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# Gonadotrophin replacement for induction of fertility in hypogonadal men



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Congenital hypogonadotrophic hypogonadism (CHH) is a rare form of infertility caused by deficient secretion or action of gonadotrophin-releasing hormone. There is no consensus regarding the optimal approach to fertility treatment in CHH men. In most cases, appropriate hormonal treatment with human chorionic gonadotrophin with or without follicle stimulating hormone will induce testicular development, spermatogenesis and fertility. Recent studies have examined sequential treatment with FSH pre-treatment to optimize fertility outcomes in severely affected CHH patients. This paper reviews historical and recent literature to summarize the current evidence on therapeutic approaches for CHH men seeking fertility.

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## Introduction

The episodic secretion of gonadotrophin-releasing hormone (GnRH) from specialized hypothalamic neurons is a key neuroendocrine regulator of the human reproductive axis [1]. Pulsatile GnRH stimulates the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary which in turn triggers testicular development in males as well as testosterone production and reproductive capacity (i.e. spermatogenesis). Accordingly, developmental disorders disrupting the GnRH network can result in congenital hypogonadotrophic hypogonadism (CHH) – a rare condition clinically characterized by absent or partial puberty and infertility [2,3]. In contrast to primary testicular disease, CHH is characterized by hypogonadotropic hypogonadism, and is usually a treatable form of male infertility. However, approaches to induce fertility in these men vary and optimal regimens have yet to be definitively determined. Thus, the focus of this review will be to provide physiologic context for gonadotrophin replacement and summarize the current evidence on therapeutic approaches for CHH men seeking fertility.

### *The reproductive axis*

In humans, the activity of GnRH-induced gonadotrophin secretion changes throughout development. GnRH neurons originate from the olfactory placode and migrate along olfactory guidance fibers to reach the arcuate nucleus of the hypothalamus before birth [2]. In early male fetal life (weeks 10–15) maternal hCG drives the testicular production of testosterone (T) that spurs growth of the phallus and early testicular descent [4]. Then, from the second half of fetal life T production is stimulated via endogenous GnRH-induced LH secretion from the pituitary. This phase is critical for final inguinoscrotal testicular descent and phallic growth. The hypothalamic-pituitary-gonadal (HPG) axis remains active during the first six months of life with hormone levels approaching adult levels [5]. This so-called “mini-puberty” also represents an important window of opportunity to identify CHH among males exhibiting maldescended testes with or without micropenis as low serum T and LH measurement can be utilized to identify congenital GnRH deficiency [6].

The HPG axis appears to be relatively quiescent during childhood yet subtle, important testicular growth occurs as evidenced by studies in primates as well as histologic studies of boys dying in traumatic accidents [7]. The onset of puberty is initially marked by sleep-entrained, pulsatile GnRH-induced LH secretion [8]. The resulting increased serum gonadotrophin and T levels progressively extend into the day as puberty progresses and these hormone dynamics begin a cascade of events culminating in reproductive maturity. The gonadotrophins exert differential effects on the compartments of the testes. Broadly, LH stimulates maturation of the interstitial Leydig cells that secrete T and insulin-like factor 3 (INSL3). Intra-testicular T, in concert with FSH, acts on the Sertoli cells to induce and maintain spermatogenesis. While testosterone's role in male reproduction is well-established, the physiologic role of INSL3 remains unclear yet it is a marker of Leydig cell activity and may have anti-apoptotic effects on germ cells [9].

Follicle stimulating hormone (FSH) is central for development of the tubular compartment, where spermatogenesis occurs. Specifically, FSH stimulates the proliferation of immature Sertoli cells that secrete inhibin B (IB) and antimüllerian hormone (AMH). The FSH-induced proliferation of immature Sertoli cells has far-reaching effects on fertility potential as mature Sertoli cells can support a species-specific number of germ cells [10] and determine final seminiferous tubule length [11,12]. Further, approximately 90% of testicular volume is accounted for by the seminiferous tubules thus testicular volume (TV) is a critical indicator of fertility. AMH is secreted by immature Sertoli cells and is downregulated by T. Accordingly, it is normally high in early puberty and falls with rising serum androgen levels [13,14]. Importantly, during the mini-puberty, Sertoli cells do not express the androgen receptor [15]. Therefore, despite the high intra-testicular T levels induced by LH, Sertoli cells remain immature and so the early neonatal period is a proliferative window for immature Sertoli and germ cells. As puberty progresses, the increasing intra-gonadal T production from the Leydig cells ends the proliferative phase as Sertoli cells mature and the cords develop a lumen noting the transition to tubule, and spermatogenesis is initiated [15]. Notably, T levels at the site of production are much higher than peripheral circulating levels and this is a requisite for spermatogenesis. A fact that was elegantly

demonstrated in a study of gonadotrophin-deficient men wherein FSH combined with LH-induced T could stimulate spermatogenesis yet FSH and exogenous T could not [16]. Therefore, FSH and LH-induced T play complementary roles and both are needed to achieve quantitatively and qualitatively normal spermatogenesis [17].

### *Congenital hypogonadotrophic hypogonadism (CHH)*

Disruption of the complex developmental processes regulating GnRH neuron fate specification, migration, or function can result in congenital hypogonadotrophic hypogonadism (CHH). This rare genetic disorder (1/4'000–10'000) [18] is caused by deficient GnRH secretion or action and presents classically as absent puberty and infertility. Importantly, CHH occurs in a spectrum of severity along with number of associated phenotypes that appear at variable rates including skeletal anomalies, cleft lip and palate, synkinesia (mirror movements), sensorineural deafness, cerebellar ataxia and renal agenesis. In particular, the association of CHH with the inability to smell (anosmia) is termed Kallmann syndrome (KS) and is apparent in approximately half of cases [3].

CHH patients typically present in adolescence or early adulthood with absent puberty (i.e. prepubertal testes) [19]. This is evident on physical examination as a testicular volume (TV) of 4 mL is commonly used among adult endocrinologists as a cutoff to indicate the clinical onset of puberty. CHH is clinically heterogeneous and presentation is based on the degree of GnRH deficiency. More severe forms of CHH are characterized by cryptorchidism and micropenis at birth and subsequently a complete absence of pubertal development (i.e. prepubertal testes). Others have partial forms as evidenced by some degree of spontaneous testicular development with TV > 4 mL [20]. The biochemical profile in CHH consists of low T levels in the setting of low or inappropriately normal serum gonadotrophins. Additional causes must be excluded (i.e. normal hypothalamo-pituitary region imaging and no space occupying lesions), and otherwise normal anterior pituitary function must be confirmed as well as normal ferritin levels [19].

