Management of Male Hypogonadotrophic Hypogonadism

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THE development of sexual maturity and reproductive function in the male is dependent upon appropriate secretion of hormones that orchestrate the sophisticated relationship between the hypothalamus, pituitary, and testes — the hypothalamic-pituitary-gonadal (HPG) axis. A state of hypogonadism, i.e. inadequate testicular function, is manifested by deficiencies in secretion of androgens, with a wide range of effects on a number of physiological processes, including gametogenesis. Any dysfunction in the HPG axis can result in hypogonadotrophic hypogonadism, where a decrease in endocrine and/or gametogenic function of the testes results in retardation of puberty and reproductive insufficiency [1].

The hypothalamus is the centre of the reproductive axis. Messages received from the central nervous system and from the gonads are integrated by the hypothalamus to regulate the production and secretion of gonadotrophin releasing hormone (GnRH), which is released in a pulsatile manner that is essential for stimulating the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary. The anterior pituitary produces LH and FSH in response to pulses of GnRH stimulation, and in the male LH and FSH both bind to specific receptors on the Leydig and Sertoli cells within the testis. This stimulation regulates the release of androgens required for pubertal development, and subsequently for the initiation and maintenance of male reproductive function and spermatogenesis. Feedback mechanisms play an important role in maintaining the reproductive axis: testosterone inhibits LH secretion, and inhibin secreted by Sertoli cells regulates FSH secretion. A reduction in negative feedback results in an increased secretion of FSH, and, in a manner similar to that found in the female with diminished ovarian function, serum FSH levels in the male act as an indicator of germinal epithelial function in the testis [2, 3].

Episodic secretion of GnRH is an essential part of this process. The hypothalamic pituitary gonadal axis is first activated during the fetal/neonatal period, when an increase in testosterone stimulates completion of the inguinoscrotal phase of testicular descent and further growth of the penis. This is accompanied by a wave of Sertoli and germ cell proliferation in the testis. A second wave of increased HPG axis activity then occurs during the early stages of puberty, with a second wave of Sertoli cell proliferation. This wave of proliferation is followed by terminal differentiation, and Leydig cells begin to secrete testosterone, allowing the normal development of puberty [4].

A disruption in any of these events, from fetal to adult life, can lead to hypogonadism, and this can be manifested in a wide variety of clinical symptoms, according to the time of onset and the severity of the resulting hormone deficiency. In many cases, the symptoms may not be recognised before puberty. Hypogonadism can be classified into three categories, which must be considered in establishing the diagnosis required for correct management and treatment:
1. Primary: congenital Leydig cell aplasia or damage to Leydig cells impairs testosterone secretion and/or damages the seminiferous tubules, with consequent oligospermia and elevated FSH and LH (hypergonadotrophic hypogonadism). Klinefelter’s syndrome is the most common cause of primary hypogonadism, where the 47XXY karyotype is associated with seminiferous tubule dysgenesis [5]. Testicular trauma, surgery, mumps, toxins, radiation and chemotherapy can also cause testicular failure with hypergonadotrophic hypogonadism.

2. Disorders of the hypothalamus or pituitary impair gonadotrophin secretion (hypogonadotrophic hypogonadism, HH). The primary feature of HH is a failure in the HPG feedback system, in that low testosterone levels fail to elicit a reciprocal increase in gonadotrophins.

3. Androgen resistance: a defect in the androgen receptor gene causes an inadequate response to available androgens, resulting in a variety of phenotypic manifestations.

The aetiology of hypogonadotrophic hypogonadism may be primary/idiopathic, genetic/congenital or secondary, with resulting primary or secondary testicular failure. Primary hypogonadism selectively affects the secretion or function of GnRH, and may be due to a variety of different defects. A few genetic causes have been identified, such as Kallman’s Syndrome, DAX-1 gene mutation and GPR54 gene mutation, but many have not been clearly defined; autosomal dominant, autosomal recessive and sex-linked inheritance have been proposed. In Kallman’s syndrome there is congenital agenesis of the olfactory lobes, so that fetal GnRH neurosecretory neurons fail to migrate from the olfactory placode to the hypothalamus [5]. Secondary HH and testicular failure can be due to congenital or acquired pathology at the level of the hypothalamus or pituitary, including tumors, neoplastic therapy, vascular disorders, sarcoid, infections, trauma, AIDS [6], malnutrition, or the stress of acute illness such as chronic renal insufficiency or anorexia nervosa [6, 7]. In some cases, the aetiology is unknown, and males with isolated/idiopathic HH may present either with constitutional delay of puberty, or later, with infertility as the primary feature.

