Effects of an Early Postnatal Treatment of Hypogonadotropic Hypogonadism with a Continuous Subcutaneous Infusion of Recombinant Follicle-Stimulating Hormone and Luteinizing Hormone

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Background: The neonatal-midinfancy surge in pulsatile gonadotropin secretion is attributable to an increase in GnRH pulse amplitude and is associated with a rapid expansion of Leydig and Sertoli cell populations with concomitant surges in testosterone, inhibin, and anti-Mullerian hormone production as well as an increase in testicular volume. Boys with congenital hypogonadotropic hypogonadism (HH) do not activate these processes. A potential cause for azoospermia and infertility in adult life is deficient proliferation of immature Sertoli cells before and during puberty due to the absence of FSH.

Objective: The objective of the study was to investigate whether early postnatal continuous sc infusion of gonadotropins could mimic the physiological growth of testes and to evaluate responses of the Leydig and Sertoli cells to early gonadotropin replacement.

Design and Methods: Two neonates (P1 with hypotuitarism and P2 with HH) with micropenis and microorchidism were treated for 6 months with high doses of recombinant LH and FSH (a gift of Luveris and Gonal-F from Serono, Lyon, France) delivered sc with an insulin pump.

Results: Gonadotropin continuous sc infusion increased mean serum LH and FSH to normal or supranormal levels. Mean testosterone increased from undetectable levels to 7.6 and 5.2 nmol/liter, respectively, in P1 and P2. Inhibin B and anti-Müllerian hormone increased to normal levels. Mean testicular volume increased from 0.45 to 0.57 ml at birth to 2.10 ml at 7 months. Stretched penile length increased from 8 to 30 mm (P1) and 12 to 48 mm (P2).

Conclusions: The present regimen induced physiological postnatal testes growth and high-normal activation of Leydig and Sertoli cells. **(*J Clin Endocrinol Metab* 93: 2202–2205, 2008)**

At birth hypogonadotropic hypogonadism (HH) is revealed by a micropenis with or without cryptorchidism (1, 2). HH in the male infant can be isolated or associated with other pituitary hormone deficiencies (congenital hypopituitarism). The infantile GnRH-gonadotropin spurt allows to make the diagnosis of HH by using this brief window of normally increased FSH, LH, testosterone, and inhibin B values (3). If the diagnosis of euosmic HH is missed in infancy, it will be made only much later during adolescence or even adulthood. The diagnosis of HH in the male newborn is supported by detecting low levels of plasma LH, FSH, testosterone, and inhibin B and lack of LH response to the iv administration of GnRH (2, 4–6). Our current knowledge of HH genetics can be separated into mutations associated with multiple pituitary hormone deficiencies, including

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Abbreviations: AMH, Anti-Mullerian hormone; CSI, continuous sc infusion; hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; rh, recombinant human.

gonadotropin deficiency (*e.g.* Prophet of Pit-1, HESX1, LIM class of homeodomain protein-3, plant homeodomain-like finger gene-6) and isolated HH [*e.g.* Kallmann syndrome, FGFR1 (KAL2), GNHR, G protein-coupled receptor 54 (KiSS1-derived peptide receptor), small nuclear ribonucleoprotein polypeptide SmN (Prader-Willi), leptin, leptin receptor, dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1].

Infants with HH and micropenis require testosterone therapy to increase the length of their penis. Some clinicians have used repeated injections of human chorionic gonadotropin (hCG). A single study has proposed that male infants with hypothalamic hypogonadism be given a course of recombinant human (rh) FSH and rhLH injections (7). This treatment increased testicular size, presumably because FSH increased the number of Sertoli cells (as reflected in an increase in plasma inhibin B levels), without increasing testosterone concentrations.

Prepubertal treatment of HH with rhFSH stimulates inhibin B production and testicular growth (8). The treatment of many patients with HH condition is attempted only at adolescence or early adulthood. In addition to androgen replacement therapy, fertility has become an increasingly important concern. In adolescents or adult patients treated with monotherapy or combinations of hCG, human menopausal gonadotropins (hMG), and rhFSH (9–13), results depend on pretreatment testicular size and whether or not there has been cryptorchidism.

Patients and Methods

Patient 1 was the first child of nonconsanguinous parents. The 46,XY patient was referred at 2 wk because of micropenis (7 mm). Testes were intrascrotal but very small (< 10 mm diameter). He had a total lack of LH and FSH as well as testosterone (Table 1). Inhibin B and anti-Mullerian hormone (AMH) were below the lower normal limit (10) (Table 1). A single dose (100 IU/kg) of hCG increased testosterone to 0.08 nmol/liter. IGF-I level was 14 ng/ml, prolactin 45 ng/ml, fasting cortisol 3 μg/dl with ACTH 6 pg/ml, and T_4 5.1 μg/dl with TSH 4.7 μU/ml. MRI found an interrupted stalk and hypoplastic pituitary with ectopic functional neurohypophysis. The child received 4 mg hydrocortisone, 20 μg L-(T_4), and 0.5 mg Saizen rhGH per day. At the age of 8 wk, he began treatment with continuous sc infusion (CSI) at a mean infusion rate of 56 IU rhLH and 67 IU of rhFSH per day. CSI was well tolerated and stopped at 25 wk of life.