### *Predictors of outcome to fertility treatment*

CHH is a treatable form of male infertility and approaches to stimulate gonadal development and fertility include pulsatile GnRH therapy [21,22] or injections of exogenous gonadotrophins [23,24]. Both of these approaches have proven effective in inducing spermatogenesis in the majority of patients [17–25]. Regardless of treatment modality, important predictors of fertility outcomes have emerged (Table 1). Perhaps not surprisingly, initial TV is a key predictor of outcome [27–32] and is also a significant predictor of pregnancy [33]. Notably, CHH men with absent pubertal development consistently have poorer fertility outcomes. These men have the most severe form of GnRH deficiency and lack the beneficial stimulatory effects of mini-puberty and the resulting proliferation of immature Sertoli cells and germ cells. This is reflected biochemically in low serum inhibin B (IB) levels (i.e. < 60 pg/mL) [20,34]. Thus, careful assessment of testicular size (and consideration of any prior gonadotrophin exposure) is important for assessing chances for developing fertility among CHH men (see Practice points).

Several studies have noted the higher incidence of maldescended testes (cryptorchidism) among CHH men with small testes [29,30] (Table 1). Indeed, cryptorchidism (unilateral or bilateral) is an important prognostic factor for male fertility in CHH men [23,29,35,36] as well as in the general population where it affects approximately 2–5% of full-term neonates [37]. Testicular descent will occur spontaneously in approximately  $\frac{3}{4}$  of newborns with maldescended testes and the majority will occur in the first 6-months of life. However, for those in whom the testes do not descend during the first year, the impact on gonocyte survival and long term fertility can be dramatic (particularly in cases of bilateral cryptorchidism) and orchidopexy should be performed within the first year of life [37]. This is relevant for CHH men as cryptorchidism is often associated with the condition. Importantly, men with bilateral cryptorchidism have lower serum IB levels, smaller TV, and lower sperm counts [38] and are six-times more likely to be infertile compared to unilateral or normally descended testes [39]. The impact of cryptorchidism on fertility is determined by a combination of factors including location as higher lying testes (abdominal or above the inguinal ring) have the most severe depletion of germ cells

**Table 1**

Summary of studies identifying predictors of outcomes to fertility-inducing treatment in men with CHH.

Author (year)	Treatment regimen (duration)	Cases <sup>a</sup> (# CHH)	Sperm positive	TV < 4 mL sperm +	TV > 4 mL sperm +	Negative predictor	Comments
Burris et al. (1988)	hCG: 2'000 U 3×/wkly (24 months)	22 (22 CHH)	15/22 (68%)	6/11 (55%)	9/11 (82%)	TV < 4 mL	<ul style="list-style-type: none"> <li>• hCG mono-therapy – outcomes better for larger initial TV</li> </ul>
Pitteloud et al. (2002)	Pulsatile GnRH: 25–600 ng/kg every 2 h (12–24 months)	76 (70 CHH)	51/70 (87%)	43/52 (83%)	18/18 (100%)	TV < 4 mL IB < 60 pg/mL	<ul style="list-style-type: none"> <li>• Cryptorchidism more common in CHH men with TV &lt; 4 mL</li> <li>• 3/6 bilateral cryptorchidism remained azoospermic</li> <li>• 24/31 (77%) have sperm by 12 mos, 42/51 (82%) by 24mos</li> </ul>
Miyagawa et al. (2005)	hCG: 3'000 U 2× weekly FSH: 75 IU 2× weekly (12–24 months)	36 (29 CHH)	9/18 (50%)	4/11 (36%)	5/7 (71%)	TV < 4 mL	<ul style="list-style-type: none"> <li>• Cryptorchidism more common in CHH men with TV &lt; 4 mL</li> <li>• Positive correlation between initial TV and sperm count</li> </ul>
Liu et al. (2009)	hCG: 1'500–2'000 U 2×/wk FSH (post 6 mos hCG): 75–150 IU 3×/wk (23 ± 2 months)	75 (51 CHH)	85–90%	n/a	n/a	TV < 4 mL priorT	<ul style="list-style-type: none"> <li>• Time to response effected by initial TV</li> <li>• Positive effect of prior cycles</li> </ul>
Warne et al. (2009)	hCG: 1'000 U 3×/wk or 2'000 U 2×/wk FSH (post 3–6 mos hCG): 150–300 3×/wk (18 months)	81 (77 CHH)	68/81 (84%)	n/a	n/a	TV < 4 mL BMI > 30	<ul style="list-style-type: none"> <li>• Time to response effected by initial TV</li> <li>• No effect of prior treatment</li> <li>• All non-responders were CHH men with initial TV &lt; 4 mL</li> </ul>

CHH: congenital hypogonadotropic hypogonadism; TV: testicular volume; hCG: human chorionic gonadotrophin, GnRH: gonadotrophin-releasing hormone; IB: inhibin B; FSH: follicle stimulating hormone; n/a: not assessed.

<sup>a</sup> Other subjects included patients with either adult-onset hypogonadotropic hypogonadism or gonadotropin deficiency (i.e. combined pituitary hormone deficiency).

[40]. Further, early surgical correction is important as demonstrated in a recent study comparing orchidopexy at 9 months compared to 3 years of age that revealed compelling evidence for earlier intervention in terms of TV as well as number of germ cells and Sertoli cells [40]. Therefore, current recommendation is for surgical intervention between 6 months and 1 year of age [37,41].

Outcomes for orchidopexy can vary as the procedure of ligating infantile testis can be technically challenging and thus surgical trauma may also be a confounding factor for outcomes [37]. Moreover, as reviewed by Biers and Malone [42], adjuvant hormone treatment is a plausible approach to augment testicular size prior to surgical intervention and has been examined in several studies. Although the use of hCG treatment alone is the standard of care, it is shadowed by some findings suggesting apoptotic and inflammatory changes in germ cells [43,44] thus considerable controversy remains (see Research agenda). For CHH infants lacking mini-puberty, a novel concept of neonatal combined gonadotrophin treatment has emerged as a means to stimulate testicular growth (and IB production) and augment penile size in CHH infants [45]. The initial studies have been promising and raise important questions about the timing and type of treatment offered to CHH patients.

In one large patient series, prior androgen exposure was found to be associated with diminished likelihood of attaining sperm thresholds for conception among CHH men [31] (Table 1). This study stands in contrast to previous reports [46] that found no effect of prior androgen therapy on fertility. As this was not a randomized study, the observed association may reflect that more severe cases of GnRH deficiency may be identified earlier and thus received treatment with androgens (T). Notably,