**Diagnosis**

**History and examination**

The background physiology behind male hypogonadotrophic hypogonadism clearly indicates that the history and symptoms of the condition will depend on the patient’s age at the time of onset, and therefore it is essential to establish the diagnosis, define the aetiology, and identify any associated features in order to manage the patient appropriately [8]. A complete medical and family history, in combination with physical examination will help to define whether the aetiology is based upon the hypothalamus or pituitary, and whether the defect is structural, functional, infiltrative or inflammatory, and congenital or acquired. Relevant features include state of external genitalia such as small testes or micropenis, a history of cryptorchidism or migrating testes, renal abnormalities, visual field defects, cleft lip, anosmia or hyposmia, and a history of chronic illness or trauma. The patient’s growth pattern can help to distinguish between IHH and delayed development, and bone age measurements are helpful in determining appropriate treatment for the younger age group.

In childhood, androgen deficiency has few consequences and may not be recognised, unless it is associated with growth retardation or other anatomic or endocrine abnormalities [9]. Boys with congenital hypogonadotropic hypogonadism usually show normal growth, but sometimes manifest micropenis or cryptorchidism before the expected time of puberty. Males who do not produce sufficient testosterone at the appropriate adolescent age may experience behavioural and cognitive impairment. Patients at late adolescent have poor muscle development, small testes, phallus and prostate, scant pubic and axillary hair, long arms and legs due to delayed epiphyseal closure, gynecomastia, and a high-pitched voice. A boy who has no pubertal development by the age of 14 may have constitutional delay of puberty, and there is often a family history of delay in sexual development in a parent or sibling. These boys usually have short stature during childhood and/or adolescence, with a growth pattern that parallels the lower percentile groups of the growth chart. When the bone age is plotted on the growth curve, it essentially equals the percentile group of the genetic target; the majority will
have evidence of sexual maturation by the age of 18 yrs and ultimately reach their genetic target for height. Other pathology must be excluded, i.e., growth hormone deficiency, hypothyroidism, primary or secondary hypogonadism — before a diagnosis of constitutional delay is reached.

In an adult, the androgen deficiency of hypogonadism may produce clinical symptoms and signs that are often subtle and slow to fully evolve — they may be denied by the patient, and ignored by the physician. Manifestations depend upon the degree and length of deficiency, and include a progressive decrease in muscle mass, loss of libido, impotence, oligospermia/azoospermia, hot flushes, and poor ability to concentrate. With long-standing hypogonadism, testicular atrophy, fine wrinkling of the skin around the eyes and lips and sparse body hair may been seen [10].

**Investigations**

Approximately 85% of testicular mass consists of germinal tissue, and reduced germinal cell mass is reflected by small, smooth and soft testes. Testicular volume (TV) may therefore be used as an indicator of probable time of onset of the disorder: prepubertal testes are smaller than 4 ml in volume and <2 cm in length, testes at the early puberty are 4–15 ml and >2 cm long, and adult testes are usually between 20–30 ml, and 4.5 to 6.5 cm long by 2.8 to 3.3 cm wide [11, 12]. Kumar et al. [13] studied testicular biopsies of 8 adult IHH patients with prepubertal testes (<4 ml), with no previous gonadotropin therapy and with no history of cryptorchidism. The testes of all patients showed seminiferous cords separated by interstitium but typical adult Leydig cells were absent. The cords contained only Sertoli cells and early type A spermatogonia.

In boys with delayed puberty, a full endocrine assessment must be carried out in order to identify any associated deficits in growth hormone, thyroid and adrenal function, or possible medical complications of chronic systemic disorders and treatment. Hyperprolactinaemia, due to a prolactinoma or as a side effect of certain medications can also cause HH. A small TV may also be due to androgen resistance,
where defects in the androgen receptor gene prevent an adequate response to normal secretion of androgens, resulting in a variety of phenotypic variations according to the nature of the genetic defect.

In isolated LH deficiency, patients have a monotropic loss of LH, while FSH remains normal. At puberty, these boys have testicular growth, since the bulk of the testis is made up of seminiferous tubules that respond to FSH. However, the absence of LH causes Leydig cell atrophy and testosterone deficiency; the patients do not develop secondary sexual characteristics, and continue to grow because of lack of epiphyseal closure.

An initial blood test should include testosterone, prolactin, FSH and LH levels. Testosterone and LH levels vary in a diurnal pattern, and from the second half of puberty are higher at night than during the day; blood is generally drawn in the morning, when the levels are highest [14]. Testosterone levels increase throughout puberty from <0.7 nmol/L to between 10.5 and 41.5 nm/L or from <10 ng/dL to between 250 and 1100 ng/dL, and its secretion is pulsatile as well as circadian.

Testosterone circulates bound to carrier proteins in the blood, sex-hormone binding globulin (SHBG) and albumin, and any alteration in these protein levels will alter total testosterone measurements. Low testosterone levels stimulate an increase in SHBG by the liver. In young adult men, approximately 2% of testosterone is in the free form, 30% is bound tightly to SHBG, and 68% is weakly bound to albumin [5].

LH and FSH are normally secreted in pulses of 90-to 120-minute intervals, and therefore these hormones should be measured in nocturnal blood samples at 20-minute intervals for 2 hours to document the presence or absence of the normal pulses. Serum LH is usually <0.5 IU/L and FSH <1 IU/L in boys and <3.5 IU/L in girls before puberty, are higher at night in the latter half of puberty, and have a pulsatile fluctuation between 5 and 20 IU/L in adults.

