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## 64<sup>th</sup> International Days of Clinical Endocrinology - Henri-Pierre Klotz :

### Hormones & Fertility

**Puberty induction with recombinant gonadotropin : what impact on future fertility ?**

**Induction de la puberté par les gonadotrophines recombinantes : quel impact sur la fertilité ultérieure ?**

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#### **Résumé/ Abstract :**

On désigne par hypogonadismes hypogonadotropes congénitaux (HHC) un ensemble de maladies rares caractérisées par une sécrétion inadéquate des gonadotrophines LH (hormone lutéinisante) et FSH (hormone folliculo-stimulante) durant les périodes d'activation physiologiques de l'axe gonadotrope. L'anomalie est présente dès la vie fœtale et persiste le plus souvent toute la vie. Cliniquement, l'hypogonadisme hypogonadotrope se manifeste par des signes cliniques néonataux (micropénis, cryptorchidie chez les garçons dans environ la moitié des cas). Le diagnostic n'est parfois évoqué que devant une absence ou un arrêt de maturation pubertaire chez l'adolescent, souvent mal toléré physiquement et psychiquement.

Historiquement, les hypogonadismes hypogonadotropes congénitaux diagnostiqués à la puberté sont traités par testostérone en injections intramusculaires. Ces injections permettent le développement des caractères sexuels secondaires, sans augmentation du volume testiculaire dans les formes sévères (déficit en FSH), et un pic statural pubertaire. Au cours

des vingt dernières années, des études ont souligné le rôle bénéfique d'un traitement inducteur de la puberté par gonadotrophines recombinantes pour la fertilité future. C'est ce que nous allons développer.

Congenital hypogonadotropic hypogonadism (CHH) is a group of rare diseases characterized by inadequate secretion of the gonadotropins LH (luteinizing hormone) and FSH (follicle stimulating hormone) during the physiological activation periods of the gonadotropic axis. The disease ? (anomaly) is present from fetal life and usually persists throughout life. Clinically, hypogonadotropic hypogonadism is associated with neonatal clinical signs (micropenis, cryptorchidism in boys in about half of the cases). The diagnosis is sometimes only evoked in the presence of an absence or arrest of pubertal maturation in the adolescent, which is often poorly tolerated physically and psychologically. Different therapeutic options for pubertal induction have been described, but we lack the necessary larger randomized trials to define the best approaches for both sexes. Historically, congenital hypogonadotropic hypogonadism diagnosed at puberty is treated with testosterone injections. These injections allow the development of secondary sexual characteristics, without an increase in testicular volume in severe forms (FSH deficiency), and a pubertal statural peak. During the last twenty years, studies have underlined the beneficial role of recombinant gonadotropins to induce puberty in this population for future fertility. This is what we will develop.

**Key Words:** hypogonadotropic hypogonadism, GnRH deficiency, delayed puberty, testosterone, recombinant gonadotropin, fertility / Hypogonadisme hypogonadotrope, retard pubertaire, gonadotrophines recombinantes, fertilité.

## 1. Physiology

### 1.1 Ontogeny of the hypothalamic-pituitary-gonadal

The ontogeny of the hypothalamic-pituitary-gonadal axis takes place in 3 phases: a first phase of incomplete functional development during foetal life and the first months of postnatal life, followed by a phase of regression and partial quiescence during childhood, ending with a phase of complete maturation during puberty (1).

From the 7th of development, in human male embryos, the undifferentiated gonad begins to develop into a testis. Testicular differentiation is characterized by the formation of the seminiferous cords, i.e. epithelial cords composed of germ cells (spermatogonia) and somatic cells (Sertoli cells).

Testosterone production by fetal Leydig cells begins in the 7th week of pregnancy and peaks around 12-14 weeks (2). It is initially under the control of placental hCG (which stimulates LH/CG receptors on the surface of the Leydig cells). At 12 weeks, as placental hCG levels begin to fall in the fetal circulation, the gonadotropic cells of the fetal pituitary take over and begin to secrete gonadotropins (3). Fetal LH secretion gradually increases in parallel with the decrease in hCG.