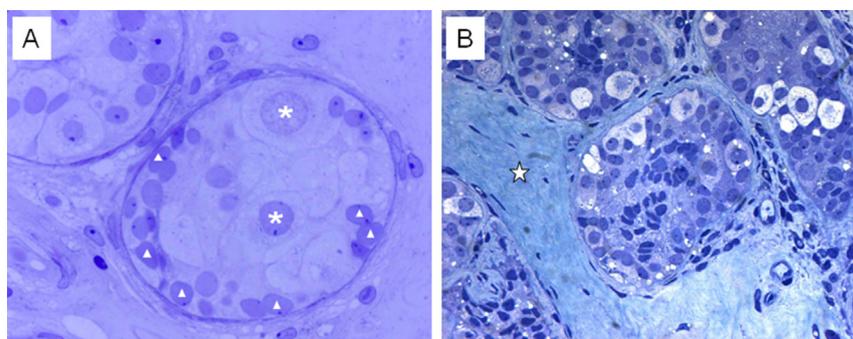
peritubular fibrosis has been noted in following androgen therapy [47] and hCG can induce similar histological changes [48]. Such histologic observations have also been noted in GnRH deficient patients [49]. Further, in an expanded series of CHH patients with prepubertal testes and no history of cryptorchidism, 7/11 (64%) had thickened basement membranes and 8/11 (73%) exhibited increased interstitial fibrosis and all but one had received prior androgen therapy (Fig. 1). This remains an important question for the field because while pubertal induction has been demonstrated with hCG [26] and combined gonadotrophins [50], yet the vast majority of cases receive androgen treatment in adolescence/early adulthood, prior to gonadotrophin treatment for fertility induction. Therefore, addressing the possible long-term fertility impact of prior androgen treatment and identifying the optimal timing for gonadotrophin treatment is critical (See Research points). The existing data regarding predictors of outcome (Table 1) underscore the importance of careful history and clinical examination prior to starting fertility-inducing treatment as well as counseling patients to establish realistic expectations regarding the likelihood of positive outcomes as fertility is not achieved in all cases.

### CHH genetics and future fertility

The majority of CHH cases are sporadic which seems to be in keeping with a condition that impairs fertility. However, a third of the cases display a familial pattern of inheritance [3]. Cases have been described as following autosomal dominant, autosomal recessive, or X-linked inheritance as well as oligogenic forms [51]. CHH is genetically heterogeneous with more than 20 loci identified to date acting alone or in synergy. Despite the growing number of CHH-associated loci, only about 35–40% of cases are currently accounted for by mutations in the identified genes. Gene screening can be guided by clinical presentation providing clues for identifying the underlying molecular basis [52]. Notably, patients with an X-linked form of CHH who harbor mutations in *KAL1* tend to have the poorer response to treatment [53] and genetic counseling should be offered to patients/couples pursuing fertility treatment [54]. Moreover, targeted mutation screening in affected offspring in addition to neonatal hormonal profiling [6], could aid an early diagnosis and potentially be useful for guiding clinical decision making and early initiation of treatment (see Research agenda).

### Approaches to fertility treatment I: pulsatile GnRH therapy

As CHH patients exhibit isolated GnRH deficiency, a logical approach is to replace GnRH. However, this is complicated by the fact that physiologic GnRH secretion is episodic and continuous



**Fig. 1.** Testis histology of CHH men with no pubertal development prior to fertility induction. A total of 11 CHH men with prepubertal testes (TV < 4 mL) and no history of maldescended testes underwent baseline testicular biopsy prior to fertility induction. All subjects exhibited signs of immature testes, absent Leydig cells, small seminiferous cords (approximately 100 micra in diameter) with scarce immature Sertoli cells and few spermatogonia [49]. (A) Light micrograph (1000×) showing immature testis with few spermatogonia (asterisks) and Sertoli cells (triangles). (B) Light micrograph (600×) demonstrating increased interstitial fibrosis (star). On sequential treatment (rFSH alone followed by 24-months of pulsatile GnRH) the subject in panel A had testicular growth from 1.5 → 5.9 mL (via ultrasound), achieved maximal sperm count of  $1.7 \times 10^6$ /mL and conceived twins via IVF. The subject in panel B received 24-months of pulsatile GnRH (LH + FSH) resulting in testicular growth from 0.2 → 3.4 mL (via ultrasound). He achieved a maximal sperm count of  $7.0 \times 10^6$ /mL and was able to conceive naturally [82].

administration results in pituitary de-sensitization and suppression of gonadotrophin secretion [1]. Thus, either intravenous or subcutaneous GnRH must be administered in a pulsatile fashion via mini-infusion pump [21]. A variety of protocols have been described all of which aim to deliver a physiologic regimen (i.e. every 2 h) with GnRH dosing titrated to induce serum gonadotrophin levels capable of stimulating serum T levels in the adult range and spermatogenesis [21,22,29,55,56]. Pulsatile GnRH successfully induces puberty [21,22] and fertility with approximately 80% of men being able to develop spermatogenesis on long-term treatment. CHH men with some degree of spontaneous puberty (TV > 4 mL) typically develop sperm more rapidly than those with prepubertal testes (6–12 months vs. 18–24 months, respectively) [29]. However, this approach is limited by the fact that it requires particular expertise that is typically offered at specialized centers and few options for microinfusion devices. Notably, pulsatile GnRH and exogenous gonadotrophin therapy have comparable outcomes [25–27].

#### *Approaches to fertility treatment II: human chorionic gonadotrophin therapy (hCG)*

Human chorionic gonadotrophin (hCG) has been used as a therapeutic approach for inducing fertility in CHH [23,24]. One advantage of exogenous gonadotrophin treatment is that it can be effective for hypothalamic as well as pituitary disorders yet this mono-therapy approach has important limitations. Mono-therapy with hCG is best used to stimulate spermatogenesis in those CHH men with larger testicular size [23,24,28,57]. This includes those CHH men with some degree of spontaneous testicular development as well as those with the so-called fertile eunuch syndrome [58]. These men exhibit testicular development yet are not virilized and typically have eunuchoidal proportions (armspan exceeding height by >6 cm). The enfeebled GnRH secretion in these men is sufficient to stimulate enough LH and FSH secretion to induce seminiferous tubule development (as evidenced by increased TV and spermatogenesis) yet not sufficient enough to stimulate serum T at levels needed for full-virilization. Thus, fertile eunuchs are distinguished by their active spermatogenesis and can typically achieve fertility with hCG therapy alone and in some instances T only [59].

Thus, mono-therapy with hCG therapy is a viable treatment option for those CHH men on the mild end of the phenotypic spectrum (i.e. partial to near-full testicular development). Importantly, hCG mono-therapy is much less successful in men who lack testicular development [23,28,34,57]. In the seminal paper by Finkel and colleagues, only 1/6 CHH men with frankly hypogonadal serum T levels and prepubertal testes developed sperm on hCG alone [23]. Burriss and colleagues treated a larger cohort of 22 CHH men (11 with absent puberty and 11 with partial puberty) with long-term (12–24 months) hCG treatment [28]. They observed that men with smaller initial testicular size were less likely to develop sperm with 6/11 (55%) vs. 9/11 (81%) (Table 1). Similar results were observed in an Italian study of 17 CHH men on long-term hCG mono-therapy (up to 10 years) reporting 5/8 (60%) vs. 8/9 (90%) respectively [24]. More recently, a large Chinese study of CHH men (nearly all of whom had prepubertal testes) provided hCG treatment alone for up to 18 months stimulating sperm production in only 34/84 (41%) [60]. Thus, hCG mono-therapy is not the ideal treatment for men who lack testicular development (i.e. TV < 4 mL) as these studies indicate that only about half of such CHH men are able to develop sperm on hCG mono-therapy. In such cases, combined gonadotrophin therapy has achieved better outcomes.