In adult males, low serum testosterone and elevated FSH and LH suggest a failure of germinal epithelium in the seminiferous tubules, i.e. primary testicular failure (hypergonadotropic hypogonadism), whereas low testosterone and low or normal gonadotrophins suggest a hypothalamic or pituitary disorder. A semen analysis provides an excellent index of seminiferous tubule function in adolescents or adults. In acquired HH, a prolactin level and pituitary imaging studies should be carried out in order to investigate the possibility of a pituitary tumour. Thyroid, adrenal and growth hormone tests are also indicated.

Children of short stature with delayed pubertal development show low testosterone and low gonadotrophins — this may be a feature of constitutional delay as well as HH.

In the prepubertal period, it may be difficult to differentiate between HH and constitutional delay of puberty, and endocrine challenge tests using either hCG or GnRH can be useful in determining the aetiology of the syndrome [15, 16]. The GnRH test measures pituitary function: a bolus of 100 µg GnRH given by rapid IV injection directly stimulates the pituitary to release LH and FSH, which are measured in serum every 20–30 minutes for 2 hrs. In childhood, the response is predominantly an increase in FSH with little or no increase in LH. During puberty, LH and FSH rise more or less equally by two to threefold. In adulthood, LH rises two to fivefold over baseline, while FSH rises between 20–50%. If the patient has pituitary insufficiency (hypopituitarism), this test elicits an inadequate to absent increase in gonadotrophins. Patients with hypothalamic disease may have a normal or very low rise in FSH and LH; an inadequate rise may be due to atrophy of gonadotrophin-secreting neurons in the pituitary as a result of insufficient endogenous stimulation by GnRH. In patients with hypothalamic disease, such as Kallmann’s syndrome, the repeated pulsatile administration of GnRH may restore gonadotroph secretion to normal.

With the advent of highly purified recombinant proteins and improved assay techniques it has been possible to accurately measure mullerian inhibiting substance (MIS) and inhibin B levels in various patient populations including male hypogonadotrophic hypogonadism [17–19]. Pitteloud et al. [19] compared IHH patients according to the absence or presence of some prior pubertal development and found significant differences in inhibin B, and MIS levels. IHH patients with a history of some prior pubertal development had significantly lower MIS and significantly higher Inhibin B levels than those individuals with no prior pubertal development.

Men who have idiopathic seminiferous tubule failure, or secondary failure due to disease or disorders affecting the testes (infection, trauma, cryptorchidism, chemotherapy/radiotherapy, vascular damage, alcoholism) may have oligo- or azoospermia associated with
infertility, with FSH levels that may be elevated or normal. Serum testosterone and LH are usually normal, but the GnRH stimulation test may cause an excessive rise in LH [20].

Human chorionic gonadotrophin (hCG) has a structural subunit that is also common to LH, and therefore hCG stimulates Leydig cells to produce testosterone. The hCG test is a useful indicator of testicular function. A single dose of hCG, 5000 IU/1.7 m² in adults, or 3000 IU/m² in children, should induce at least a doubling in testosterone levels within 3 to 4 days if Leydig cells are functional [16, 21].

Clomiphene citrate (CC) is a weak oestrogen agonist that interrupts the negative feedback loop and thereby stimulates release of gonadotrophins from the pituitary. In the CC challenge test, 100 mg of CC is given orally for 5–7 days; if the hypothalamic-pituitary axis is intact, LH levels should double, and an FSH rise of between 20–50% is expected [22].

The sensitivity of standard RIA endocrine assays previously limited the value of these challenge tests [15], particularly in prepubertal boys; however, more sensitive assays (IRMA, immunoradiometric assays, and TR-FIA, time-resolved fluoroimmunoassay) have increased the discriminative power of GnRH and hCG testing and improved their diagnostic accuracy [16]. In suspected patients aged 10 years and older, a combination of GnRH and hCG testing provide excellent diagnostic value.

Management

Gonadotrophin or GnRH therapy to induce or restore secondary sexual development and fertility due to androgen deficiency is effective only in hypogonadotrophic hypogonadism, and therefore this diagnosis must be firmly established before treatment is considered. Initial assessment should distinguish between hyper- and hypogonadotrophic hypogonadism. Having made the diagnosis, it is essential to identify the needs of the patient and design a specific treatment tailored towards their needs, with a physiological rather than a pharmacological approach. Treatment with testosterone alone can develop and maintain secondary sexual characteristics and enhance libido, but TV is reduced and direct or indirect administration of gonadotrophins is required to induce spermatogenesis. Treatment options include GnRH by pump, or hCG and a preparation containing FSH.