It is difficult to assess the specific involvement of FSH but it would appear that the proliferation of Sertoli cells in the second trimester of pregnancy is related to the stimulation of the fetal testis by FSH (progressive increase in FSH during the 2nd trimester). As

evidenced by the presence of FSH receptors on fetal testicular sections but also by the increase in inhibin B observed in male fetuses.

During the 3rd trimester of pregnancy, gonadotropins decrease, probably due to negative feedback from placental hormones (oestradiol, progesterone) and inhibin B. Indeed, sex steroid receptors appear on the fetal pituitary during the 2nd trimester (4). This drop in gonadotropins at the end of pregnancy could explain the decrease in the number of Leydig cells at this period, as well as the stagnation of Sertoli cell proliferation.

Hypothalamic GnRH also plays an important role. Indeed, its presence is detectable by immunohistochemistry as early as 4.5 weeks of gestation. GnRH neurons from the olfactory placode appear in the fetal hypothalamus as early as 9 weeks of gestation but connections with the portal system do not appear until 16 weeks of gestation. Studies in sheep have shown that pulsatile LH secretion begins as early as the antenatal period and can be inhibited by administration of GnRH. It is possible that there is also an antenatal pulsatile secretion of GnRH from the human hypothalamus.

## 1.2 Neonatal development

After birth, during the first 6 months of life, a period of significant hormonal activity, called mini puberty is observed in the newborn boy (due to the cessation of negative feedback from placental steroids), resulting in an increase in testicular volume. A disinhibition of pulsatile GnRh secretion is then observed allowing a transient reactivation of the gonadotropic axis.

Testosterone secretion is high at birth and then falls rapidly during the first week of life to levels around 0.2 ng/ml (0.7 nmol/l). From 10-15 days of life, testosterone levels rise in response to the increase in LH levels, reaching peak values at 3 months of life (3 ng/ml) allowing Leydig cell proliferation. These high values are not accompanied by virilisation.

In response to the increase in FSH levels, there is also a proliferation of Sertoli cells, which increase in number 5 to 6 times in the neonatal period resulting in an increase in AMH, inhibin B and testicular volume due to an increase in the size of the seminiferous cords (5).

Germ cells proliferate from 28<sup>th</sup> weeks of gestation to 1000 days of life with a peak between 50 and 150 days of life characterizing the high mitotic activity present at this period. The gonocytes transform into spermatogonia under the action of testosterone. The spermatogonia remain blocked in the pre-meiotic phase and are reactivated at the time of puberty.

These intra-testicular cellular events thus occur in a critical time window where stimulation by gonadotropins seems to be crucial for the proper development of Sertolian function and spermatogenesis at puberty.

This phase of cell proliferation is followed by a phase of quiescence. The Leydig cells will regress and enter apoptosis. The Sertoli cells will continue to proliferate but at a low level until the beginning of puberty. The germ cells will remain in the spermatogonial stage until the initiation of spermatogenesis at puberty.

### 1.3 Physiology of puberty

Puberty is a transitional phase between childhood and adulthood, and allows the acquisition of reproductive functions. It is also accompanied by physical, psychological, cognitive and socio-cultural changes.

The age of onset of puberty in boys is variable, ranging from 9 to 14 years. It is influenced by complex interactions between genetics, nutrition, environment, and socio-economic factors.

After a period of quiescence in childhood, the increase in frequency and amplitude of GnRH secretion is the initiating signal for puberty. This increase in pulsatile GnRH secretion leads to

activation of the gonadotropic axis, with increased secretion of LH and FSH, well before the onset of clinical signs of puberty.