#### *Approaches to fertility treatment III: combined gonadotrophin therapy*

Combined gonadotrophin therapy (hCG + FSH) is another treatment regimen often used for CHH patients. This is based on the principle that hCG serves as a long-acting substitute for LH and that FSH can be replaced using purified urinary FSH preparations or recombinant FSH. The earliest FSH preparations (i.e. human menopausal gonadotrophins, hMG) had both FSH and LH activity as they were derived from menopausal urine. Subsequently, highly purified urinary FSH preparations were developed [61], and in the 1990s recombinant FSH (rFSH) formulations emerged that had no LH cross-reactivity [62]. While initial studies employing gonadotrophins administered them via intramuscular injection, these medications are now routinely delivered safely and effectively via subcutaneous injection [63–65]. These less invasive injections have much greater patient acceptability and likely help

contribute to adherence. Moreover, the emergence of long-acting FSH preparations [66] now on the market may also hold promise for CHH men undergoing fertility induction.

The first patient series demonstrating the effectiveness of combined gonadotrophin therapy for inducing fertility in CHH emerged in the mid-1980s [23,35,67]. Over the next 30 years a number of clinical series have been published reporting outcomes from individual centers [30,56,64,68–72] yet results are difficult to compare as agents and dosing schedules are highly variable. For instance, the time to develop sperm in the ejaculate ranges from 3 to 19 months with median times in the range of 9–12 months for combined treatment (using different regimens) [31,32,50,61,73]. In 2005, a Japanese group reported outcomes on a series of 36 patients including 29 CHH men receiving a standard long-term treatment [30]. Only 36% of the CHH men with prepubertal testes developed sperm compare to 71% with some testicular development (Table 1) and those men with TV < 4 mL were much more likely to have had cryptorchidism (39% vs 8%). These findings were in line with prior reports that mal-descended testes limit favorable fertility outcomes and such patients require longer treatment to achieve spermatogenesis [29,36,74,75]. Subsequently, in 2009 Liu and colleagues performed a detailed analysis of large cohort of 75 patients (51 of whom had CHH) who received a standardized treatment regimen [31]. Importantly, half of treatment cycles (58/116) resulted in pregnancy and a only a minority of which (7/58, 12%) resulted from *in vitro* fertilization. Using a variety of elegant statistical analytic approaches they identified the median time to develop sperm in the ejaculate was 7 months and median time to conception was 28 months [33]. Further, analysis of patients receiving multiple rounds of gonadotrophin treatment revealed that spermatogenesis was achieved 2–3 fold faster in subsequent courses compared to the previous treatment period. In line with prior studies, the authors identified that smaller initial testicular volume was a negative predictor [27,28,30,69] (Table 1). Later in 2009, a combined analysis of several international studies evaluating a structured combined gonadotrophin treatment regimen [73,76,77] (Table 1) was published again confirming smaller baseline TV as a negative outcome predictor [32]. Overall, 84% of participants in the combined analysis developed sperm and 69% achieved concentrations  $\geq 1.5 \times 10^6/\text{mL}$  which has been used in a number of studies as a concentration for achieving pregnancy [61,64,73].

A common practice has been to initiate treatment with hCG with injections i.e. 1000–2000 U two to three times per week depending on available dosing formulations. Concomitant treatment with FSH can be initiated immediately, but typically, hCG mono-therapy is continued for 3–6 months at which time FSH is added (i.e. 75–150 IU every other day/three times per week) [78]. Approximately 80% of men will be able to develop sperm [31,32] and the vast majority achieve maximum testicular volume and develop sperm in the ejaculate with 12–18 months of treatment (see Practice points).

#### *Approaches to fertility treatment IV: sequential treatment protocols*

As reviewed above, mono-therapy with hCG has produced less than optimal outcomes for patients without testicular development and outcomes improve with the addition of FSH. However, prepubertal TV remains a strong negative predictor of outcome. As 90% of testicular volume is determined by the seminiferous tubules, factors promoting tubule development (i.e. proliferation of immature Sertoli and germ cells) are critical for optimizing spermatogenic capacity [11,12]. Therefore, a plausible approach for improving fertility in the most severely affected CHH men is to provide unopposed FSH treatment to proliferate immature Sertoli cells prior to androgen-induced maturation [15]. Accordingly, a sequential treatment approach could be employed in an attempt to recapitulate the hormonal dynamics of puberty wherein FSH rises prior to increased serum levels of LH-induced T. Raivio and colleagues first demonstrated the beneficial effects of rFSH on serum inhibin B (IB) and TV in three prepubertal boys with gonadotrophin deficiency (one case each of Kallmann syndrome, panhypopituitarism and post-treatment for craniopharyngioma) [79]. This resulted in testicular growth and the increased serum IB following rFSH administration was presumed to reflect FSH-induced Sertoli cell proliferation. Moreover, rFSH has been administered to gonadotrophin-deficient patients of diverse etiologies (CHH, congenital hypopituitarism, or post-surgery for intracranial tumors) in several studies all of which demonstrated testicular growth and increased serum IB and AMH levels [34,79–81]. In 2007, Raivio and colleagues reported a long term follow up of a series of 14 prepubertal boys with gonadotrophin deficiency of different etiologies (including 4 CHH, 2 of whom had a history of

cryptorchidism) all of whom received pre-treatment with variable rFSH regimens prior to pubertal induction with hCG [80]. Importantly, three of the four CHH men developed sperm suggesting that those men with the most severe GnRH deficiency (TV < 4 mL, cryptorchidism  $\pm$  microphallus) may benefit from rFSH priming prior to hCG induced maturation.

In 2013, the results of an open-label randomized study were published that compared 4-months of rFSH treatment prior to 24 months of pulsatile GnRH (LH + FSH) ( $n = 7$ ) versus standard treatment (24 months of pulsatile GnRH) ( $n = 6$ ) in CHH men with prepubertal testes [82]. To control for confounding factors patients with without cryorchidism and gonadotrophin therapy were excluded. rFSH treatment induced normal serum IB levels and TV doubled in 4 months while histologic studies revealed Sertoli cells and spermatogonia proliferation as well as cytoskeletal rearrangements. Importantly, all men receiving rFSH pre-treatment developed sperm in their ejaculate (7/7) compared to 4/6 in the GnRH-only group. Moreover, the pre-treated group trended towards higher maximal sperm counts. Given the difficulty to recruit severely affected CHH patients without cryptorchidism or prior gonadotropin treatment, this study was not sufficiently powered to reach statistical significance and would require an estimated 28 subjects in each treatment arm to do so. Regardless, these initial studies demonstrate that sequential treatment approach including pre-treatment with FSH is successful in inducing testicular growth and fertility in CHH men with prepubertal testes. To definitively demonstrate superiority of this approach, a larger prospective multicenter study would be required. Further, a similar treatment approach could potentially be employed for those CHH men with a history of mal-descended testes who are among the least responsive to gonadotrophin therapy (see Research agenda).

