993;5:83–6.

Matsumoto AM, Karpas AE, Bremner WJ. Chronic human chorionic gonadotropin administration in normal men: evidence that follicle-stimulating hormone is necessary for the maintenance of quantitatively normal spermatogenesis in man. J Clin Endocrinol Metab. 1986;62:1184–92.

Matsumoto AM, Snyder PJ, Bhasin S, Martin K, Weber T, Winters S, Spratt D, Brentzel J, O'Dea L. Stimulation of spermatogenesis with recombinant human follicle-stimulating hormone (follitropin alfa; GONAL-f): long-term treatment in azoospermic men with hypogonadotropic hypogonadism. Fertil Steril 2009;92:979–990.

McNicholas T, Ong T. Review of Testim gel. Expert Opin Pharmacother. 2006;7:477–84.

Nelson D, Ho J, Pacaud D, Stephure D. Virilization in two pre-pubertal children exposed to topical androgen. J Pediatr Endocrinol Metab. 2013;24:1–5.

Netchine I, Sobrier ML, Krude H, Schnabel D, Maghnie M, Marcos E, Duriez B, Cacheux V, Moers A, Goossens M, Gruters A, Amselem S. Mutations in LHX3 result in a new syndrome revealed by combined pituitary hormone deficiency. Nat Genet. 2000;25:182–6.

Nieschlag E. Clinical trials in male hormonal contraception. Contraception. 2010;82:457–70.

Nieschlag E, Nieschlag S. Testosterone deficiency: a historical perspective. Asian J Androl. 2014;16:161–8.

Nieschlag E, Vorona E. Medical consequences of doping with anabolic androgenic steroids: effects on reproductive functions. Eur J Endocrinol. 2015;173:47–58.

Nieschlag E, Behre HM, Bouchard P, Corrales JJ, Jones TH, Stalla GK, Webb SM, Wu FC. Testosterone replacement therapy: current trends and future directions. Hum Reprod Update. 2004;10:409–19.

Nieschlag E, Bouloux P-MG, Stegmann BJ, Shankar RR, Guan Y, Tzontcheva A, McCrary Sisk C, Behre H. Efficacy and safety of the long-acting Corifollitropin Alfa in combination with hCG in adult men with hypogonadotropic hypodonadism. Reprod Endocr Biol. 2017;15:17.

Nieschlag E, Büchter D, Von Eckardstein S, Abshagen K, Simoni M, Behre HM. Repeated intramuscular injections of testosterone undecanoate for substitution therapy in hypogonadal men. Clin Endocrinol. 1999a;51:757–63.

Nieschlag E, Simoni M, Gromoll J, Weinbauer GF. Role of FSH in the regulation of spermatogenesis: clinical aspects. Clin Endocrinol. 1999b;51:139–46.

Nieschlag E, Wang C, Handelsman DJ, Swerdloff RS, Wu FCW, Einer-Jensen N, Waites GMH, editors. Guidelines for the use of androgens. Geneva: WHO Special Programme of Research/ Development and Research Training in Human Reproduction; 1992.

Phillip M, Arbelle JE, Segev Y, Parvari R. Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone. N Engl J Med. 1998;338:1729–32.

Pitteloud N, Dwyer AA, DeCruz S, Lee H, Boepple PA, Crowley WF Jr, Hayes FJ. Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab. 2008;93:784–91.

Pitteloud N, Hayes FJ, Dwyer A, Boepple PA, Lee H, Crowley WF Jr. Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2002;87:4128–36.

Pitteloud N, Quinton R, Pearce S, Raivio T, Acierno J, Dwyer A, Plummer L, Hughes V, Seminara S, Cheng YZ, Li WP, Maccoll G, Eliseenkova AV, Olsen SK, Ibrahimi OA, Hayes FJ, Boepple P, Hall JE, Bouloux P, Mohammadi M, Crowley W. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. J Clin Invest. 2007;117:457–63.

Quaynor SD, Kim HG, Cappello EM, Williams T, Chorich LP, Bick DP, Sherins RJ, Layman LC. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. Fertil Steril. 2011;96:1424–30.

Quinton R, Cheow HK, Tymms DJ, Bouloux PM, Wu FC, Jacobs HS. Kallmann's syndrome: is it always for life? Clin Endocrinol. 1999;50:481–5.

Raivio T, Falardeau J, Dwyer A, Quinton R, Hayes FJ, et al. Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007;357:863–73.

Rajagopal A, Vassilopoulou-Sellin R, Palmer JL, Kaur G, Bruera E. Symptomatic hypogonadism in male survivors of cancer with chronic exposure to opioids. Cancer. 2004;100:851–8.

Rochira V, Zirilli L, Genazzani AD, Balestrieri A, Aranda C, Fabre B, Antunez P, Diazzi C, Carani C, Maffei L. Hypothalamic-pituitary-gonadal axis in two men with aromatase deficiency: evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback. Eur J Endocrinol. 2006;155:513–22.