Patient 2 was an Asian child adopted by French parents. The patient was referred at the age of 14 wk because of micropenis (12 mm). Testes were intrascrotal and small (<10 mm diameter). The karyotype was 46,XY. LH, FSH, and testosterone were all undetectable and inhibin B and AMH persistently below the normal range (10) (Table 1). A single dose (100 IU/kg) of hCG increased testosterone to 0.47 nmol/liter. Other pituitary axes were normal. No mutation of KAL1 nor FGFR1 was found (Dr. C. Dodé, Laboratory of Molecular Genetics, Cochin). At 20 wk, the child began treatment with CSI at a mean infusion rate of 50 IU rhLH and 125 IU r FSH per day. CSI was well tolerated and stopped at 48 wk of life.

Drugs

The treatment started after written consent from both parents under institutional review board auspices. rhLH and rhFSH (Luveris 37.5 IU and Gonal-F 37.5 IU) were obtained from Serono (Geneva, Switzerland) and dissolved in solvent for infusion with a Paradigm insulin pump (Minimed, Northridge, CA) whose catheter was changed every 48–72 h. No adverse effects were observed at the infusion site.

Assays

Serum FSH, LH, testosterone, AMH, and inhibin B were measured as reported (14). Normal 95% ranges at 3 months (14) are presented in Table 1. Testes length and volume were evaluated using sonography.

Results

rhLH and rhFSH CSI increased serum gonadotropins to high values (14) (Table 1). Serum testosterone, inhibin B, and AMH reached concentrations in the high normal range (Table 1). Mean testicular length increased from 7 mm at birth to 25 mm at 7 months (P1) and from 8 to 21 mm at 7 months (P2). Testicular volume increased from 0.57 ml (P1) and 0.45 ml (P2) at birth to 2.10 ml at 7 months (P1 and P2). Stretched penile length increased from 8 mm to 21 mm (P1) and from 12 mm to 48 mm (P2).

TABLE 1. Serum concentrations of reproductive hormones before, during, and after treatment of the two studied infants with gonadotropin CSI

	Treatment period			
Patient 1	**2–6 wk**	**8–25 wk**	**30–35 wk**	**Normal values[a]**
LH (IU/liter)	0.19 ± 0.2	5.2 ± 2.4	0.16 ± 0.09	0.5–7.1
FSH (IU/liter)	0.19 ± 0.04	29.9 ± 14	0.76 ± 0.76	0.2–4
Testosterone (nmol/liter)	0.03 ± 0	7.6 ± 8	0.03 ± 0.01	0.52–4.79
Inhibin-B (pg/ml)	167 ± 32	701 ± 284	228 ± 107	125–570
AMH (pmol/liter)	394 ± 125	608 ± 221	734 ± 117	260–1157
	Treatment period			
Patient 2	**14–15 wk**	**20–48 wk**	**50–55 wk**	
LH (IU/liter)	0.03 ± 0.02	4.2 ± 1.1	0.20 ± 0.09	
FSH (IU/liter)	0.12 ± 0.01	73 ± 22	1.01 ± 0.2	
Testosterone (nmol/liter)	0.06 ± 0.01	4.8 ± 1.9	0.15 ± 0.03	
Inhibin-ß (pg/ml)	48 ± 9	426 ± 189	113 ± 10	
AMH (pmol/liter)	386 ± 44	826 ± 289	810 ± 111	

[a] Recorded at the peak of gonadotropin surge (1–3 months) in 10 normal age-matched male infants (14).

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Discussion

The natural history of the hypothalamic GnRH pulse generator-pituitary gonadotropin-gonadal apparatus in the male infant has been summarized by Grumbach (3) in a recent master review. FSH and LH levels are low in umbilical cord blood plasma owing to the inhibitory effect of the high circulating levels of placenta-derived estrogens on both the GnRH pulse generator and pituitary gonadotropes (5, 6, 15). Within a few minutes after birth, a brief surge in LH secretion is followed by an increase in the serum concentration of testosterone, which persists for about 12 h (16, 17). FSH levels during the first neonatal days are low. After the fall in circulating placentally derived estrogens during the first few days after birth, the sex steroid inhibited pituitary gonadotropin-gonadal system is activated around 1–2 wk of age (18); the increase in FSH and LH secretion evokes increased secretion of gonadal sex steroids (19). This transient surge in the hypothalamic GnRH pulse generator-pituitary gonadotropin-gonadal apparatus, sometimes called the minipuberty of early infancy (3), is associated with a pubertal concentration of circulating LH and FSH. In the male infant, serum FSH and LH levels increase during the second postnatal week, reach a maximum between 1 and 3 months and decline by about age 6 months to the low levels that are present until the onset of puberty. Serum testosterone follows the pattern of LH secretion, reaching a peak at about 3 months with a subsequent decline to prepubertal values by 6–9 months of age (5, 19, 20). Soon after birth Leydig cell numbers increase to reach a peak at about 3 months of age, followed by striking regression and apoptosis of the fetal (and infantile) Leydig cells (21–23).

