Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism

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Abstract | Congenital hypogonadotropic hypogonadism (CHH) causes pubertal failure and infertility in both women and men due to partial or total secretory failure of the two pituitary gonadotropins lutropin (LH) and follitropin (FSH) during periods of physiological activation of the gonadotropic axis. Men and women with CHH frequently seek treatment for infertility after hypogonadism therapy. Some etiologies, such as autosomal dominant or X-linked Kallmann syndrome, raise the question of hereditary transmission, leading to increasing demands for genetic counseling and monitoring of medically assisted pregnancies. Diagnosis and treatment of newborn boys is, therefore, becoming an increasingly important issue. In male individuals with complete forms of CHH, the antenatal and neonatal gonadotropin deficit leads to formation of a micropenis and cryptorchidism, which could undermine future sexual and reproductive functions. Standard treatments, usually started after the age of puberty, often only partially correct the genital abnormalities and spermatogenesis. The aim of this Review is to examine the possible additional benefits of neonatal gonadotropin therapy in male patients with CHH. Encouraging results of neonatal therapy, together with a few reports of prepubertal treatment, support the use of this novel therapeutic strategy aimed at improving sexual and reproductive functions in adulthood.

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Introduction

The term congenital hypogonadotropic hypogonadism (CHH) refers to a group of rare disorders characterized by a deficiency in gonadotropin-releasing hormone (GnRH), which results in pituitary gonadotropin deficiencies that are already present in the fetus and usually persist throughout life.[1,2] Schematically, three subgroups of CHH exist, the largest of which involves isolated gonadotropin deficiency and is composed of individuals whose entire phenotype is explained exclusively by prenatal and postnatal gonadotropin and sex-steroid deficiency. The second most frequent form is Kallmann syndrome, in which the congenital GnRH deficit is usually accompanied by partial or complete loss of olfaction and sometimes by other neurological abnormalities or malformations.[3,4] The third subgroup consists of heterogeneous 'syndromic' forms of CHH, in which the gonadotropin deficit is sometimes only a marginal feature of a predominantly endocrine, metabolic or neurological syndrome.[1] However, syndromic forms of CHH are sometimes associated with only minor clinical disorders and resemble Kallmann syndrome or normosmic CHH.[1,3–7] Management of patients with CHH has gradually improved over the past 30 years thanks to progress in hormone assays, imaging, genetics and hormonal therapy. The discovery of gene mutations underlying these disorders has led to major pathophysiologic advances and also helped to determine the probable modes of genetic transmission: X-linked, autosomal dominant, autosomal recessive, or digenic or oligogenic.[1–4] Therapeutic progress has enabled men and women with these disorders to envisage having children.[8]

Treatment of female patients is based on feminization and increasing adult height using estrogen–progestin combinations and on correction of the trophicity of the internal genitalia and uterus. Once feminization is complete, ovulation and pregnancy can be achieved by pulsatile GnRH administration or combination therapy with extractive or recombinant gonadotropins. In male patients, the virilization induced by androgen administration is often considered acceptable in nonsevere forms but, as will be discussed below, seems very unsatisfactory in those with severe forms of CHH with cryptorchidism—the failure of one or both testes to move into the scrotum as the male fetus develops—and micropenis (2.5 SD below the mean of normal for a given age). Furthermore, treatment with spermatogenesis-inducing hormones usually fails to correct infertility in men with CHH and cryptorchidism. Severe congenital gonadotropin deficiency, therefore, leads to infertility and unsatisfactory sexuality in adulthood, and these issues are becoming increasingly important for male teenagers and young men with CHH.

As potential future parents, patients with CHH are also concerned with the risk of transmitting the disorder to their children and with the anatomical consequences of the disease for their offspring, especially boys. In our experience, the most pressing questions from parents concern their future sons' pubertal development, sexuality and fertility. The aim of this Review is to discuss the

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Key points

- During fetal life, hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary gonadotropins play a key part in the development and growth of the male external genitalia
- Complete congenital hypogonadotropic hypogonadism (CHH) is associated with penile and testicular hypotrophy and, in many cases, with cryptorchidism
- Conventional treatment of patients with CHH, with testosterone or gonadotropins, is inadequately effective in terms of adult sexuality and fertility when started after the age of puberty
- Neonatal treatment corrects genital hypotrophy and improves testicular endocrine function, which might improve the response to treatments intended to induce postpubertal virilization and to restore fertility in men with CHH
- Long-term studies are needed to assess the effect of this approach on sexuality and fertility before recommending its routine use