Rogol AD. Pubertal androgen therapy in boys. Pediatr Endocrinol Rev. 2005;3:383–90.

Rohayem J, Hauffa BP, Zacharin M, Kliesch S, Zitzmann M. The "German Adolescent Hypogonadotropic Hypogonadism Study Group". Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotropic hypogonadism? -a multicentre prospective study of hCG/rFSH treatment outcomes during adolescence. Clin Endocrinol (Oxford). 2017;86:75–87.

Rohayem J, Nieschlag E, Kliesch S, Zitzmann M. Inhibin B, AMH, but not INSL3, IGF1 or DHEAS support differentiation between constitutional delay of growth and puberty and hypogonadotropic hypogonadism. Andrology. 2015;3:882–7.

Rohayem J, Sinthofen N, Nieschlag E, Kliesch S, Zitzmann M. Causes of hypogonadotropic hypogonadism predict response to gonadotropin substitution in adults. Andrology. 2016;4:87–94.

Rohayem J, Tüttelmann F, Mallidis C, Nieschlag E, Kliesch S, Zitzmann M. Restoration of fertility by gonadotropin replacement in a man with hypogonadotropic azoospermia and testicular adrenal rest tumors due to untreated simple virilizing congenital adrenal hyperplasia. Eur J Endocrinol. 2014;170:K11–7.

Sartorius G, Spasevska S, Idan A, Turner L, Forbes E, Zamojska A, Allan CA, Ly LP, Conway AJ, McLachlan RI, Handelsman DJ. Serum testosterone, dihydrotestosterone and estradiol

concentrations in older men self-reporting very good health: the healthy man study. Clin Endocrinol. 2012;77:755–63.
Schopohl J. Pulsatile gonadotrophin releasing hormone versus gonadotrophin treatment of hypothalamic hypogonadism in males. Hum Reprod. 1993;8:175–9.
Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. J Clin Endocrinol Metab. 2002;87:1613–20.
Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. N Engl J Med. 2003;349:1614–27.
Shiraishi K, Oka S, Matsuyama H. Assessment of quality of life during gonadotrophin treatment for male hypogonadotrophic hypogonadism. Clin Endocrinol. 2014;81:259–65.
Sidhoum VF, Chan YM, Lippincott MF, Balasubramanian R, Quinton R, Plummer L, Dwyer A, Pitteloud N, Hayes FJ, Hall JE, Martin KA, Boepple PA, Seminara SB. Reversal and relapse of hypogonadotropic hypogonadism: resilience and fragility of the reproductive neuroendocrine system. J Clin Endocrinol Metab. 2014;99:861–70.
Siegel ET, Kim HG, Nishimoto HK, Layman LC. The molecular basis of impaired follicle-stimulating hormone action: evidence from human mutations and mouse models. Reprod Sci. 2013;20:211–33.
Şimşek E, Montenegro LR, Binay C, Demiral M, Acıkalin MF, Latronico AC. Clinical and hormonal features of a male adolescent with congenital isolated follicle-stimulating hormone deficiency. Horm Res Paediatr. 2016;85:207–12.
Sinisi AA, Esposito D, Maione L, Quinto MC, Visconti D, De Bellis A, Bellastella A, Conzo G, Bellastella G. Seminal anti-Mullerian hormone level is a marker of spermatogenic response during long-term gonadotropin therapy in male hypogonadotropic hypogonadism. Hum Reprod. 2008;23:1029–34.
Sisk CL, Zehr JL. Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol. 2005;26:163–74.
Soliman AT, De Sanctis V. An approach to constitutional delay of growth and puberty. Indian J Endocrinol Metab. 2012;16:698–705.
Spratt DI, Carr DB, Merriam GR, Scully RE, Rao PN, Crowley WF. The spectrum of abnormal patterns of gonadotropin-releasing hormone secretion in men with idiopathic hypogonadotropic hypogonadism: clinical and laboratory correlations. J Clin Endocrinol Metab. 1987;64:283–91.
Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. 1998;18:213–5.
Swerdloff RS, Wang CL. Review of guidelines on diagnosis and treatment of testosterone deficiency. In: Nieschlag E, Behre HM, Nieschlag S, editors. Testosterone: action, deficiency, substitution. 4th ed. Cambridge: Cambridge University Press; 2012. p. 408–20.
Swerdloff RS, Pak Y, Wang C, Liu PY, Bhasin S, Gill TM, Matsumoto AM, Pahor M, Surampudi P, Snyder PJ. Serum testosterone (T) level variability in T-gel-treated older hypogonadal men: treatment monitoring implications. J Clin Endocrinol Metab. 2015;100:3280–7.
Takeda T, Takasu N, Yamauchi K, Komiya I, Ohtsuka H, Nagasawa Y, Ohara N, Yamada T. Magnetic resonance imaging of the rhinencephalon in a patient with Kallmann's syndrome. Intern Med. 1992;31:394–6.
Tarnutzer AA, Gerth-Kahlert C, Timmann D, Chang DI, Harmuth F, Bauer P, Straumann D, Synofzik M. Boucher-Neuhäuser syndrome: cerebellar degeneration, chorioretinal dystrophy and hypogonadotropic hypogonadism: two novel cases and a review of 40 cases from the literature. J Neurol. 2015;262:194–202.
Tenforde AS, Barrack MT, Nattiv A, Fredericson M. Parallels with the female athlete triad in male athletes. Sports Med. 2016;46:171–82.
Tommiska J, Jørgensen N, Christiansen P, Juul A, Raivio T. A homozygous R262Q mutation in the gonadotropin-releasing hormone receptor presenting as reversal of hypogonadotropic hypogonadism and late-onset hypogonadism. Clin Endocrinol. 2013;78:316–7.