Pulsatile GnRH treatment can be delivered subcutaneously through a portable infusion pump if there is sufficient gonadotroph reserve in the pituitary [8, 23–25]. LH, FSH and testosterone levels must be monitored every two weeks until they reach a normal range, after which they can be monitored every 2 months. GnRH can be used to initiate pubertal development, maintain virilization and sexual function, and to initiate and maintain spermatogenesis. In most patients, these effects may take from 3 to 15 months to achieve sperm production [26]. However, a small subset of IHH men, fail to reach a normal testicular volume (TV) and produce sperm on this therapy. To determine predictors of outcome in terms of TV and sperm count, Pitteloud et al. [18] studied 76 IHH men (38% with anosmia) undergoing GnRH therapy for 12–24 months. In patients who had complete prior pubertal development (and there was no evidence of cryptorchidism), response to therapy was faster, normalizing androgen production by 2 months and completing spermatogenesis by 6 months. The independent predictors of outcome of long-term GnRH therapy were: 1) the presence of some prior pubertal development (positive predictor) 2) a baseline Inhibin B less than 60 pg/ml (negative predictor); and 3) prior cryptorchidism (negative predictor). Notably, anosmia was not an independent predictor of outcome when adjusted for other baseline variables.

The use of an infusion pump for GnRH delivery is however generally both inconvenient and difficult to maintain for long periods, and the treatment often fails due to pituitary hypoplasia or downregulation of gonadotrophin receptors. In one study using the GnRH pump, Pitteloud et al. [18] reported an 8% (6/76) non-compliance rate. Treatment with injectable gonadotrophins (hCG with or without FSH) is now more commonly used to treat HH [27]. Several studies have used only hCG [28, 29], however this may preclude an optimal response in those IHH men with no prior pubertal development [12, 28, 30]. Human menopausal gonadotrophin (HMG), a urine-derived source of FSH, has been shown to induce spermatogenesis in men with HH, but this preparation also contains LH, which adds a further variable in the treatment [30]. Additionally, the urinary gonadotropin preparations such as HMG had to be administered by intramuscular injection, but recombinant preparations are now available that can be self-administered by subcutaneous
injection, with important implications for clinical management [24, 31–33].

In the presence of complete hypophysectomy [34, 35] treatment with an FSH/LH preparation (HMG) can restore spermatogenesis, but the Leydig cells are not adequately stimulated. The addition of hCG stimulates Leydig cells to produce testosterone, promoting testicular growth with restoration of potency and normal ejaculates. Using hCG instead of direct testosterone replacement yields more stable androgen levels, and there is less fluctuation in hypogonadal symptoms. hCG treatment can also stimulate sufficient intra-testicular testosterone to allow the initiation of spermatogenesis [29, 30]. Mancini et al. [36] demonstrated that hCG has a stimulating effect on the spermatogonial phase, while HMG stimulates all germinal phases. The combined treatment regimen gives complete recovery of spermatogenesis, full development of Leydig cells, repair of Sertoli cells and disappearance of hyalinisation.

Induction of secondary sexual development

When prepubertal boys are diagnosed as having hypogonadotropic hypogonadism such as Kallmann’s syndrome or combined pituitary hormone deficiency, the patients’ desirable adult height should be taken into account. Since gonadal replacement therapy, such as hCG-hMG/FSH or testosterone treatment usually induces rapid pubertal maturation even at low doses, it is often difficult to mimic the slow, normal pubertal developmental pattern. Pubertal height gain in normal height children is around 30 cm according to Japanese standard data. The method for gaining 30 cm of pubertal growth has not yet been established with hCG-hMG/FSH therapy or testosterone treatment and therefore gonadal replacement treatment tends to start later than the normal pubertal age so as to obtain the patients’ desirable adult height. There are some attempts to mimic slow normal pubertal development using testosterone ointment or low doses (500 IU) of HCG, but it should be born in mind that height and bone age at start of gonadotrophin replacement therapy is closely related to adult height.

Data from US clinical practice AACE Guidelines [10], also recommends that peripubertal boys with HH and delayed puberty can be treated with injections of hCG. Again, the stage of bone development should be taken into account, and if the patient is still growing, the initial regimen was reported to be in the range of 1,000 to 2,000 IU of hCG once a week [37]. If the bone age is significantly advanced, the dose should be given three times a week. In all cases, clinical response must be monitored, and testosterone levels measured every 2 to 3 months, with dose adjustment as required to reach an optimal schedule and to avoid side effects such as gynaecomastia. Increasing doses may have a down-regulating effect and reduce testicular stimulation, and reduced or less frequent dosing may produce a better result. The use of gonadotrophins in the treatment of adolescent boys can be important not only from the point of view of developing secondary sexual characteristics and fertility potential but also for normal psychosexual maturation [38, 39].