Clinically, the first sign of puberty is an increase in testicular volume ( $> 3$  mL, or the testicular length greater than 2.5 cm), on average around the age of 12-13 years. From birth and throughout the pre-pubertal period (i.e. up to 9-14 years, Tanner 1), the volume of the seminiferous tubules depends mainly on Sertoli cells, whereas during pubertal development (between Tanner 1 and 5) the increase in testicular volume is mainly due to germ cell proliferation.

Biologically, LH rises gradually. LH values  $>0.6$  IU/L (in immunofluorometry) are in favour of pubertal onset.

Under the effect of LH, testosterone rises. A testosterone level at 8 o'clock  $> 0.2$  ng/mL is predictive of pubertal onset within 12 to 15 months. Statural growth accelerates for testosterone levels  $> 1$  ng/mL (6).

As a result of the increase in FSH, inhibin B also increases, with inhibin B levels during puberty positively correlated with FSH levels.

In parallel with the increase in testosterone levels, AMH levels decrease .

Histologically, at puberty, FSH causes Sertoli cell proliferation and LH allows the reappearance of mature Leydig cells. The concentration of testosterone increases in the testes before increasing in the bloodstream, causing maturation of Sertoli cells characterized by an increase in cell size and a cessation of proliferation and a decrease in AMH (1). The proliferation of Sertoli cells and their increase in size are probably responsible for the moderate increase in testicular volume that marks the onset of puberty. Subsequently, the increase in testicular volume to its final size depends on spermatogenesis. The seminiferous

tubules acquire a lumen and their diameter increases. The sperm appears between 12 and 15 years of age, for a Tanner stage 3, preceding the pubertal peak by 6 months.

The main objectives of puberty-inducing treatment are the onset of secondary sexual characteristics, the achievement of a good peak in pubertal growth, the optimisation of the chances of future fertility, the reduction of long-term complications of hypogonadism (such as allowing a good peak in bone mass). For example, bone mass is acquired from childhood to adulthood, but 50% of this bone mass is acquired between the onset of puberty and the end of adolescence, according to a curve following the statural growth curve. This acquisition is completed after the age of 20, so the quality of the bone formed during puberty determines the subsequent risk of osteoporosis and fracture in adulthood. Last but not least, the psychological well-being of these patients is improved (7).

Delayed puberty can be responsible for significant psychological suffering. Thus, it has been shown that patients with HHC have lower self-esteem, poor perception of their body image, impaired psychosexual development and sometimes sexual identity disorders. Anxiety and depression are more common in adolescents with HHC (7).

## 2. Diagnostic

In the neonatal period, the diagnosis is most often suspected on clinical findings or family history (e.g. a characteristic hypogonadotropic mutation). The Leydig markers (testosterone and INSL3) and Sertolic markers (AMH, inhibin B) are low compared to the reference standards (5). The window for diagnosis is between 15 days and 4 months of life (1).

In childhood, the diagnosis is difficult to make because of the physiological hypogonadism of this period. Undetectable FSH levels, complete lack of response to GnRH test, very low AMH and/or inhibin B, could be indicative of HHC.



In adolescence, total plasma testosterone is low for the age. Baseline FSH and LH are also low or normal low. The response to the GnRH test is variable and depends on the severity of the gonadotropin deficiency, e.g. it may show no response in profound HH but be normal in partial HH. (8,9). Its diagnostic value is questionable. The inhibin B assay can also be informative. Indeed, a value of <35 pg/mL is associated with HHC in 100% of cases in Tanner 1 patients in the study by Coutant et al (10). However, the range of inhibin B values is wide, with some patients having inhibin B values comparable to those of adolescents with normal puberty(11). Inhibin B levels correlate with testicular volume and are therefore a good marker of the severity of hypogonadotropic hypogonadism (12).

The main differential diagnosis for patients presenting to the endocrinopediatric clinic is simple pubertal delay. Classically, in simple pubertal delay, there is a delay in growth and bone age (<13 years) and a family history of pubertal delay. However, the diagnosis of simple pubertal delay is unlikely in cases of associated micropenis and/or cryptorchidism, extra-gonadal signs of HHC or after the age of 18 (13).