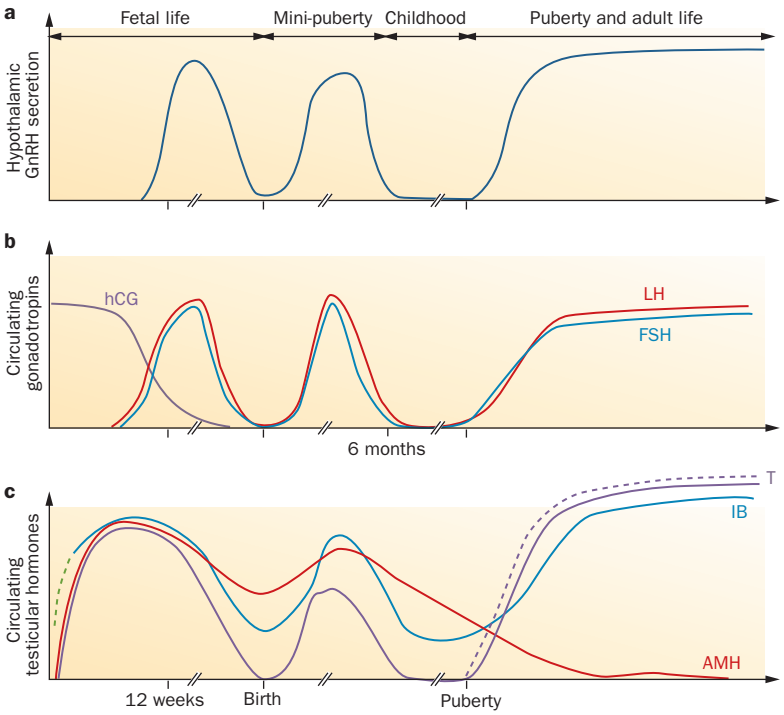

Figure 1 | Schematic of the activation of the hypothalamic–pituitary–testicular axis during fetal and postnatal life in humans. During fetal, early neonatal and pubertal development, **a** | pulsatile hypothalamic secretion of GnRH stimulates **b** | pituitary gonadotropin biosynthesis and secretion that, in turn, stimulates **c** | testicular steroid and peptidic hormone production. The dotted line represents spermatogenesis, which occurs only after puberty. Abbreviations: AMH, anti-Müllerian hormone; FSH, follitropin; GnRH, gonadotropin-releasing hormone; hCG, human choriogonadotropin; IB, inhibin B; LH, lutropin; T, testosterone.

consequences of CHH for the development of the external male genitalia and, now that CHH can be diagnosed at birth, the possible beneficial effect of early hormone therapy on adult status.

Ontogenesis of the gonadotropic axis

Antenatal masculinization of the internal and external genitalia depends on adequate production of testosterone by the fetal testicles and on the correct action of this androgen on its target organs. During early male fetal life, testosterone production by Leydig cells is first detected at week 8 of gestation, reaching its maximum at 12–14 weeks (Figure 1).[9,10] Human fetal testosterone production is initially triggered by placental human choriogonadotropin (hCG) stimulation of LH/CG receptors expressed on the surface of Leydig cells.[11,12] The key role of LH/CG receptors during this period of male genital development is demonstrated by the absent or defective masculinization of external genitalia (feminine phenotype or partial disorder of sex development) that is due to mutations that cause complete or partial loss of LH/CG receptor function, respectively.[13]

Placental hCG production, as assessed on the basis of assays of maternal serum and amniotic fluid, is maximal between weeks 8 and 12 of gestation.[9,10,14,15] At this stage, placental hCG levels in the fetal compartment are adequate to stimulate the fetal testicles. Indeed, hCG is detectable in fetal blood, albeit at levels lower than in amniotic fluid.[9,10,15] At week 12, when the level of placental hCG in the fetal circulation starts to fall,[9,10,15] LH and FSH start to be secreted by fetal pituitary gonadotrope cells (Figure 1). This secretion has been demonstrated in humans by the detection of pituitary transcripts for both the common α subunit and the specific β subunits of these two dimeric gonadotropins and by immunoassay of the hormones in fetal blood.[9,10,15] It was thus shown that the human male fetal pituitary gland is capable of producing dimeric LH and FSH.[9,10] LH secretion by fetal pituitary gonadotrope cells rises gradually, ensuring continued fetal testicular steroidogenesis despite the decline in hCG. Thus, initial masculinization of the external genitalia (perineum, scrotum, penis) and their antenatal growth seem to be initially dependent on fetal testicular testosterone secreted first under the control of placental hCG and then of pituitary LH.