Induction of spermatogenesis

HH is a rare cause of infertility, but it is specifically responsive to appropriate hormone treatment. In men who wish to achieve fertility, initial testicular volume and prior treatment determine the response to treatment [12, 40] as well as the presence of cryptorchidism [41]. Although the resulting sperm counts are usually below the normal range (<20 million per ml), pregnancies can still be achieved. Burris et al. [42] defined the minimal number of sperm needed for conception, in men with isolated hypogonadotropic hypogonadism (IHH) who became sperm-positive during gonadotropin therapy. Twenty-two of 24 men (92%) proved fertile, initiating a total of 40 pregnancies. The mean (+/– standard error of the mean) sperm concentration at the time of conception was16.7 +/– 4.0 × 10^6/ml. However, 71% of pregnancies were conceived when the mean sperm concentration was less than 20 × 10^6/ml; in 16%, the mean sperm concentration was less than 1 × 10^6/ml. A value of 1.5 × 10^6/ml is commonly used in clinical reports as representative of a sperm concentration compatible with pregnancy [43–47]. Recently the use of assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI) has also been applied in HH men undergoing gonadotrophin therapy [48–50] with better sperm quality rates and pregnancy being obtained with prolonged gonadotrophin treatment [48].

A TV of less than 4 ml indicates that the onset of HH occurred before pubertal development, and these patients usually require therapy with both hCG and FSH to induce spermatogenesis. Men with partial
gonadotropin deficiency, or who have previously been stimulated with hCG to induce pubertal development may initiate and maintain sperm production with hCG only. Men with postpubertal acquired HH and who have previously had normal production of sperm may also initiate and maintain spermatogenesis with hCG treatment only. Therapy with hCG is generally begun at 1000 to 2000 IU two to three times a week, and testosterone levels should be monitored monthly to determine whether any adjustments are needed to normalize the levels. Normal levels of testosterone may require 2 to 3 months of treatment. When testosterone levels are in the normal range, testicular growth and semen assessments should be monitored monthly. If spermatogenesis has not been initiated within 6 to 12 months of hCG therapy, an FSH preparation should be added to hCG, at a dose of 75 IU–150 IU depending on body weight, three times a week. If pregnancy occurs, the regimen can be switched to hCG only, to allow continued spermatogenesis for subsequent potential pregnancies. For long-term maintenance of secondary sexual characteristics and fertility, it is important to consider age and compliance. A combination of hCG and FSH can be continued, with a lowered dose and frequency during periods when another pregnancy is not desired. If no further pregnancies are desired, the patient can be switched to testosterone therapy, or long-term hCG therapy can be continued in conjunction with appropriate contraceptive measures, if needed.

Clinical trials using Gonadotrophins

I) hCG/HMG therapy

Tanaka et al. [51] assessed the effects of hCG-hMG treatment in 13 boys with hypopituitarism associated with combined growth hormone and gonadotropin deficiency. Four of the patients had been previously treated with testosterone enanthate by intramuscular injection, and one patient had been unsuccessfully treated with a GnRH pump. The hCG-hMG treatment was started after an interval of at least one month after these pre-treatments. Treatment was started at a mean age of 20.4 years, initiated with hCG 5000 IU twice a week and hMG once a week. Four patients achieved normal spermatogenesis with this dose after 6 to 32 weeks of treatment. The dose was increased to hCG 5000 IU twice a week and HMG 75 IU twice a week in nine patients who did not achieve a normal sperm count, and one of these patients fathered a normal infant after 62 months of treatment. Of those remaining, four achieved normal sperm counts, two were oligospermic, one was azoospermic, and one was not evaluated. Testis volume increased in all patients, and overall 9 out of the 13 achieved normal sperm counts. Although all of the patients had been assessed by hCG and GnRH challenge tests, there was no correlation between the responses to treatment and the test results; neither test was predictive for spermatogenesis after hCG-hMG treatment. The authors recommended starting with the second (higher) dosage schedule in order to obtain early spermatogenesis.

Miyagawa et al. [4] reviewed 30 years of experience at University affiliated male infertility centres in order to determine the outcome of long-term therapy with hCG and human menopausal gonadotropin (hMG). Medical charts of 36 men aged 11 to 42 years were analysed retrospectively: a total of 29 men (81%) were diagnosed with primary HH (including 5 patients with Kallmann’s syndrome), and 7 (19%) with secondary HH. The patients were stratified according to testicular volume; 23 had a TV ≤ 4 ml, and 13 had an average TV of 7.5 ± 3.5 ml. Therapy was initiated with hCG 3000 IU and hMG 75 IU administered by intramuscular injection twice a week for 12 to 48 months. Treatment with hCG/HMG for 12 to 240 months (average 56 ± 11) resulted in sperm production for 36% of the patients with small testes, and 71% of those with large testes. Peak testosterone levels were observed at 12–24 months. After obtaining maximum testicular development, the doses were decreased to biweekly injections of 3000 IU hCG and 150 IU hMG if the patients wished to continue with this therapy. Alternatively, they were treated with biweekly injections of 125 or 250 mg of testosterone enanthate to maintain virilization (Table 2).

