Recombinant Human FSH Treatment Outcomes in Five Boys With Severe Congenital Hypogonadotropic Hypogonadism

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Context: Recombinant human FSH (r-hFSH), given to prepubertal boys with hypogonadotropic hypogonadism (HH), may induce Sertoli cell proliferation and thereby increase sperm-producing capacity later in life.

Objective: To evaluate the effects of r-hFSH, human chorionic gonadotropin (hCG), and testosterone (T) in such patients.

Design and Setting: Retrospective review in three tertiary centers in Finland between 2006 and 2016.

Patients: Five boys: *ANOS1* mutation in two, homozygous *PROKR2* mutation in one, *FGFR1* mutation in one, and homozygous *GNRHR* mutation in one. Prepubertal testicular volume (TV) varied between 0.3 and 2.3 mL; three boys had micropenis, three had undergone orchidopexy.

Interventions: Two boys received r-hFSH (6 to 7 months) followed by r-hFSH plus hCG (33 to 34 months); one received T (6 months), then r-hFSH plus T (29 months) followed by hCG (25 months); two received T (3 months) followed by r-hFSH (7 months) or r-hFSH plus T (8 months).

Main Outcome Measures: TV, inhibin B, anti-Müllerian hormone, T, puberty, sperm count.

Results: r-hFSH doubled TV (from a mean $\pm$ SD of 0.9 ± 0.9 mL to 1.9 ± 1.7 mL; $P < 0.05$) and increased serum inhibin B (from 15 ± 5 ng/L to 85 ± 40 ng/L; $P < 0.05$). hCG further increased TV (from 2.1 ± 2.3 mL to 8.6 ± 1.7 mL). Two boys with initially extremely small testis size (0.3 mL) developed sperm (maximal sperm count range, 2.8 to 13.8 million/mL), which was cryopreserved.

Conclusions: Spermatogenesis can be induced with gonadotropins even in boys with HH who have extremely small testes, and despite low-dose T treatment given in early puberty. Induction of puberty with gonadotropins allows preservation of fertility.

Abbreviations: AMH, anti-Müllerian hormone; CHH, congenital hypogonadotropic hypogonadism; hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; inhB, inhibin B; KS, Kallmann syndrome; r-hFSH, recombinant human FSH; T, testosterone; TV, testicular volume.

Congenital hypogonadotropic hypogonadism (CHH) is a rare and heterogeneous genetic disorder diagnosed typically in adolescence due to delayed puberty [1]. CHH is caused by impaired production, secretion, or action of GnRH [2]. Approximately half of patients with CHH exhibit impaired sense of smell, a condition termed Kallmann syndrome (KS), and the other half have normosmic HH [2]. CHH can also rarely present as a part of a wider syndrome [*e.g.,* CHARGE (Coloboma, Heart defects, Atresia of the choanae, Retarded growth and development, Genital hypoplasia, and Ear anomalies and/or deafness) and Waardenburg syndrome] [3]. Estimates of CHH and KS incidence are scarce; studies based on French and Sardinian military screening suggest varied incidences from 1 in 10,000 for CHH [4] and 1 in 84,000 for KS [5] in men, whereas in Finnish population the incidence of KS is estimated at 1:30,000 for males and 1:125,000 for females [6].

Adolescent boys with CHH require hormonal treatment to induce puberty. The goals in treatment are to promote virilization, height growth, sexual function, bone health, psychological and emotional well-being, and future fertility [2]. Although boys with CHH achieve virilization with exogenous testosterone, testicular maturation and induction of spermatogenesis require treatment with gonadotropins or pulsatile GnRH [7–9]. However, in the most severe forms of GnRH deficiency, characterized by cryptorchidism and small adult testicular volume (TV) (<4 mL), the outcomes of the above-mentioned fertility-inducing treatments tend to be poor [10].