Terminal inguinoscrotal testicular descent, which occurs around weeks 27–35 of fetal life, is also androgen-dependent and seems to be controlled mainly by pituitary LH.[9,10,16,17] The insulin-like 3 peptide (INSL3), which is secreted by fetal Leydig cells, also seems to have an important role in fetal testicular migration, alongside testicular testosterone.[18]

The effect of FSH on testicular function during human fetal development is poorly documented,[10,19] largely because of the difficulty of distinguishing, in humans, the respective effects of the two pituitary gonadotropins. However, binding experiments,[20] transcript assays[21] and immunohistochemical methods[22] have shown that the human fetal testicle expresses FSH receptors, and fetal blood FSH levels increase during the course of the second trimester (Figure 1).[12] Hence, Sertoli cell proliferation,[21] observed from the second trimester, is possibly linked to stimulation of the fetal testicles by pituitary FSH. This hypothesis is also supported by the increase in inhibin B levels observed in the human male fetus;[23] by the known stimulatory effect of FSH on testicular growth and Sertoli cell proliferation; and by the increase in sertolian peptide secretion during the neonatal period (the other period of early testicular development; see below).

During the third trimester, circulating levels of the two fetal pituitary gonadotropins fall as term approaches (Figure 1), probably owing to the appearance of negative

feedback mechanisms[24] exerted by placental estradiol and progesterone and by inhibin B. This negative feedback might be secondary to the appearance of sex-steroid receptors in the fetal pituitary during the second trimester.[25,26] The resulting fall in gonadotropin levels observed towards the end of gestation[10,23] could explain the reduction in the number of Leydig cells during this period, as well as the low circulating testosterone concentrations on the day of birth (Figure 1) and, thus, the stagnation of fetal Sertoli cell proliferation towards the end of gestation.[10,21]

Hypothalamic GnRH plays a key part in the positive control of postpubertal pituitary gonadotropin secretion.[27] This neurohormone and the neurons that secrete it have, however, also been the focus of attention during human fetal life.[28–31] GnRH has been detected in human embryonic brain extracts as early as at 4.5 weeks of gestation.[30,31] GnRH neurons, originating from the olfactory epithelium, have been detected in the fetal hypothalamus by 9 weeks of gestation, but functional connections between these neurons and the portal system only seem to appear at around 16 weeks (Figure 1).[30,31] Studies of ovine fetuses have shown that pulsatile LH secretion begins as early as midgestation and that it can be blocked by chronic administration of a GnRH agonist.[32–34] This finding supports the possibility of active antenatal pulsatile GnRH secretion by the human hypothalamus.[32]

The important role of fetal gonadotrope cell stimulation by GnRH in pituitary LH secretion and, subsequently, in fetal testicular steroidogenesis and male genital development, has also been shown in primates, sheep and rodents by using pharmacological approaches. The effects of hypothalamic GnRH on pituitary function were blocked by antenatal administration of GnRH analogues, which effectively reduced fetal pituitary gonadotropin secretion, appeared to inhibit terminal inguinoscrotal testicular migration and led to a reduction in neonatal testicle and penis size.[33–35]

Antenatal gonadotropin deficiency

Studies of natural human disease models have confirmed the key role of antenatal GnRH and fetal pituitary gonadotropin secretion on male genital development. One such model is the fetus with anencephaly and an XY karyotype.[35–37] These fetuses show inadequate secretion of pituitary hormones, especially gonadotropins, and exhibit unambiguous male genital development. It was, thus, deduced that initial masculinization of the urogenital sinus into the scrotum and penis was independent of pituitary gonadotropin secretion. However, these fetuses were found to have penile and testicular hypotrophy.[35–37] One hypothesis put forward at that time was that placental hCG secretion in early gestation was adequate for early masculinization of the external genitalia but that, with the physiological reduction in hCG concentrations in the fetal compartment, subsequent masculinization and growth of the external genitalia was unable to continue because the physiological rise in LH did not occur. This absence of pituitary gonadotropins would, therefore, compromise penile growth and inguinoscrotal testicular migration, which are dependent on gonadotropins and androgens.[35–37]

CHH is a 'purer' natural pathological model than the anencephalic fetus, which is relatively complex. CHH has served as a model to determine the specific role of the gonadotrope axis in the development of the testicles and external genitalia, because it only affects physiological GnRH and/or pituitary gonadotropin secretion during fetal life, without having a clinically significant effect on other pituitary secretions.[1] This model confirms the key roles of antenatal hypothalamic GnRH secretion, pituitary GnRH receptors and the two pituitary gonadotropins in terminal fetal development of the male genitalia. It has also served, as discussed below, to specify the role of the gonadotropic axis in immediate postnatal testicular activation and, thus, in the terminal growth of the human external genitalia. Indeed, almost all known genetic forms of isolated CHH due to deficient hypothalamic GnRH secretion or to pituitary resistance to this neuropeptide exhibit the cardinal clinical signs of antenatal pituitary gonadotropin deficiency, namely cryptorchidism and micropenis (Table 1).[38–58] These models have also revealed the essential role of two hypothalamic neurohormones and their receptors in GnRH secretion and, therefore, in gonadotropin secretion during fetal life: kisspeptin and its receptor KiSS1R (also known as GPR54) and neurokinin B and its receptor NK3R.[48–55] Cryptorchidism and micropenis have also been observed in patients with Kallmann syndrome[56,57,59–82] or more complex syndromic forms.[83–85] It must, however, be emphasized that not all patients with CHH necessarily manifest cryptorchidism and micropenis; the frequency of these features varies depending on the underlying genetic cause. For instance, cryptorchidism has been shown to be more frequent in individuals with CHH due to a *KAL1* mutation than in those with mutations in the *FGFR1* (previously known as *KAL2*) gene (Table 1).[57]