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction. Nat Genet. 2009;41:354–8.

Valdes-Socin H, Rubio Almanza M, Tomé Fernández-Ladreda M, Debray FG, Bours V, Beckers A. Reproduction, smell, and neurodevelopmental disorders: genetic defects in different hypogonadotropic hypogonadal syndromes. Front Endocrinol (Lausanne). 2014;5:109.

Wabitsch M, Ballauff A, Holl R, Blum WF, Heinze E, Remschmidt H, Hebebrand J. Serum leptin, gonadotropin, and testosterone concentrations in male patients with anorexia nervosa during weight gain. J Clin Endocrinol Metab. 2001;86:2982–8.

Waldstreicher J, Seminara SB, Jameson JL, Geyer A, Nachtigall LB, Boepple PA, Holmes LB, Crowley WF Jr. The genetic and clinical heterogeneity of gonadotropin-releasing hormone deficiency in the human. J Clin Endocrinol Metab. 1996;81:4388–95.

Wang C, Harnett M, Dobs AS, Swerdloff RS. Pharmacokinetics and safety of long-acting testosterone undecanoate injections in hypogonadal men: an 84-week phase III clinical trial. J Androl. 2010;31:457–65.

Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, Weber T, Berman N, Hull L, Swerdloff RS. Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. J Clin Endocrinol Metab. 2004;89:2085–98.

Warne DW, Decosterd G, Okada H, Yano Y, Koide N, Howles CM. A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle-stimulating hormone and human chorionic gonadotropin. Fertil Steril. 2009;92:594–604.

Wehkalampi K, Widén E, Laine T, Palotie A, Dunkel L. Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. J Clin Endocrinol Metab. 2008;93:723–8.

World Health Organization (WHO). WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010.

Wilson DA, Hofman PL, Miles HL, Unwin KE, McGrail CE, Cutfield WS. Evaluation of the buserelin stimulation test in diagnosing gonadotropin deficiency in males with delayed puberty. J Pediatr. 2006;148:89–94.

Wu W, Cogan JD, Pfaffle RW, Dasen JS, Frisch H, O'Connell SM, Flynn SE, Brown MR, Mullis PE, Parks JS, Phillips JA 3rd, Rosenfeld MG. Mutations in PROP1 cause familial combined pituitary hormone deficiency. Nat Genet. 1998;18:147–9.

Young J, George JT, Tello JA, Francou B, Bouligand J, Guiochon-Mantel A, Brailly-Tabard S, Anderson RA, Millar RP. Kisspeptin restores pulsatile LH secretion in patients with neurokinin B signaling deficiencies: physiological, pathophysiological and therapeutic implications. Neuroendocrinology. 2013;97:193–202.

Zacharin M, Sabin MA, Nair VV, Dagabdhao P. Addition of recombinant follicle-stimulating hormone to human chorionic gonadotropin treatment in adolescents and young adults with hypogonadotropic hypogonadism promotes normal testicular growth and may promote early spermatogenesis. Fertil Steril. 2012;98:836–42.

Zhu J, Choa REY, Guo MH, Plummer L, Buck C, Palmert MR, Hirschhorn JN, Seminara SB, Chan YM. A shared genetic basis for self-limited delayed puberty and idiopathic hypogonadotropic hypogonadism. The Journal of Clinical Endocrinology & Metabolism 2015;100:E646–E654.

Zitzmann M, Faber S, Nieschlag E. Association of specific symptoms and metabolic risks with serum testosterone in older men. J Clin Endocrinol Metab. 2006;91:4335–43.

Zitzmann M, Nieschlag E. Androgen receptor gene CAG repeat length and body mass index modulate the safety of long-term intramuscular testosterone undecanoate therapy in hypogonadal men. J Clin Endocrinol Metab. 2007;92:3844–53.