Prepubertal testis size comprises interstitial tissue and seminiferous cords, formed by somatic Sertoli cells enveloping spermatogonia. In puberty, the seminiferous cords grow in diameter and obtain lumen as Sertoli cells enter a mature, nonproliferative state to support and nurture the developing spermatogenic cells [11]. Subsequently, the number of Sertoli cells in adulthood correlates with sperm output [12]. The stage for future spermatogenesis is, however, already set before puberty. Because only 10% of the Sertoli cell number is reached within the neonatal period, proliferation of immature Sertoli cells continues in the minipuberty of infancy, and the final proliferation phase occurs in early puberty [13, 14]. At this time, Sertoli cells also differentiate and stop proliferating, which is linked to their increased expression of androgen receptors and increasing intratesticular testosterone levels [15, 16]. Consequently, a decline in the high circulating levels of anti-Müllerian hormone (AMH) secreted by immature, prepubertal Sertoli cells occurs, which reflects androgen-mediated differentiation of Sertoli cells [17]. However, the role of exogenously administered testosterone in this process is unclear.

More than 20 years ago, we introduced the concept of treating boys with prepubertal onset of HH by using recombinant human FSH (r-hFSH) [18], and 10 years later we reported the long-term outcome of this treatment modality in a heterogeneous group of patients [19]. Subsequently, Dwyer *et al.* [20] showed data on men with CHH suggesting proliferation and maturation of Sertoli cells in response to r-hFSH, but their randomized study did not reach conclusive evidence for the superiority of r-hFSH pretreatment on sperm parameters. Although the long-term outcomes of r-hFSH pretreatment are promising, and the European consensus statement on CHH [2] suggests that it may benefit most severely affected patients (*i.e.,* those with small testis size and history of cryptorchidism), there is no conclusive evidence on the possible benefits of this treatment. At the same time, it is unclear whether exogenous testosterone (T), widely used in the induction of puberty in patients with CHH, induces premature differentiation of Sertoli cells and thereby reduces sperm-producing capacity.

In this study, we describe biochemical and clinical markers of puberty and testicular function during and after r-hFSH treatment in five prepubertal patients with CHH who

have a molecular genetic diagnosis. Three of the boys had very small prepubertal testis size, and three boys had a history of cryptorchidism, both known risk factors for poor spermatogenesis later in life [10, 21]. In addition, we describe clinical and hormonal data pertinent to Sertoli cell function in boys with CHH treated with exogenous T.

1. Patients and Methods

A. Patients

Data were retrospectively collected from electronic medical records of five boys with CHH treated in Children's Hospital of Helsinki University Hospital, Turku University Hospital, or Kuopio University Hospital between 2006 and 2016. Patients included in this study are characterized in detail in Tables 1 and 2 and in the Results section. The diagnosis of CHH was based on clinical history, absence of the sense of smell (in patients with KS), low gonadotropin and sex steroid levels for age, and low responses in GnRH stimulation tests. Brain MRI was performed in four of five boys. In all boys, the diagnoses were confirmed by molecular genetic analyses. Genetic testing of patient 4 was carried out commercially in an accredited laboratory (SYNLAB MVZ Humane Genetik); all other patients were tested in a research setting.

During treatment, the boys visited a pediatric endocrinologist at 1- to 8-month intervals. At each visit, their stage of puberty was assessed according to the Tanner system, testis length and width were measured with a ruler to the nearest millimeter, and TV was calculated (length $\times$ width^2 $\times$ 0.52)]; TV is reported as the volume of one testis or the mean of both testes when possible. Prepubertal penile length < -2.5 SDs was defined as micropenis [22].

At each visit, blood samples were drawn for serum gonadotropins, T, AMH, and inhB concentrations. Serum AMH was measured by Beckman Coulter AMH Gen II ELISA with a lower limit of detection of 0.08 µg/L. Intra- and interassay coefficients of variation were from 2.5% to 5.4% and from 4.5% to 5.6%, respectively. LH and FSH were measured with Elecsys® Electrochemiluminescence immunoassay (Roche Diagnostics). The lower limit of detection was 0.10 mIU/mL for both LH and FSH; intra- and interassay coefficients of variation were 2% and 3% for LH and 2.5% and 3% for FSH, respectively. Testosterone was measured by liquid chromatography/tandem mass spectrometry (API 2000 LC/MS/MS System; Applied Biosystems) with a detection limit of 0.05 nmol/L. Serum inhB was measured as described recently in detail [23].