The existence of micropenis in the setting of isolated LH deficiency due to a mutation in the gene encoding the LH subunit β indicates that this gonadotropin has the main role in antenatal penile growth and testicular migration,[86–88] by stimulating INSL3 and testosterone secretion from Leydig cells.[89,90] However, in addition to LH deficiency, the majority of fetuses with CHH probably have a concurrent FSH deficiency.

The specific consequences of the FSH deficit in humans are not currently clear, because it is difficult to dissociate them from the consequences of the antenatal LH deficit. It is possible that the FSH deficiency affects antenatal proliferation of Sertoli cells and, possibly, germ cells.[21] This hypothesis is in keeping with the marked reduction in testicular volume and in circulating inhibin B and anti-Müllerian hormone (AMH) concentrations observed in male neonates with CHH relative to those with a normal gonadotrope axis. It is also compatible with the well-established stimulatory effect of FSH on testicular volume and testicular inhibin B secretion at different stages of life.[19,91,92] Moreover, FSH seems to have an important role in establishing normal adult fertility throughout antenatal and postnatal life, independently of LH, as shown by the reduction in testicular volume and defective spermatogenesis observed in men with loss-of-function

Table 1 | Isolated CHH, Kallmann syndrome and syndromic CHH associated with cryptorchidism and/or micropenis

Gene	Transmission	Phenotype	Cryptorchidism	Micropenis	References
Normosmic CHH					
GNRH1	Autosomal recessive	Isolated CHH	+	+	38,39
GNRHR	Autosomal recessive	Isolated CHH	+	+	40–49
KISS1R	Autosomal recessive	Isolated CHH	+	+	50–52
TAC3	Autosomal recessive	Isolated CHH	+	+	53–55,58
TACR3	Autosomal recessive	Isolated CHH	+	+	53–55,58
Kallmann syndrome					
KAL1	X-linked	CHH Anosmia/hyposmia	++	++	59–69
FGFR1	Autosomal dominant	CHH Anosmia/hyposmia	+	+	56,70–75
FGF8	Autosomal recessive, digenic or oligogenic	CHH Anosmia/hyposmia	+	+	76,77
*PROK2**	Autosomal recessive, digenic or oligogenic	CHH Anosmia/hyposmia	+	+	78–82
*PROKR2**	Autosomal recessive, digenic or oligogenic	CHH Anosmia/hyposmia	+	+	78–82
WDR11	NR	CHH Anosmia/hyposmia	+	NR	83
NELF	NR	CHH Anosmia/hyposmia	+	NR	84
Complex syndromic CHH					
CHD7	Autosomal dominant	CHARGE or Kallmann	+	+	5,6
PROP1	Autosomal recessive	Combined pituitary deficiencies or isolated CHH	+	+	85

*Compared with monoallelic *PROK2* or *PROKR2* mutations, biallelic *PROK2* or *PROKR2* mutations are associated with more frequent cryptorchidism and micropenis.[7,82] Abbreviations: CHARGE, coloboma, heart defect, choanal atresia, retarded growth and development, genital hypoplasia, ear abnormalities and/or hearing loss defect; CHH, congenital hypogonadotropic hypogonadism; NR, not reported; +, lower penetrance; ++, higher penetrance.

mutations in the genes encoding the FSH subunit β and the FSH receptor.[93–95]

Postnatal gonadotropic axis

As in male fetuses, the first 6 months of life represent a period during which the hormonal activity of the hypothalamic–pituitary axis and testes is important (Figure 1).[96–105] This active phase, which is possibly related to the interruption of the negative feedback effect of both placental sex steroids and peptides on pituitary gonadotropin secretion, is reflected physically by an increase in testicular volume due to seminiferous tubule elongation[98] and by an increase in penis length.[99] During this period, pituitary LH and FSH levels rise, leading to an increase in circulating levels of testosterone, inhibin B[22,100–102] and AMH (Figure 1),[22,102] which can attain levels observed in adult men or even higher. Concomitantly, Sertoli cells proliferate and a degree of germ cell development occurs.[103–105] Interestingly, however, spermatogenesis does not occur in male neonates, despite neonatal FSH and LH secretion, possibly owing to absent androgen signaling in Sertoli cells.[22,106]