II) hCG/uFSH therapy

MHH patients have also been treated with hCG in combination with a urinary derived FSH preparation [39, 43, 44]. Burgues et al. [43], studied 60 men with hypogonadotrophic hypogonadism, of which, 16 suffered from Kallmann’s syndrome, 19 from idiopathic hypogonadotrophic hypogonadism and 25 from hypopituitarism. They received uFSH (150 IU × three/week) and HCG (2500 IU × two/week) for at least 6 months and underwent periodic assessments of testicular function. Testosterone concentrations increased and all but one patient reached values in the normal
range. At the end of treatment, 48 patients (80.0%) had achieved a positive sperm count. The maximum sperm concentration during treatment was 24.5 × 10^6/ml (mean +/– SEM). The median time to induce spermatogenesis was 5 months. There was no significant difference in the spermatogenic response between the different diagnostic groups. The rate of complete responses in patients with isolated HH (63%) was similar to that observed in patients with multiple pituitary deficiencies (68%). Treatment efficacy was however significantly related to the onset of hypogonadism. Thus, in patients diagnosed with hypogonadism before puberty the response rate at the end of treatment was 59.6%, whereas 100% of patients with postpubertal hypogonadism showed an adequate response at 6 months (P<0.01). Likewise, the maximum sperm concentration in patients with postpubertal onset hypogonadism (37.9 × 10^6/ml) was significantly higher (P<0.04) than that observed in the other patients (22.3 × 10^6/ml). The spermatogenic response was not significantly related to previous treatments, although there was a trend to an improved response in patients treated with gonadotrophins. Nine out of 10 (90%) patients who had received gonadotrophins before entering the study achieved spermatogenesis as compared to 78% in those who had not (P<0.3).

A multicentre study in Europe [44] was also carried out using uFSH in combination with hCG in inducing spermatogenesis in 28 men with primary, complete isolated hypogonadotrophic hypogonadism. The primary efficacy end point was a sperm density of at least 1.5 × 10^6/mL. Twenty-five (89.3%) patients achieved spermatogenesis; 18 (64.3%) of which achieved a density of >1.5 × 10^6/mL.

Barrio et al. [39] studied 7 prepubertal males with isolated HH (IHH) with a mean age of 15.44 (SD of 1.97) years and 7 prepubertal males with panhypopituitarism-associated HH (PHH) with a mean age of 18.1 (SD of 3.24) years. HCG at a dose of between 1,000–1,500 IU and uFSH 75–100 IU were administered together every alternate weekday from the beginning of therapy until complete induction of puberty and spermatogenesis was achieved. All patients achieved normal sexual maturation and normal or nearly normal adult male levels of testosterone. Maximum increase in testicular size was achieved after 32 months (average 10.4 ml) in the IHH group and 31 months (average 15.3 ml) in the PHH group. Four patients in the IHH group and six patients in the PHH group achieved a normal testicular volume (>=12 mL), [52]. There was no significant difference in the maximum testicular volume between the 2 groups. Positive sperm production was assessed in four of five patients with isolated HH and in three of three patients with panhypopituitarism associated HH. Poor sperm production was related to the presence of cryptorchidism as previously reported by [41].

In a subsequent study, Liu et al. [33], evaluated potential predictors of response in 29 consecutive gonadotrophin-deficient men all desiring paternity who received 43 courses of therapy in one centre between 1982 and 1998. The Kaplan-Meier survival analysis estimates of median (SE) time to a sperm concentration of >0, >5 and >20 × 10^6/ml were 5.5 (1.1), 12.4 (2.3) and 29.1 (1.9) months respectively. Conception occurred in 22/43 cycles (with eight men achieving two pregnancies) with a median (SE) Kaplan-Meier estimate of 20.5 (4.7) months. The median sperm concentration at conception was 5.0 (SE 2.0; range 0.0–59.5) × 10^6/ml. By employing a multivariate model to predict these same sperm thresholds and conception it was found that larger testicular volume at start of treatment, prior gonadotrophin therapy, completion of puberty, older age, the absence of adverse fertility factors and the absence of multiple pituitary hormone deficiency predicted a favourable response. Multivariate modelling suggested that the two most important predictors of sperm output were testicular volume and pubertal status. The most important potentially modifiable predictor was prior gonadotrophin therapy that led to a shorter median time to first appearance of sperm in a subsequent treatment. Prior androgen therapy was associated with a longer time to reach a sperm concentration of 20 × 10^6/ml. However prior androgen use and partner’s age did not appear to be significant predictors of spermatogenesis and pregnancy.
III) hCG and r-hFSH therapy

Recent clinical studies have confirmed the efficacy and safety of new recombinant gonadotropin preparations as an alternative to urinary-derived hMG in the management of HH [53]. Recombinant human FSH (r-hFSH) is as effective in inducing spermatogenesis as the urinary products, but it has greater purity, higher specific activity, is more consistent in composition, and is less antigenic allowing subcutaneous administration, [54, 55]. Follitropin alfa, filled-by-mass (GONALEF®, Merck Serono, an affiliate of Merck KGa A, Darmstadt, Germany) has a high specific activity (13,645 IU FSH/mg) and a low batch-to-batch variability (<2% vs up to 20% for urinary products) [55]. It is the first r-hFSH to be filled and released in mass units, 5.5 µg being equivalent to 75 IU FSH activity according to the rate in vivo bioassay [55]. In clinical use it has been demonstrated to improve the clinical response [56–59]. The therapy also has the advantage of greater patient acceptance, as the injections can be self-administered subcutaneously.