### 3. Treatment

Therapeutic goals in the adolescent CHH male are well defined: 1. to induce virilization; 2. to reach optimal adult height; 3. to acquire normal bone mass and body composition; 4. to achieve normal psychosocial development; 5. and to gain fertility. However, available treatment regimens may not always cover all of these aspects (14).

#### 3.1 Testosterone therapy

Puberty is most often induced by intramuscular testosterone injections, because of simplicity and cost. Testosterone corrects androgenic and estrogenic deficits (by aromatisation of the

injected testosterone). There are also oral and gel forms. The pubertal induction protocol used depends on the centers and the age of the patient. In paediatrics, we often start with 50 mg of testosterone enantate each month, which is increased to 100 mg per month around 6 months of pubertal induction. The adult dosage (250mg/2-3 weeks) is gradually reached in 18-24 months (15). A gradual increase in dosage allows puberty to be mimicked, pubertal growth to be maximised (avoiding premature fusion of the growth plates) and the necessary time for psychosexual maturation to occur. In adult endocrinology patients, the main complaint is the lack of development of secondary sexual characteristics, and as there is no growth risk of statural growth, the initial dosage will be higher with a more rapid increase (250 mg per month then every 2-3 weeks)(16).

However, androgen therapy does not increase testicular volume or induce spermatogenesis. Reversibility of hypogonadism should be considered if testicular enlargement is observed with androgen therapy alone (17).

There is even evidence that prolonged testosterone treatment can inhibit spermatogenesis.

### 3.2 Recombinant Gonadotropin treatment

Because of its structural and functional homology with human LH and far longer half-life, human chorionic gonadotropin (hCG) is used to stimulate the production of endogenous testosterone from testicular Leydig cells, thereby inducing virilization, growth spurt, bone maturity, and psychological development in the same manner as exogenous testosterone. Moreover, hCG also achieves physiologically high intratesticular testosterone concentrations that—via binding to androgen receptors on Sertoli cells—act in concert with FSH to induce germ and Sertoli cell proliferation and maturation and thereby achieve spermatogenesis (18)

Since 1997, several studies testing the administration of recombinant gonadotropins to induce puberty have been published. Pituitary gonadotropins are administered by subcutaneous self-injections and their use has gained ground in clinical practice in recent years, with several novel themes having emerged (19).

Among studies that have investigated gonadotropin use for pubertal induction, few were randomized controlled trials and, overall, were heterogeneous in terms of patient numbers, ages (ranging from adolescence to adulthood), follow-up, and therapeutic protocol adopted, whether with hCG alone or in combination with FSH.

In 1997, Raivio *et al.* evaluated the effect of recombinant FSH administration (1.5 IU/kg three times a week for 12 months) in 3 children aged 12.8 to 13.2 years with authenticated gonadotropic insufficiency: 2/3 patients had isolated gonadotropic insufficiency without an identified mutation and 1/3 had acquired hypopituitarism (surgery of a craniopharyngioma at 10 years). There was an increase in FSH to normal values for age without an increase in LH and steroid hormone concentrations, a normalization of inhibin B levels to prepubertal values and an increase in testicular volume in all three children (20) (Patient with hypopituitarism: 0.8ml to 2.5ml; patient with acquired hypopituitarism 2.5 to 5ml).

In 1999, JL. Chaussain at St Vincent de Paul (Paris) and P. Rocchiccioli at Toulouse tested the administration of hCG associated with hMG (human Menopausal Gonadotropin) in 37 adolescents (13-15.2 years) with hypogonadotropic hypogonadism (21). All patients showed satisfactory genital development in relation to an increase in blood testosterone concentrations (0.26 +/- 0.04 to 5.3 +/- 0.8 ng/ml) and an increase in testicular volume (1.98 +/- 1.2 to 9 +/- 3.3 ml). Children treated with gonadotropins have, in addition to satisfactory testosterone concentrations, a good pubertal development (testicular volume). This treatment therefore

seems to have a better future benefit (fertility) than the administration of testosterone by avoiding the complications associated with atrophic testicles (21).