#### *Patient monitoring during fertility induction*

Patient care during fertility induction involves both regular assessment of testicular development and spermatogenesis as well as monitoring for unwanted effects associated with gonadotrophin therapy. Gynecomastia is the most common side effect of hCG therapy [48] and is seen in up to one third of patients. The presumed mechanism is excess LH-induced estrogen secretion which is best minimized or avoided by utilizing the lowest dose capable of maintaining low-normal serum T levels. As the half-life of hCG is approximately, 36 h [83], monitoring serum T levels should be done in the context of the pharmacokinetics. Specifically, trough levels obtained prior to the subsequent injection are most informative as they can facilitate closer dose titration to minimize unwarranted effects as well as to avoid excessive escalation of serum T levels that could result in elevated hematocrit. Typically hCG dose begins at 1'000–2'000 U every other. As TV increases, doses can often be reduced to 500–1'500 U every other day as long as the dose for maintaining trough serum T level at the lower end of the normal adult range. With the efficacy of subcutaneous hCG injections [64,65,84], invasive intramuscular injections are no longer necessary. However, inquiries about adherence should be made at patient visits because sporadic compliance may contribute to the development of hCG antibodies. As such, falling T response to hCG treatment may result from poor adherence and in rare cases, antibodies [85]. For sequential or combined treatment approaches serum measurement of FSH is warranted. We target levels of 4–6 IU/L and attempt to avoid levels >9 IU/L [82].

Additional hormone markers, such as IB and AMH levels, may prove useful from both a prognostic point of view as well as for monitoring treatment. Low baseline serum IB level (<60 pg/mL) has been demonstrated to be negative predictor of outcome [29] (Table 1). Given that CHH men have a limited Sertoli cell population (and low T levels), it is expected that serum IB levels are low and AMH levels are high [13,20]. As such, IB and AMH could be used as a proxy for the proliferation of immature Sertoli cells [9,82] during FSH treatment. In CHH men with severe GnRH deficiency, serial monitoring of serum IB indicated that levels reached a plateau after 2 months of rFSH treatment (75 IU daily) in, suggesting that 2 months could be a sufficient pre-treatment period with rFSH alone [82]. Similarly, INSL3 has been proposed as a marker of Leydig cell function and could be used to gauge Leydig cell response to treatment much in the same way as IB for Sertoli cells [86].

Clinically, assessment of testicular volume using a Prader orchidometer is an important metric of response to fertility treatment [87]. Typically TV of 8–10 mL indicates spermatogenesis yet it is important to note that men on fertility inducing treatment who attain limited testicular growth (i.e. volume of 4–5 mL by Prader orchidometer) can have sperm in the ejaculate [27,28,53]. Further, CHH

men with TV as small as 3 mL via ultrasonography can have active spermatogenesis and in some cases are able to conceive naturally [82] (Fig. 1). The vast majority of CHH men will not achieve normal sperm counts as defined by the World Health Organization (i.e. >20 million/mL) [88] yet low sperm counts do not preclude fertility [89]. Indeed, median sperm counts for conception range from 3 to 8 million/mL [24,27,28,33] and median time to reach sperm has been reported at 5 months [33] thus seminal fluid analysis can commence within a few months of starting treatment. However, testis volume is the single most important predictor of outcome and those with smaller testes or a history of maldescended testes can take considerably longer with 12–24 months often needed for patients with bilateral cryptorchidism [22,27,31,63,65]. Therefore, when discussing fertility plans with patients, it is recommended that treatment is initiated at least 6–12 months prior to the time at which fertility is desired.

Fertility-inducing treatment is typically continued after conception into the 2nd trimester due to the possibility of miscarriage early in the pregnancy. If the couple desires to conceive again quickly, hCG alone can be continued. This can effectively maintain spermatogenesis yet sperm counts tend to progressively fall over time [90]. Importantly, Liu and colleagues noted that a prior gonadotrophin treatment resulted in a 2–3 fold shorter time to the appearance of first sperm on subsequent gonadotrophin treatment regimens [31]. So, while it appears that repeated gonadotrophin treatment does not have a negative effect on fertility, it is important to remember that this does not guarantee success on subsequent cycles. Therefore, we recommend that patients be given the option to store sperm and transition to testosterone replacement in between periods of gonadotrophin treatment (see Research agenda). Further, for those adolescents receiving pubertal induction via gonadotrophin therapy, cryopreservation prior to transitioning to testosterone replacement could be done as an insurance policy of sorts for future fertility.

#### *Assisted reproductive technology (ART)*

The field of assisted reproductive technology (ART) had rapidly advanced and it is now possible to achieve conception in men with severely impaired sperm counts or quality. Clinically, before pursuing expensive and often invasive ART one should not neglect to evaluate fertility of the female partner as an important factor. ART approaches range from relatively less invasive intra-uterine insemination (IUI), to *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI). ICSI was first used in CHH as an approach to shorten treatment duration [91]. Yet authors recommended delaying ART intervention until maximal testicular development has been achieved. The success rates of ICSI are high with fertilization rates of 50–60% and pregnancy rates of ~30% per cycle [92–95]. If no sperm are present in the ejaculate it is possible to utilize epididymal aspiration or testicular biopsy as alternative approaches. Notably, detailed assessment of sperm quality in a group of CHH men undergoing combined gonadotrophin treatment revealed that CHH per se does not seem to impede DNA integrity or increase the risk for chromosomal aberrations [96]. Questions have been raised regarding the possible increased risk for some birth defects with ART [97,98] yet this remains controversial and there is no clear consensus on this matter.

#### **Summary**

Men with CHH are a patient population with a treatable form of male infertility. The existing data support that approximately 80% of CHH men are able to develop sperm in the ejaculate with either combined or sequential gonadotrophin treatment (rFSH followed by hCG + rFSH treatment). CHH men with partial testicular development typically develop sperm within 6-months of starting treatment. Important factors affect fertility outcomes including degree of spontaneous testicular development and history of maldescended testes. For the most severe cases (i.e. TV < 4 mL), a sequential approach including FSH followed by FSH + hCG (or pulsatile GnRH) appears to be highly effective to induced spermatogenesis. However, a large multicenter study is needed to definitively confirm if this novel approach results in superior fertility outcomes. Additional questions remain including the issue neonatal treatment in cases of absent mini-puberty (i.e. cryptorchidism and micropenis) or whether using gonadotrophins for pubertal induction is preferable to inducing secondary sexual characteristics with exogenous androgens. In addition to fertility outcomes, a recent report provides initial support for

the notion that gonadotrophin therapy may help ameliorate some of the psychosocial aspects of CHH and improve some health-related quality of life domains [99]. Last, the growing understanding of genetics of CHH may also reveal insights into fertility potential in CHH and could lead to more personalized approaches to treatment.