When CHH is suspected in a male neonate, the diagnosis can be confirmed by hormone assays before the age of 6 months—the only period of childhood during which testosterone and gonadotropin deficiencies can be demonstrated.[101,107,108] Given the 'physiological' gonadotropin deficiency that characterizes childhood after the first 6 months of life (Figure 1), the only signs of CHH later in childhood are cryptorchidism and micropenis, especially if these defects are associated with signs pointing to a particular cause of CHH (for instance, anosmia or mirror movements).[108] However, CHH cannot be confirmed by gonadotropin and testosterone assays. Men with complete CHH in addition to fetal gonadotropin deficiency have absent postnatal activation of LH and FSH secretion. Together, these abnormalities account for the reduction in Sertoli cell mass, reflected by small testicular volume and markedly low serum inhibin B levels, and for the hypotrophy of the external genitalia observed in adulthood (Figure 2).[57,82,92,109,110]

Risk of inheritability

In nonsyndromic, normosmic forms of CHH, transmission is usually autosomal recessive.[1–4,38–55] In this genetic context and in the absence of consanguinity, the risk of transmission seems to be very small, considering the probably very low frequency of the mutated alleles in the general population. By contrast, when the mode of CHH transmission is autosomal dominant, as in the case of *FGFR1* and *CHD7* mutations, the risk is theoretically 50%, albeit with variable penetrance (Table 1).[4–6] A similar high-risk situation arises in Kallmann syndrome, in which transmission occurs via the X chromosome

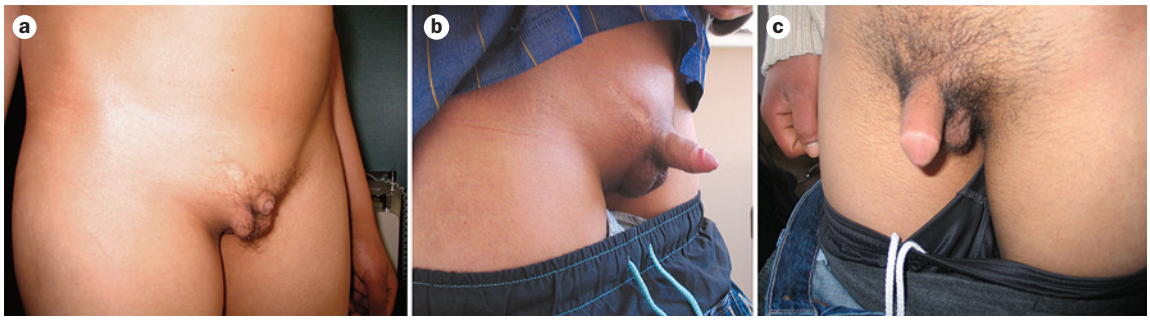

Figure 2 | Genital aspect in a young man with Kallmann syndrome, surgically cured cryptorchidism and micropenis **a** | at presentation, when he was aged 18 years, then **b** | 6 months and **c** | 16 months after testosterone enanthate administration (250 mg every 3 weeks, intramuscularly). Written consent for publication was obtained from the patient.

(*KAL1* mutations).[3–4,59–69] In this situation, family studies are crucial and must be routinely proposed in order to identify female carriers. CHH diagnosis at birth is becoming more frequent, as infertility treatment of these patients is increasingly more effective, and therapeutic management of children born with CHH will, therefore, become an essential aspect of the overall management of couples carrying these mutations. Finally, it must be stressed that genetic counseling of patients with CHH is far more complex in cases of digenic or oligogenic transmission,[2–4,82] in which the entire set of genetic events responsible for the phenotype is rarely known. Ongoing progress in the identification of genes responsible for CHH should improve the management of these infertile patients.