Bouloux et al. [45] conducted a study in 26 azoospermic men aged 16–48 (mean 25.9 ± 7.7) with severe IHH. Mean TV was ≤4 ml, there was no evidence of pituitary or hypothalamic lesion, and no illness or use of drugs that could affect testicular function. Patients were pre-treated for up to 6 months with hCG (Profasi®, Merck Serono, an affiliate of Merck KGa A, Darmstadt, Germany) 2000 IU twice weekly. The dose was titrated after 2 months to maintain testosterone levels within the normal range, and patients then received 18 months of treatment with hCG in combination with r-hFSH (GONALEF®) 150 IU three times a week, which could be increased to 225 IU after 9 months. The primary efficacy point was a sperm concentration compatible with fertility, of at least 1.5 × 10⁶/ml. Secondary end points, including mean testicular volume and secondary sexual characteristics were also assessed. The drug was well tolerated, and the results of this study showed that r-hFSH is effective in initiating spermatogenesis in the majority of patients with IHH. The primary end point, a sperm concentration of at least 1.5 × 10⁶/ml was achieved in 63% of patients, and some degree of spermatogenesis was achieved in 79%. There was also a significant improvement sperm motility and morphology, and successful pregnancies were achieved in four out of seven couples who wished to conceive. The median time to response in this study was 9 months. Four patients however remained azoospermic.

Warne et al. [46] conducted a meta-analysis of 4 clinical trials conducted in Europe, Australia, USA and Japan, in order to assess the safety and efficacy of long-term treatment with r-hFSH (GONALEF®) for induction of spermatogenesis in a large number of men (n = 100) with HH. The analysis also considered the impact of baseline characteristics on efficacy outcome. The four studies were phase III, open-label, non-comparative multicentric studies conducted in Europe (32 patients), USA (36 patients), Japan (22 patients) and Australia (10 patients). All 4 studies involved 3 to 6 months of pre-treatment to normalize serum testosterone, using hCG 1000 IU 3×/week or 2000 IU 2×/week, adjusted according to individual response. This was followed by 18 months of treatment with r-hFSH 150 IU 3×/week, with concomitant hCG. The primary efficacy endpoint was a sperm density of at least ≥1.5 × 10⁶/ml. Assessments were carried out every 3 months, and if the patients remained azoospermic after 6 months, the dose of FSH could be increased up to a maximum of 300 IU 3×/week. Both drugs were self-administered by subcutaneous injection.

A total of 100 men were enrolled for pre-treatment with hCG, and 81 entered the treatment phase with hCG and FSH. 68 patients achieved spermatogenesis during the treatment phase, and the primary efficacy endpoint of sperm concentration ≥1.5 × 10⁶/ml was achieved in 56 out of the 81 men (69.1%). The median time to reach this endpoint was 9 months in the European study, and 6 months in the other studies. Total sperm count per ejaculate increased steadily throughout the 18-month period, and ejaculate volume, percentage of sperm with normal morphology and progressive motility also increased during treatment. All men showed an increase in mean testicular volume.

This pooled data from 4 clinical studies represents the largest international database of patients with MHH available to date, and the self-administration of follitropin alfa in combination with hCG was successful in treating the majority of the patients. Spermatogenesis was successfully induced in over 85% of men treated, and over two-thirds of the men achieved a sperm count of ≥1.5 × 10⁶/ml (Table 3). The two baseline parameters that were found to have a significant prognostic value on efficacy outcome were mean testicular volume (Fig. 1) and BMI (Table 4). Patients with a BMI ≥30 kg/m² had a lower response (64.3%) compared to those with a BMI <30 kg/m² (88.1%).
Both injections were well tolerated, with only mild adverse events reported. More than 99% of the men reported no local site reactions.

GONALEF® received approval in the European Union for the treatment of male HH based on the results of the above studies conducted in Europe, Australia and the USA. Despite the established efficacy of this intervention, it is possible that populations in other regions may respond differently to treatment because of genetic variability. Okada et al. [47] reported on a
prospective, non-comparative open label study at 13 University clinical sites in Japan. Twenty-two Japanese men aged 15–55 (mean age 31.5) with azoospermia due to HH and a mean TV ≤ 6 ml were enrolled in this trial. During the pre-treatment phase, patients received hCG (Profasi®) 1000 IU subcutaneously three times per week for 12–24 wks, in order to normalize testosterone levels. Assessment was carried out every 4 weeks between weeks 12 and 24, and the dose of hCG could be increased to 5000 IU after week 8 if serum testosterone levels had not reached the normal range. Administration of hCG was continued until a semen sample was produced, and serum testosterone levels were shown to be normal at two consecutive assessments. Eighteen patients then entered the treatment phase of the study, and were given concomitant treatment with hCG s.c 2 to 3 times per week at the last dose used in pre-treatment, and r-hFSH (GONALEF®) 150 IU s.c. three times weekly for up to 72 weeks. If the patient remained azoospermic after 24 weeks, the dose could be increased by 75 IU, and a further dose increase was permitted at week 48 if the patient continued to be azoospermic. The aetiology of the men who entered treatment was idiopathic in 15 (83.3%), organic brain lesions in 2, and Kallmann’s Syndrome in 1. Seventeen patients completed 72 weeks of treatment.