### Practice points

- The neonatal mini-puberty is a window of opportunity to diagnose CHH and this period may have far-reaching effects on fertility.
- Initial testicular volume (<4 mL), history of cryptorchidism and low inhibin B levels (<60 pg/ml) are critical negative predictors of spermatogenesis.
- Prior treatment with rFSH followed by rFSH + hCG (or pulsatile GnRH) appears to improve fertility in a subset of CHH men with severe GnRH deficiency.
- Men with CHH and prepubertal testes (with/without cryptorchidism) often require combined gonadotrophin stimulation and often extended treatment (18–24 months) to achieve fertility.
- Approximately 80% of CHH can attain spermatogenesis and low sperm counts do not preclude fertility in these patients.
- Assisted reproductive technologies can be helpful in men with severely compromised sperm count and or quality.
- Professional genetic counseling is recommended for CHH patients pursuing fertility.

### Research agenda

- Evidence is conflicting on the potential benefit of gonadotrophin treatment prior to surgical intervention for cryptorchidism and warrants further investigation.
- Initial studies point to beneficial effects of early gonadotrophin treatment during infancy or for pubertal induction raising important questions about the optimal timing for treatment.
- Some evidence suggests prior androgen exposure negatively impacts fertility outcomes, this should be clarified on a larger scale.
- The use of rFSH pretreatment prior to combined gonadotrophin treatment in men with no testicular development (i.e. TV < 4 mL) with/without cryptorchidism should be assessed in a large, multicenter study.
- Further genetic elucidation of CHH could possibly contribute to more personalized treatment approaches concerning particular genotypes that have more/less favorable outcomes to fertility-inducing treatment.

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### References

- [1] Belchetz PE, Plant TM, Nakai Y, et al. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* 1978;202:631–3.
- [2] Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2009;5:569–76.
- [3] Mitchell AL, Dwyer A, Pitteloud N, et al. Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. *Trends Endocrinol Metab* 2011;22:249–58.
- [4] Hutson JM, Southwell BR, Li R, et al. The regulation of testicular descent and the effects of cryptorchidism. *Endocr Rev* 2013;34:725–52.
- [5] Kuri-Hanninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Horm Res Paediatr* 2014;82:73–80.

- [6] Grumbach MM. A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab* 2005;90:3122–7.
- [7] Muller J, Skakkebaek NE. Quantification of germ cells and seminiferous tubules by stereological examination of testicles from 50 boys who suffered from sudden death. *Int J Androl* 1983;6:143–56.
- [8] Boyar RM, Rosenfeld RS, Kapen S, et al. Human puberty. Simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. *J Clin Investig* 1974;54:609–18.
- [9] Rey RA, Grinspon RP, Gottlieb S, et al. Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. *Andrology* 2013;1:3–16.
- [10] Russell LD, Ren HP, Sinha Hikim I, et al. A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components, emphasizing those related to the sertoli cell. *Am J Anat* 1990;188:21–30.
- [11] Orth JM, Gunsalus GL, Lamperti AA. Evidence from sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of sertoli cells produced during perinatal development. *Endocrinology* 1988;122:787–94.
- [12] Sharpe RM, Turner KJ, McKinnell C, et al. Inhibin B levels in plasma of the male rat from birth to adulthood: effect of experimental manipulation of sertoli cell number. *J Androl* 1999;20:94–101.
- [13] Young J, Rey R, Couzinet B, et al. Antimullerian hormone in patients with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1999;84:2696–9.
- [14] Hero M, Tommiska J, Vaaralahti K, et al. Circulating antimullerian hormone levels in boys decline during early puberty and correlate with inhibin B. *Fertil Steril* 2012;97:1242–7.
- [15] Rey RA, Musse M, Venara M, et al. Ontogeny of the androgen receptor expression in the fetal and postnatal testis: its relevance on sertoli cell maturation and the onset of adult spermatogenesis. *Microsc Res Tech* 2009;72:787–95.
- [16] Schaison G, Young J, Pholsena M, et al. Failure of combined follicle-stimulating hormone-testosterone administration to initiate and/or maintain spermatogenesis in men with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1993;77:1545–9.
- [17] 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.
- [18] Fromantin M, Gineste J, Didier A, et al. Impuberism and hypogonadism at induction into military service. Statistical study. *Problemes actuels d'endocrinologie de Nutr* 1973;16:179–99.
- [19] Young J. Approach to the male patient with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2012;97:707–18.
- [20] Pitteloud N, Hayes FJ, Boepple PA, et al. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2002;87:152–60.
- [21] Hoffman AR, Crowley Jr WF. Induction of puberty in men by long-term pulsatile administration of low-dose gonadotropin-releasing hormone. *N Engl J Med* 1982;307:1237–41.
- [22] Delemarre-Van de Waal HA. Induction of testicular growth and spermatogenesis by pulsatile, intravenous administration of gonadotrophin-releasing hormone in patients with hypogonadotropic hypogonadism. *Clin Endocrinol* 1993;38:473–80.
- \*[23] Finkel DM, Phillips JL, Snyder PJ. Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. *N Engl J Med* 1985;313:651–5.
- [24] Vicari E, Mongioi A, Calogero AE, et al. Therapy with human chorionic gonadotrophin alone induces spermatogenesis in men with isolated hypogonadotropic hypogonadism—long-term follow-up. *Int J Androl* 1992;15:320–9.
- [25] Liu L, Banks SM, Barnes KM, et al. 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.
- [26] Schopohl J, Mehlretter G, von Zumbusch R, et al. Comparison of gonadotropin-releasing hormone and gonadotropin therapy in male patients with idiopathic hypothalamic hypogonadism. *Fertil Steril* 1991;56:1143–50.
- [27] Buchter 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.
- [28] Burriss AS, Rodbard HW, Winters SJ, et al. Gonadotropin therapy in men with isolated hypogonadotropic hypogonadism: the response to human chorionic gonadotropin is predicted by initial testicular size. *J Clin Endocrinol Metab* 1988;66:1144–51.
- \*[29] Pitteloud N, Hayes FJ, Dwyer A, et al. Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2002;87:4128–36.
- [30] Miyagawa Y, Tsujimura A, Matsumiya K, et al. Outcome of gonadotropin therapy for male hypogonadotropic hypogonadism at university affiliated male infertility centers: a 30-year retrospective study. *J Urol* 2005;173:2072–5.
- \*[31] Liu PY, Baker HW, Jayadev V, et al. 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.
- \*[32] Warne DW, Decosterd G, Okada H, et al. 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.
- \*[33] Liu PY, Gebiski VJ, Turner L, et al. Predicting pregnancy and spermatogenesis by survival analysis during gonadotrophin treatment of gonadotrophin-deficient infertile men. *Hum Reprod* 2002;17:625–33.
- [34] Young J, Chanson P, Salenave S, et al. Testicular anti-mullerian hormone secretion is stimulated by recombinant human FSH in patients with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2005;90:724–8.
- [35] Ley SB, Leonard JM. Male hypogonadotropic hypogonadism: factors influencing response to human chorionic gonadotropin and human menopausal gonadotropin, including prior exogenous androgens. *J Clin Endocrinol Metab* 1985;61:746–52.