Hormone therapy

Virilization

Virilizing hormone therapy is always indicated for adolescents or adults with CHH to avoid the suffering caused by pubertal failure. In theory, virilization can be achieved either with pulsatile GnRH or with hCG, alone or combined with FSH.[111–113] In practice, given the costs and constraints of treatment with these hormones, most physicians prefer to use testosterone, in the form of an injectable ester.[111–113] The precise protocol depends on the age at diagnosis and local practice. Given the lack of randomized comparative trials in patients with CHH, no single androgen regimen is clearly superior, and pediatric and adult endocrinologists must, therefore, be cautious when counseling patients or their parents. Pediatric endocrinologists, who see these patients at a young age, frequently begin treatment with low and gradually increasing doses of testosterone.[111–113] Their main concerns are, on one hand, the risk of premature bone maturation, which could compromise growth capital and, on the other hand, the risk of precocious sexual activity that could affect the patient's personal and familial equilibrium. Adult endocrinologists see patients with CHH later in life, when the main complaint is the lack of pubertal development. In this case, the therapeutic approach can be more incisive, with higher initial androgen doses. Another concern is to ensure that osteoporosis, if present, does not persist or worsen.[114] These two—pediatric and adult—approaches have not been compared with respect to long-term quality of life among men with CHH, particularly in terms of sexuality and relationships. Few published data exist on the efficacy of androgen therapy in this specific population,[115] and studies examining its effect on quality of life and sexuality are lacking, particularly in men with severe CHH, micropenis and cryptorchidism (J. Young, unpublished work).[116] In fact, micropenis in men with CHH currently tends to be considered more as a clinical sign, distinguishing CHH from constitutional delayed puberty, than as a factor predictive of the effect of androgen therapy on the quality of sexuality in adulthood. The relationship between micropenis and sexual dysfunction in adult life is supported by a previous study from our research group, in which patients with partial androgen insensitivity syndrome (PAIS) were interviewed: the patients regularly stated that their severely reduced penis size had a major negative effect on their self-confidence when engaging in sexual activity with a partner. From late childhood to the end of adolescence, a period of active sexual concerns in boys, penile length remained below 50 mm in all 15 patients studied, 14 of whom stated that it impaired their initiation of sexual activity.[117]

Fertility

The infertility of men with CHH is due to absent sperm production. Spermatogenesis can only be induced by long-term pulsatile GnRH administration via a pump or by several subcutaneous or intramuscular gonadotropin injections per week.[118–142] Several studies conducted over the past 30 years have assessed these treatments in men with hypogonadotropic hypogonadism (either congenital or acquired after birth or puberty).[118–142] However, owing to the heterogeneity of these studies, the efficacy of these treatments in the specific subpopulation of patients with CHH is difficult to analyze. Indeed, most studies included men with CHH, men in whom hypogonadotropic hypogonadism occurred after puberty and men with hypogonadotropic hypogonadism of unknown cause.[120–128,143–145] The CHH subgroups were often very small. In addition, many of the studies that included patients with CHH were biased by the exclusion of patients who were likely to have a poor treatment response, such as patients with cryptorchidism,[122,127,133,135,138,140] and patients with severe CHH in whom treatment with hCG alone had failed to normalize serum testosterone concentrations.[119]

Table 2 | Neonatal, prepubertal or peripubertal hormonal therapy in boys with hypogonadotropic hypogonadism

References	Year	*n*	Age at the start of treatment	Hormonal therapy	Clinical outcome	Hormonal outcome
Raivio *et al.*[148]	1997	3	12.8–13.2 years	rhFSH	Increased testicular volume	Increased serum inhibin B levels
Bouvattier *et al.*[149]	1999	37	13.0–15.2 years	hCG and hMG	Increased testicular volume	Increased serum testosterone levels
Main *et al.*[152]	2000	3	4–18 months	Testosterone (suppositories)	Increased penis length	Increased serum testosterone levels
Main *et al.*[151]	2002	1	7.9 months	rhLH and rhFSH	Increased testicular volume and penis length	Increased serum inhibin B and estradiol levels
Raivio *et al.*[150]	2007	14	10.4–17.7 years	rhFSH then hCG	Increased testicular volume*	Increased serum inhibin B levels*
Bougnères *et al.*[153]	2008	2	2–5 months	rhLH and rhFSH	Increased testicular volume and penis length	Increased serum testosterone, inhibin B and AMH levels

*During rhFSH; further testicular volume and genital development increased during combined rhFSH and hCG treatment. Abbreviations: AMH, serum anti-Müllerian hormone; FSH, follitropin; hCG, human choriogonadotropin; hMG, human menopausal gonadotropin; LH, lutropin; rh, recombinant human.

Despite these limitations, several factors are evident. First, even if low sperm concentration does not always preclude fertility in men with CHH,[123] the sperm count rarely normalizes in these patients (based on WHO criteria) despite long-term gonadotropin combination therapy.[123,128,129,141] Second, the rise in sperm count occurs far later in men with CHH than in men with hypogonadotropic hypogonadism of postpubertal onset.[121,140] Third, pre-therapeutic testicular volume is an important factor in treatment outcome: the smaller the testicular volume (which is generally very low in those with complete CHH), the more difficult it is to achieve a testicular volume increase, to normalize the sperm count and to achieve a pregnancy.[123,124,126,128,131,139] Finally, several studies of patients with CHH indicate that cryptorchidism is the main risk marker of poor prognosis.[140,141]