In 2007, Raivio et al. published a retrospective study of 14 children or adolescents (10.4-17.7 years) with gonadotropic insufficiency treated with rhFSH alone and then with a combination of rhFSH and hCG. Pre-pubertal administration of rhFSH (1.5 IU/kg three times a week from 2 months to 2.8 years depending on response) resulted in an increase in testicular volume ( $0.9 \pm 0.6$  ml to  $1.8 \pm 1.1$  ml ( $p < 0.005$ )) and inhibin B levels ( $27 \pm 14$  to  $80 \pm 57$  pg/ml ( $p < 0.01$ )) suggesting immature Sertoli cell proliferation. Patients with HHC had a poorer response to rhFSH ( $2.3 \pm 1.2$  vs  $0.8 \pm 0.2$  ml,  $p = 0.08$ ) and required a longer duration of treatment ( $>1$  year) suggesting the important role of early secretions of LH, FSH and testosterone on testicular response to gonadotropins later in life. (22). Despite an initial reduced testicular volume, Spermatogenesis was achieved in 6 out of 7 boys who provided semen samples, with a maximal sperm count ranging from 2.9 to 92 million/ml (median 8.5 million/ml).

A subsequent randomized controlled study (see below) by Dwyer et al. in 2013 showed similar results in young adults (18). CHH patients with history of cryptorchidism were excluded from these studies, but it is precisely these individuals with depleted Sertoli and germ cell mass who potentially stand to derive greatest advantage from an initial phase of FSH monotherapy. In all these studies, patients undergoing pretreatment with rhFSH necessarily delayed the beginning of hCG-induced virilization, which is physically and psychologically undesirable. However, another option would have been to deploy exogenous testosterone, of which relatively little would

In 2017, Rohayem *et al.* studied a relatively large group of adolescents with delayed puberty, most of them with absent puberty ( $n = 34$ ) (325). The adolescents received low dose hCG

(250-500 IU twice weekly) with increasing increments of 250-500 IU every 6 months, and rFSH was added once serum T achieved targeted pubertal level (5.2 nmol/L). This treatment led to a substantial increase in TV (bi-testicular volumes:  $5 \pm 5$  to  $34 \pm 3$  ml) and induction of spermatogenesis in 91% of patients (23)

hCG monotherapy induces testicular growth (23) and spermatogenesis (24) especially in patients with a postpubertal onset of CHH (basal Testicular Volume (TV) > 4 mL) (25), but combination therapy with FSH achieves notably better results in males starting off with prepubertal TV, with significantly more patients showing evidence of spermatogenesis and at greater sperm densities and higher final TV (19).

A study performed in our department (unpublished data) on 19 patients with CHH treated with hCG+ RhFSH for a period of 18 to 24 months; Patients received hCG, 2500 to 5000 IU per week, in 1 or 2 injections. They also received recombinant FSH at 150 IU x 3 injections per week subcutaneously. The adaptation of doses was left to the discretion of the child's referring endocrinologist. We showed an increase in testicular volume but with a normal goal reached only in 3/19 patients (4/19 patients, stage G3, 7/19 patients stage G2 and 7/19 patients stage G1 (<3ml)). The initial and maximal testicular volume was significantly lower in patients with a history of cryptorchidism. However, the increase in VT under hCG reached the testosterone goal in 12/19 patients at M12 and 11/14 patients at M15. 50% of patients reached the goal with an inhibin b level > 100 pg/ml (no significant difference between cryptorchid and non-cryptorchid patients). Regarding growth, patients had completed the 2 years of treatment with a mean peak statural height of  $11.2 \pm 5.5$  cm. The patients were on average -1 DS from their genetic target height. In terms of weight, 80% of the treated patients maintained their body weight. In terms of fertility, 12 patients had at least one spermogram. 75% of the patients had obtained spermatogenesis at M24 despite a non-normalized testicular

volume (notably 2 patients with VT at 2ml). 50% of the patients had oligospermia and the 3 patients with bilateral cryptorchidism had severe oligospermia.