- [36] Kirk JM, Savage MO, Grant DB, et al. Gonadal function and response to human chorionic and menopausal gonadotrophin therapy in male patients with idiopathic hypogonadotrophic hypogonadism. *Clin Endocrinol* 1994;41:57–63.
- \*[37] Chan E, Wayne C, Nasr A, et al. *alFRCS* for Canadian Association of Pediatric Surgeon Evidence-Based Resource. Ideal timing of orchiopexy: a systematic review. *Pediatr Surg Int* 2014;30:87–97.
- [38] Trsinar B, Muravec UR. Fertility potential after unilateral and bilateral orchidopexy for cryptorchidism. *World J Urol* 2009;27:513–9.
- [39] Lee PA, O'Leary LA, Songer NJ, et al. Paternity after unilateral cryptorchidism: a controlled study. *Pediatrics* 1996;98:676–9.
- [40] Kollin C, Stukenborg JB, Nurmio M, et al. Boys with undescended testes: endocrine, volumetric and morphometric studies on testicular function before and after orchidopexy at nine months or three years of age. *J Clin Endocrinol Metab* 2012;97:4588–95.
- [41] Ritzen EM, Bergh A, Bjercknes R, et al. Nordic consensus on treatment of undescended testes. *Acta Paediatr* 2007;96:638–43.
- [42] Biers SM, Malone PS. A critical appraisal of the evidence for improved fertility indices in undescended testes after gonadotrophin-releasing hormone therapy and orchidopexy. *J Pediatr Urol* 2010;6:239–46.
- [43] Dunkel L, Taskiran S, Hovatta O, et al. Germ cell apoptosis after treatment of cryptorchidism with human chorionic gonadotropin is associated with impaired reproductive function in the adult. *J Clin Invest* 1997;100:2341–6.
- [44] Heiskanen P, Billig H, Toppari J, et al. Apoptotic cell death in the normal and cryptorchid human testis: the effect of human chorionic gonadotropin on testicular cell survival. *Pediatr Res* 1996;40:351–6.
- \*[45] Bouvattier C, Maione L, Bouligand J, et al. Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2012;8:172–82.
- [46] Burger HG, de Kretser DM, Hudson B, et al. Effects of preceding androgen therapy on testicular response to human pituitary gonadotropin in hypogonadotropic hypogonadism: a study of three patients. *Fertil Steril* 1981;35:64–8.
- [47] de Kretser DM, Kerr JB, Paulsen CA. The peritubular tissue in the normal and pathological human testis. An ultrastructural study. *Biol Reprod* 1975;12:317–24.
- [48] Maddock WO, Nelson WO. The effects of chorionic gonadotropin in adult men: increased estrogen and 17-ketosteroid excretion, gynecomastia, leydig cell stimulation and semi-niferous tubule damage. *J Clin Endocrinol Metab* 1952;12:985–1014.
- [49] Kumar PA, Pitteloud N, Andrews PA, et al. Testis morphology in patients with idiopathic hypogonadotropic hypogonadism. *Hum Reprod* 2006;21:1033–40.
- [50] Barrio R, de Luis D, Alonso M, et al. Induction of puberty with human chorionic gonadotropin and follicle-stimulating hormone in adolescent males with hypogonadotropic hypogonadism. *Fertil Steril* 1999;71:244–8.
- [51] Sykiotis GP, Plummer L, Hughes VA, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci U. S. A* 2010;107:15140–4.
- [52] Costa-Barbosa FA, Balasubramanian R, Keefe KW, et al. Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. *J Clin Endocrinol Metab* 2013;98:E943–53.
- [53] Sykiotis GP, Hoang XH, Avbelj M, et al. Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. *J Clin Endocrinol Metab* 2010;95:3019–27.
- [54] Au MG, Crowley Jr WF, Buck CL. Genetic counseling for isolated GnRH deficiency. *Mol Cell Endocrinol* 2011;346:102–9.
- [55] Aulitzky W, Frick J, Galvan G. Pulsatile luteinizing hormone-releasing hormone treatment of male hypogonadotropic hypogonadism. *Fertil Steril* 1988;50:480–6.
- [56] Spratt DI, Finkelstein JS, O'Dea LS, et al. Long-term administration of gonadotropin-releasing hormone in men with idiopathic hypogonadotropic hypogonadism. A model for studies of the hormone's physiologic effects. *Ann Intern Med* 1986;105:848–55.
- [57] Kung AW, Zhong YY, Lam KS, et al. Induction of spermatogenesis with gonadotrophins in Chinese men with hypogonadotropic hypogonadism. *Int J Androl* 1994;17:241–7.
- [58] McCullagh EP, Beck JC, Schaffenburg CA. A syndrome of eunuchoidism with spermatogenesis, normal urinary FSH and low or normal ICSH: (fertile eunuchs). *J Clin Endocrinol Metab* 1953;13:489–509.
- [59] Pitteloud N, Boepple PA, DeCruz S, et al. The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor. *J Clin Endocrinol Metab* 2001;86:2470–5.
- [60] Yang L, Zhang SX, Dong Q, et al. Application of hormonal treatment in hypogonadotropic hypogonadism: more than ten years experience. *Int Urol Nephrol* 2012;44:393–9.
- [61] European Metrodin HP Study Group. Efficacy and safety of highly purified urinary follicle-stimulating hormone with human chorionic gonadotropin for treating men with isolated hypogonadotropic hypogonadism. *Fertil Steril* 1998;70:256–62.
- [62] Mannaerts B, Fauser B, Lahlou N, et al. Serum hormone concentrations during treatment with multiple rising doses of recombinant follicle stimulating hormone (Puregon) in men with hypogonadotropic hypogonadism. *Fertil Steril* 1996;65:406–10.
- [63] Jones TH, Darne JF. Self-administered subcutaneous human menopausal gonadotrophin for the stimulation of testicular growth and the initiation of spermatogenesis in hypogonadotropic hypogonadism. *Clin Endocrinol* 1993;38:203–8.
- [64] Burgues S, Calderon MD, Spanish Collaborative Group on Male Hypogonadotropic Hypogonadism. Subcutaneous self-administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotropic hypogonadism. *Hum Reprod* 1997;12:980–6.
- [65] Saal W, Happ J, Cordes U, et al. Subcutaneous gonadotropin therapy in male patients with hypogonadotropic hypogonadism. *Fertil Steril* 1991;56:319–24.
- [66] Verboost P, Sloot WN, Rose UM, et al. Pharmacologic profiling of corifollitropin alfa, the first developed sustained follicle stimulant. *Eur J Pharmacol* 2011;651:227–33.
- [67] Okuyama A, Nakamura M, Namiki M, et al. Testicular responsiveness to long-term administration of hCG and hMG in patients with hypogonadotropic hypogonadism. *Horm Res* 1986;23:21–30.