The primary efficacy endpoint was a sperm count of ≥1.5 × 10⁶/ml. Secondary endpoints assessed included ejaculate volume, total sperm count, morphology and motility, as well as testis volume, secondary sexual characteristics, endocrinology, and fathering a child.

Sixteen of the men (88.9%) who entered the treatment phase achieved the primary efficacy endpoint, with sperm counts ≥1.5 × 10⁶/ml. The median time to reaching this endpoint was 36.4 weeks (range 10.1–60.4 weeks). Seven patients were trying to initiate a pregnancy with their partner, and this was achieved in two cases.

The injections were administered subcutaneously by self-injection, and were well tolerated with very few local site reactions.

**Final recommendations and conclusions**

Male hypogonadotrophic hypogonadism is a rare medical condition of varied aetiology, but it is one that can now be effectively treated with combinations of hCG and r-hFSH. As mentioned above, making an accurate diagnosis is of paramount importance, and the specific needs of individual patients must be considered so that treatment can be appropriately designed to address those needs. The response to treatment is largely determined by presence of some prior pubertal development, presence of cryptorchidism, BMI, testicular volume at initiation of therapy and prior gonadotrophin treatment [18, 19, 33, 43, 46]. It has been proposed that pubertal gonadotrophin therapy that culminates in appropriate testis development may facilitate later spermatogenesis [60]. Tachiki et al. [61] have also reported a longer time in inducing spermatogenesis in cases of acquired gonadotrophin deficiency where treatment is delayed for more than 2 years from the onset of the condition. Similarly, initial testicular volume at start of treatment is a significant predictor of shorter time to achieve spermatogenesis [33]. Prior androgen treatment does not seem to be detrimental to attainment of spermatogenesis [33] although it may take longer due to smaller initial testicular volume, from the initiation of gonadotrophin therapy than in patients already on hCG therapy.

Fertility is usually achieved via GnRH or gonadotrophin treatment, but this may not be the immediate goal, and hCG/FSH can also be used for normalization of development [39, 60] as well as for normal psychosexual maturation [38, 39].

When considering the details of gonadotrophin treatment regimes, patients can be categorised into three different subpopulations:

1. Adult MHH patients who require fertility can be treated with a combination of hCG (1000 IU up to 5000 IU three times a week) and routinely 150 IU

<table>
<thead>
<tr>
<th>Baseline variable</th>
<th>Sperm density &gt;0.0 × 10⁶/mL, %</th>
<th>Sperm density ≥1.5 × 10⁶/mL, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean testicular volume</td>
<td></td>
<td></td>
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<tr>
<td>0 mL</td>
<td>58</td>
<td>40</td>
</tr>
<tr>
<td>3 mL</td>
<td>85</td>
<td>69</td>
</tr>
<tr>
<td>11 mL</td>
<td>100</td>
<td>98</td>
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<tr>
<td>Body mass index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30 kg/m²</td>
<td>64.3</td>
<td>42.9</td>
</tr>
<tr>
<td>&lt;30 kg/m²</td>
<td>88.1</td>
<td>74.6</td>
</tr>
</tbody>
</table>

**Table 4.** Calculated probabilities of achieving spermatogenesis and a sperm density of ≥1.5 × 10⁶/mL for mean testicular volume and body mass index in MHH patients treated with hCG/r-hFSH (GONALEF®) [46]
r-hFSH three times per week, with doses titrated according to a monitored response. The frequency of treatment may be decreased when a sperm count compatible with pregnancy (>1.5 million/ml) is achieved. Other factors such as the age of the female partner should also be taken into consideration in terms of when to stop therapy.

2. Patients who are still adolescent should be managed according to their stage of bone development. The ideal regimen is hCG alone initially, with the possibility of adding rhFSH. If the patient is still growing with young bone age and desires taller adult height, treatment should be administered once a week with low dose hCG or testosterone ointment, and if the bone age is near adult, the dosage may be increased to three times weekly.

3. Patients who require long-term treatment for maintenance of secondary sexual characteristics and fertility form a third sub-population. Therapy consists of a combination of hCG and FSH, and there may be situations in which testosterone replacement could be considered if fertility is not required. In this group, it is important to consider age and compliance. Patients who already have a child may be treated with a lower dose of hCG and FSH for an interim period before fertility is again required.

The features of MHH syndromes may often be subtle, but the symptoms can have a serious impact on the quality of life of patients that are otherwise apparently in good health, especially with respect to psychological relationships with their partners. Whereas treatment with the older urinary gonadotrophins required intramuscular injection, long-term therapy with subcutaneous r-hFSH has been demonstrated to be a safe and effective treatment for normalization of development and induction of secondary sexual characteristics and spermatogenesis.

References