Circulating inhibin B increases soon after birth to reach a peak at 4–12 months, decreasing to a nadir from 3 to 9 yr and increasing again with the onset of puberty (20). Its profile reflects the continued wave of Sertoli cell proliferation (which begins in the fetus) during infancy, which continues at a low level during childhood and increases again with the onset of puberty and persists until late puberty when terminal differentiation of Sertoli cells occurs (24). In HH, the plasma concentration of inhibin B remains low (25, 26). In males, AMH/Mullerian-inhibiting substance, the earliest Sertoli cell hormone secretion in the human fetus, is present in cord blood; rises rapidly in concentration during the first month, reaching a peak level at about 6 months of age; and then slowly declines during childhood, falling to low levels in puberty (27, 28). Its secretion is mainly constitutive.

In summary, the neonatal-midinfancy surge in pulsatile gonadotropin secretion is attributable to an increase in GnRH pulse amplitude and is associated with a rapid expansion of Leydig and Sertoli cell populations with concomitant surges in testosterone, inhibin, and AMH production as well as an increase in testicular volume due to increase in seminiferous tubular cell mass (90% made of Sertoli cells) and tubule length (23, 29). Sertoli cell number, including postnatal proliferation, is a determinant of spermatogenic function (reviewed in Ref. 30). The total number of germ cells increases up to about 100 d (31), indicating mitotic activity, and then decreases mainly by apoptosis (32). According to these phenomena, the suggestion reviewed by Grumbach (3) is that the transient postnatal to midinfancy function of the GnRH pulse generator in the male infant is related to future spermatogenic function and fertility (25, 26, 30).

In an attempt to mimic the physiological development by replacing gonadotropins early in life, the current treatment doses were initially inspired by a case of infantile HH treated with LH and FSH injections (7) and then derived empirically from our own observations. The treatment was successful in inducing testicular and penile growth. The dosage augmented LH and FSH to the normal range for 3-month-old boys. The results were comparable with those from studies in adult volunteers, showing a significant increase in serum concentrations of FSH and LH within 12 h and a more rapid decline of LH than of FSH (33). Serum testosterone values were high throughout treatment, and thus, unlike the gonadotrope, Leydig cells do not seem to need intermittency of trophic hormone delivery. The dose of FSH was effective in increasing both FSH and inhibin B concentrations to normal. In adults, serum inhibin B concentrations are negatively correlated with FSH, but this feedback mechanism is not observed in the first 2 yr of life (20).

In young adults with congenital HH, the combined rhFSH/hCG stimulation of the testis suppresses AMH secretion and lowers inhibin levels, whereas rhFSH alone increases AMH and inhibin (34). This is interpreted as a suppressive effect of LH-driven testicular androgens outweighing the stimulation of Sertoli cells by rhFSH. This did not occur in the two reported infants, despite the strong LH stimulation of testosterone secretion, suggesting that the suppressive effect of testicular androgens is not functional during the first months of life. This resistance of postnatal Sertoli cells to androgens is reminiscent of observations in postnatal mouse whose AMH is not inhibited by intratesticular testosterone due to the lack of androgen receptors in Sertoli cells (35).

Another observation is the relative resistance of the postnatal Sertoli cells of our HH infants to rhFSH because very high serum concentrations of FSH (8-fold the upper limit of normal in P1 and 18-fold in P2) were needed to obtain inhibin B and AMH levels within the normal range. For comparison, FSH concentrations around 11–14 IU/liter suffice to induce inhibin B and AMH increases in adult HH patients (35). In conclusion, early postnatal treatment of HH with CSI of rhFSH and rhLH induced testicular and penile growth and augmented serum concentrations of testosterone, inhibin B, and AMH, closely mimicking the physiological development that occurs early after birth. This treatment increased testicular size, presumably by FSH-induced increase of the number of Sertoli cells, and revealed a complete resistance of Sertoli cells to the effect of androgens and a degree of resistance of these cells to FSH, compared with mature Sertoli cells. It remains to be seen whether early gonadotropin replacement will improve the reduced fertility potential often seen in adult patients with congenital HH despite hormonal treatment.

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P.B. designed the study, set gonadotropin dosage, and wrote the manuscript; M.F. started CSI in P1 and C.B. in P2; L.P. collected the data; N.L. performed the hormone measurements and provided studies in normal infants. D.R. referred patient 2.

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