- [68] 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.
- [69] Ishikawa T, Ooba T, Kondo Y, et al. Assessment of gonadotropin therapy in male hypogonadotropic hypogonadism. *Fertil Steril* 2007;88:1697–9.
- [70] Farhat R, Al-zidjali F, Alzahrani AS. Outcome of gonadotropin therapy for male infertility due to hypogonadotrophic hypogonadism. *Pituitary* 2010;13:105–10.
- [71] Oldereid NB, Abyholm T, Tanbo TG. Spermatogenesis and fertility outcome in male hypogonadotrophic hypogonadism. *Hum Fertil* 2010;13:83–9.
- [72] Milsom S, Duggan K, O'Sullivan S, et al. Treatment of infertility with hypogonadotropic hypogonadism: 10-year experience in Auckland, New Zealand. *Aust N Z J Obstet Gynaecol* 2012;52:293–8.
- [73] Bouloux P, Warne DW, Loumaye E, et al. Efficacy and safety of recombinant human follicle-stimulating hormone in men with isolated hypogonadotrophic hypogonadism. *Fertil Steril* 2002;77:270–3.
- [74] Bouvattier C, Tauber M, Jouret B, et al. Gonadotropin treatment of hypogonadotropic hypogonadal adolescents. *J Pediatr Endocrinol Metab* 1999;12:339–44.
- [75] Bouloux PM, Nieschlag E, Burger HG, et al. Induction of spermatogenesis by recombinant follicle-stimulating hormone (puregon) in hypogonadotrophic azoospermic men who failed to respond to human chorionic gonadotropin alone. *J Androl* 2003;24:604–11.
- [76] Liu PY, Turner L, Rushford D, et al. Efficacy and safety of recombinant human follicle stimulating hormone (Gonal-F) with urinary human chorionic gonadotrophin for induction of spermatogenesis and fertility in gonadotrophin-deficient men. *Hum Reprod* 1999;14:1540–5.
- [77] Matsumoto AM, Snyder PJ, Bhasin S, et al. 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;92:979–90.
- [78] Han TS, Bouloux PM. What is the optimal therapy for young males with hypogonadotropic hypogonadism? *Clin Endocrinol* 2010;72:731–7.
- \*[79] Raivio T, Toppari J, Perheentupa A, et al. Treatment of prepubertal gonadotrophin-deficient boys with recombinant human follicle-stimulating hormone. *Lancet* 1997;350:263–4.
- \*[80] Raivio T, Wikstrom AM, Dunkel L. Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. *Eur J Endocrinol* 2007;156:105–11.
- [81] Bougneres P, Francois M, Pantalone L, et al. Effects of an early postnatal treatment of hypogonadotropic hypogonadism with a continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone. *J Clin Endocrinol Metab* 2008;93:2202–5.
- \*[82] Dwyer AA, Sykiotis GP, Hayes FJ, et al. Trial of recombinant follicle-stimulating hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2013;98:E1790–5.
- [83] Padron RS, Wischusen J, Hudson B, et al. Prolonged biphasic response of plasma testosterone to single intramuscular injections of human chorionic gonadotropin. *J Clin Endocrinol Metab* 1980;50:1100–4.
- [84] Jones TH, Darne JF, McGarrigle HH. Diurnal rhythm of testosterone induced by human chorionic gonadotrophin (hCG) therapy in isolated hypogonadotrophic hypogonadism: a comparison between subcutaneous and intramuscular hCG administration. *Eur J Endocrinol* 1994;131:173–8.
- [85] Sokol RZ, McClure RD, Peterson M, et al. Gonadotropin therapy failure secondary to human chorionic gonadotropin-induced antibodies. *J Clin Endocrinol Metab* 1981;52:929–32.
- [86] Trabado S, Maione L, Bry-Gauillard H, et al. Insulin-like peptide 3 (INSL3) in men with congenital hypogonadotropic hypogonadism/Kallmann syndrome and effects of different modalities of hormonal treatment: a Single-Center study of 281 patients. *J Clin Endocrinol Metab* 2013;99:E268–275.
- [87] Anawalt BD. Approach to male infertility and induction of spermatogenesis. *J Clin Endocrinol Metab* 2013;98:3532–42.
- [88] Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update* 2010;16:231–45.
- [89] Burriss AS, Clark RV, Vantman DJ, et al. A low sperm concentration does not preclude fertility in men with isolated hypogonadotrophic hypogonadism after gonadotropin therapy. *Fertil Steril* 1988;50:343–7.
- [90] Depenbusch M, von Eckardstein S, Simoni M, et al. Maintenance of spermatogenesis in hypogonadotrophic hypogonadal men with human chorionic gonadotropin alone. *Eur J Endocrinol* 2002;147:617–24.
- [91] Yong EL, Lee KO, Ng SC, et al. Induction of spermatogenesis in isolated hypogonadotrophic hypogonadism with gonadotrophins and early intervention with intracytoplasmic sperm injection. *Hum Reprod* 1997;12:1230–2.
- [92] Zorn B, Pfeifer M, Virant-Klun I, et al. Intracytoplasmic sperm injection as a complement to gonadotrophin treatment in infertile men with hypogonadotrophic hypogonadism. *Int J Androl* 2005;28:202–7.
- [93] Resorlu B, Abdulmajed MI, Kara C, et al. Is intracytoplasmic sperm injection essential for the treatment of hypogonadotrophic hypogonadism? A comparison between idiopathic and secondary hypogonadotrophic hypogonadism. *Hum Fertil* 2009;12:204–8.
- [94] Fahmy I, Kamal A, Shamloul R, et al. ICSI using testicular sperm in male hypogonadotrophic hypogonadism unresponsive to gonadotrophin therapy. *Hum Reprod* 2004;19:1558–61.
- [95] Bakircioglu ME, Erden HF, Ciray HN, et al. Gonadotrophin therapy in combination with ICSI in men with hypogonadotrophic hypogonadism. *Reprod Biomed Online* 2007;15:156–60.
- [96] Krabchi K, Berthaut I, Chantot-Bastaroud S, et al. Quality assessment of induced spermatogenesis in hypogonadotrophic hypogonadic men treated with gonadotrophins. *Reprod Biomed Online* 2011;22:277–83.
- [97] Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N. Engl J Med* 2002;346:725–30.
- [98] Reefhuis J, Honein MA, Schieve LA, et al. Assisted reproductive technology and major structural birth defects in the United States. *Hum Reprod* 2009;24:360–6.
- [99] Shiraishi K, Oka S, Matsuyama H. Assessment of quality of life during gonadotrophin treatment for male hypogonadotrophic hypogonadism. *Clin Endocrinol* 2014;81:259–65.