In recent studies, emphasis has been placed on the effect that initial treatment for CHH has on the later efficacy of treatments for azoospermia in men with CHH, as the response to treatments for infertility appears to improve with previous exposure of the patient to gonadotropins or GnRH.[140,141] However, some clinicians consider that prior androgen therapy or very late gonadotropin therapy are associated with a poorer subsequent treatment response compared with no prior therapy.[141] A noteworthy fraction of men with documented CHH (between 12% and 50% depending on the series) have no sperm in their ejaculate despite long-term gonadotropin therapy.[138,139] As a result, some patients are offered more invasive treatments such as testicular sperm extraction under general anesthesia, with a view to perform intracytoplasmic spermatozoid injection (ICSI).[146,147] The pregnancy rate achieved with gonadotropin combination therapy depends on the types of patients treated and on the use of additional assisted reproduction methods (*in vitro* fertilization or ICSI), but appears to be below 30% if patients have CHH.[140,141] This low rate contrasts with the excellent efficacy of gonadotropin combination therapy in postnatal and postpubertal acquired hypogonadotropic hypogonadism, in which the pregnancy rate is nearly 100% in the absence of an additional female factor of infertility.[140,141] This large difference could be explained by the fact that the testes of patients with postnatally acquired hypogonadotropic hypogonadism have already been 'primed' by pituitary gonadotropins during fetal life, the neonatal period and puberty, before onset of gonadotropin deficiency. Thus, despite the progress made in recent years, only a minority of men with complete CHH are able to have children after receiving current hormonal treatments, which is why attention is turning to the possible benefits of earlier, prepubertal or even neonatal treatment of gonadotropin deficiency.

Prepubertal hormone therapy

Treatment of male patients with CHH before the age of puberty was first reported by Raivio *et al.*[148] in 1997 (Table 2). The investigators studied the effect of recombinant human FSH administration for 12 months on testis growth in three gonadotropin-deficient boys aged 12.8–13.2 years. No increase in serum LH or sex-steroid concentrations was noted, but serum FSH and inhibin B concentrations increased to within the range observed in healthy prepubertal boys, and testis volume increased in all three cases. Interestingly, a boy with unilateral cryptorchidism had the largest relative increase in testis volume.

In 1999, Bouvattier *et al.*[149] treated 37 adolescents with hypogonadotropic hypogonadism with gonadotropins (hCG and human menopausal gonadotropin), with the aim of achieving complete virilization during the first 2 years of treatment. They observed normal sexual development with a significant increase in testicular volume and testosterone levels during therapy. The investigators pointed out, however, that less satisfactory results were obtained in adolescents with cryptorchidism and suggested that gonadotropin combination therapy might be more effective than exogenous testosterone therapy during the induction of puberty, thus avoiding the psychological problems associated with atrophic testes.

In 2007, Raivio *et al.*[150] reported long-term results of therapy in a retrospective series of 14 children or adolescents with prepubertal-onset hypogonadotropic hypogonadism who were treated with recombinant human FSH followed by FSH–hCG combination therapy. They found that prepubertal FSH treatment doubled testicular volume and increased inhibin B levels. Interestingly, CHH was associated with a smaller increase in testicular volume and inhibin B levels than postnatally acquired hypogonadotropic hypogonadism, further supporting the important role of the neonatal increase in gonadotropin levels on the testicular response to these hormones in later life.

In 2002, Main *et al.*[151] published the first report of a patient with CHH and micropenis treated with recombinant human LH and FSH during the first year of life (Table 2). The researchers treated this patient from age 7.9 to 13.7 months with recombinant human LH and FSH at respective doses of 203 IU and 21.3 IU subcutaneously twice weekly. Penile length increased from 1.6 cm to 2.4 cm and testicular volume, assessed by ultrasonography, increased by 170%. During this well-tolerated treatment, the investigators also observed an increase in LH, FSH and inhibin B levels.

Main and colleagues also reported interesting results with testosterone therapy during early infancy in three boys with hypogonadotropic hypogonadism (CHH and panhypopituitarism), in whom the diagnosis of gonadotropin deficiency was established postnatally and who showed a lack of penile growth and scrotal involution after birth.[152] All the boys received testosterone suppositories at daily doses ranging between 1 mg and 5 mg, which led to normal penile and scrotal development. However, changes in testicular volume and sertolian hormone levels (inhibin B and AMH levels) were not described.