Finally, recent studies including 1 randomized clinical trial have reported that cryptorchidism (especially if bilateral), low initial TV, and a strong genetic background (all indicators of more severe GnRH deficiency since intrauterine life) represent the major negative predictors of response to gonadotropin treatment in terms of final TV and sperm concentration achieved (26) (19).

In summary, despite the heterogeneity of the available data, some pillars of gonadotropin therapy can be distinguished. hCG promotes virilization, growth spurt, and sexual and psychosexual development, the hCG dose should start off low and then progressively increase during the course of treatment, according to regular testing of serum testosterone and estradiol and clinical assessment. The early addition of FSH grants a higher efficacy in terms of both TV increase and achievement of spermatogenesis and a much higher efficacy in achieving testicular descent (27). In this context, pretreatment with FSH for a few months may be the key to better maturation of testicular microarchitecture. Although no standard of care currently exists. A large multicenter study to evaluate the benefits and cost-effectiveness of pre-treatment with FSH in severe cases of adolescent and adult CHH is warrant.

### 3.3 Patients' follow-up and life-long replacement therapy

During pubertal induction, careful clinical monitoring is required to evaluate genital maturation and virilization, growth rate, and potential side effects. Periodic reassessment of bone age may assist the physician in determining the adequacy of hormone therapy (8). The adverse events of hormone therapy are dose dependent, with the most concerning related to testosterone and hCG being erythrocytosis and premature epiphyseal closure, but also

including aggressivity, mood swings, gynecomastia, and priapism when doses are greatly excessive. Older males may experience androgenic alopecia even at optimal doses. Local side effects of IM administration are pain and erythema at the injection site. Monitoring inhibin B and anti-Müllerian hormone represent a useful marker to predict the spermatogenic response of the testicles to gonadotropin therapy. Measurement of BMD should also be considered at baseline in patients with risk factors for low BMD and repeated once pubertal induction has been completed; in case it turns out to be low, it should be reassessed within 3 to 5 years.

TV should be periodically assessed by the clinician and by ultrasonography. When reversal is clinically suspected (eg, through rising TV or gonadotropin level), testosterone therapy should be withdrawn and levels of LH, FSH, and testosterone reassessed. Moreover, a periodic reassessment of hormonal values off therapy should be considered, even if how often and up to what age remain open to question (14).

Finally, Before switching to testosterone therapy, CHH patients previously treated with gonadotropins can perform a semen analysis to evaluate the response of spermatogenesis after treatment and to detect subjects at risk for inadequate sperm recovery later in adult life, giving them the possibility of sperm cryopreservation (despite the high costs) and a chance for future fertility. Otherwise, gonadotropin therapy can be simply reintroduced in later life when fertility is desired.

#### 4. Conclusion

Recombinant gonadotropins allow the induction of steroidogenesis and spermatogenesis. Puberty has a physiological course with maturation of the external genitalia, growth of the penis, increase in testicular volume, and attainment of an average final size. However, the testicular volume remains pre-pubertal for the majority of patients, especially for those with a history of bilateral cryptorchidism but allows the induction of spermatogenesis in a large majority of cases after 2 years of the protocol. Treatment is generally well accepted and tolerated by the patients.

It remains to be demonstrated whether the induction of spermatogenesis during a desire for parenthood is obtained as expected, in a shorter time and more effectively than in patients who have been treated with testosterone alone since adolescence. For this, a randomized controlled trial should be conducted, but the rarity of the pathology complicates matters somewhat.



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