Bougnères *et al.*[153] described an approach aimed at re-establishing the physiological postnatal gonadotropin peak, the so-called 'mini-puberty' (Table 2). They described the cases of two neonates, one with congenital hypopituitarism and the other with isolated CHH, in whom these diagnoses were suggested on the basis of findings of micropenis and cryptorchidism. The two neonates were treated for 6 months with recombinant human LH and recombinant human FSH, delivered subcutaneously via a pump. Testicular volume increased from 0.45 ml and 0.57 ml at birth to 2.10 ml at 7 months, and stretched penile length increased from 8 mm to 30 mm in one case and from 12 mm to 48 mm in the other case. Mean serum LH and FSH levels increased to normal and supranormal levels, respectively, whereas mean testosterone levels increased from undetectable to normal, and inhibin B and AMH levels also increased to levels normal for age. Whereas the doses of FSH and LH administered were similar in this report, the increase of serum FSH to supraphysiololgic levels could be related to slower metabolic clearance of FSH than of LH, resulting in a longer half-life and accumulation of FSH.[91]

Alongside these encouraging reports, it should be remembered that treatment with hCG or pulsatile GnRH has been shown to be effective to treat cryptorchidism in many neonates and in boys treated before the age of puberty.[143,154] This finding could represent a further benefit of neonatal treatment of children with CHH, as cryptorchidism is a factor of poor prognosis for adult fertility as well as a risk factor for testicular malignancy. In addition, orchidopexy—surgery to move an undescended testicle into the scrotum—is technically more difficult (with a greater risk of damage to the testis) when the testis is very small. Achievement of an increment in testis volume through presurgical gonadotropin therapy could also be beneficial for patients due to undergo orchidopexy, but the merits of this approach must be confirmed in neonates with CHH and cryptorchidism, as some publications point to a deleterious effect of isolated hCG therapy in boys with cryptorchidism.[155] However, it is noteworthy that deleterious effects of hCG on germ cells in cryptorchid testicles have only been reported in boys with idiopathic cryptorchidism (with or without primary testicular insufficiency) and not specifically in the CHH population.[143,144,154,155] The apparent deleterious effects attributed to testicular stimulation with hCG in children with cryptorchidism could, therefore, conceivably be the expression of the underlying testicular disorder,[156] as reported in patients with Klinefelter syndrome, in whom seminiferous tubule degradation with apoptosis of germ cells and Sertoli cells occurs when the testicles are stimulated by the spontaneous increase in concentration of gonadotropins during the course of pubertal development.[145]

Conclusions

Previous publications show that neonatal combined gonadotropin therapy in patients with CHH diagnosed at birth can have a beneficial effect on testicular endocrine function and on genital development. Indeed, normalization of serum testosterone levels in neonates with CHH by administration of adequate gonadotropin doses (recombinant LH or hCG) results in a marked increase in penile length, which can also be obtained by neonatal testosterone administration. In addition, the increase in inhibin B and AMH levels induced by concurrent FSH administration suggests that gonadotropin combination therapy might be superior to simple androgen therapy, as it stimulates sertolian hormone secretion, Sertoli cell proliferation and growth of seminiferous tubules, as indicated by the marked increase in testicular volume. These data are reassuring with respect to germ cell survival during combined LH/hCG and FSH administration. In boys with CHH, the dual aims of neonatal treatment with the two pituitary gonadotropins would, therefore, be to attenuate the psychological effects of testis hypotrophy and micropenis in adolescence and to improve sexuality and spermatogenesis in adulthood. It is possible that the normalization of penis size in the neonate will lead, during subsequent postpubertal virilization with exogenous testosterone or hCG, to a normal adult penis size and thus avoid the sexual disorders often reported by men with CHH and micropenis. In parallel, the increase in testicular size induced by LH and FSH, which is

linked to the increase in Sertoli cell mass, could improve the response to spermatogenesis-inducing treatments started during adolescence or adulthood. Neonatal or prepubertal correction of cryptorchidism in children with CHH might also have an additional beneficial effect on induction of spermatogenesis, which is still a matter of debate and needs to be studied specifically in the CHH population.

Formal proof of the efficacy of neonatal combined gonadotropin therapy on the sexuality and fertility of men with CHH will require prospective controlled intervention studies lasting some 15–20 years. However, on the basis of the indirect evidence available today, it seems reasonable to offer this therapeutic option to the parents of newborn boys with severe CHH, while carefully explaining current uncertainties as to its final effectiveness and long-term safety. Close clinical monitoring will be needed to assess the short-term, medium-term and long-term safety of neonatal gonadotropin therapy in boys with CHH.

Finally, these data are also a call to arms for clinical, animal[157] and basic research in this area to determine, in a definitive way, if gonadotropin administration during the 'window period' is beneficial in treating CHH in male patients.

Review criteria

A search for original articles published between 1975 and 2011 and focusing on congenital hypogonadotropic hypogonadism and Kallmann syndrome was performed in MEDLINE and PubMed. The search terms used were "Kallmann syndrome", "congenital (or idiopathic) hypogonadotropic hypogonadism and GnRH", "congenital hypogonadotropic (or idiopathic) hypogonadism and gonadotropin", "congenital (or idiopathic) hypogonadotropic hypogonadism and androgen or testosterone", "human foetal testis", "human foetal GnRH neurons" and "human foetal pituitary". All articles identified were English-language papers. We also searched the reference lists of identified articles for further papers.

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