Three patients provided semen samples after the treatment period; semen analyses were performed according to World Health Organization criteria [24]. All five patients were treated with r-hFSH [Gonal-F® (EMD Serono), Puregon® (Merck Sharpe & Dohme)] in three weekly subcutaneous doses of 0.9 to 2.1 IU/kg (range, 66.7 to 112.5 IU) for 7 to 40 months. TV and inhB were used to assess response to treatment. Patients 1 and 2 received 6 to 7 months

Table 1. Molecular Genetic Diagnoses and Clinical Findings of Five Adolescent Boys With CHH

Patient No.	DG	Mutation	Initial GnRH Test, Baseline/Maximum (IU/L)	Initial T (nM)	Initial TV (mL)	MRI Finding
1	KS	*ANOS1* c.571C>T p.(Arg191*)	LH: 0.1/1.3; FSH: 0.4/1.8	0.2	0.3	Absent olfactory bulbs
2	nCHH	Homozygous *GNRHR* c.416G>A p.(Arg139His)	LH: <0.1/<0.1; FSH: NA	0.3	0.3	Normal
3	KS	Homozygous *PROKR2* c.701G>A p.(Gly234Asp)	LH: <0.1/1.6; FSH: 0.5/3.3	<0.5	2.3	Normal
4	KS	*FGFR1* c.2059G>A p.(Gly687Arg)	LH: 0.3/5.1; FSH: 1.1/ 6.5	0.3	0.5	Hypoplastic olfactory bulbs
5	KS	*ANOS1* c.571C>T p.(Arg191*)	LH: 0.1/ NA; FSH: 0.4/ NA	0.3	0.9	NA

Abbreviations: NA, not available; nCHH, normosmic congenital hypogonadotropic hypogonadism.

Table 2. Clinical Findings and Treatment Schemes of Five Adolescent Boys With CHH

Patient No.	Age at Beginning of Treatment (y)	Treatment	Testes Position	Micropenis	Final TV (mL)	Maximum Sperm Count (million/mL)
1	14.8	r-hFSH 66.7 IU SC 3 times/wk for 6 mo → r-hFHS 66.7 IU SC 3 times/wk + hCG 500–1500 IU SC/wk for 34 mo	Bilateral retractile testes operated on at age 4 y	No	6.7	2.8
2	15.1	r-hFSH 112.5 IU SC 3 times/wk for 7 mo → r-hFHS 66.7 IU SC 3 times/wk + hCG 500–1500 IU SC/wk for 33 mo	Scrotal	No	7.2	13.8
3	16.7	T 50 mg IM 1 time/mo for 6 mo → T 50–750 mg IM 1 time/1–3 mo + r-hFSH 100 IU SC 3 times/wk for 29 mo → hCG 1000–1500 IU SC 2 times/wk for 25 mo	Scrotal	Yes	12.1	9.3
4	14.7	T 50 mg IM 1 time/mo for 3 mo → r-hFSH 75 IU SC 3 times/wk for 7 mo	R: scrotal; L: operated on at age 13.5 y	Yes	1.8	NA
5	13.3	T 50 mg IM 1 time/mo for 3 mo → T 50-100 mg IM 1 time/mo + r-hFSH 75 IU SC 3 times/wk for 8 mo	R: operated on at age 9.9 y; L: scrotal	Yes	1.3	NA

Abbreviations: IM, intramuscularly; NA, not available; SC, subcutaneously.

of r-hFSH alone, and thereafter hCG (Pregnyl®; Merck) was commenced for 33 to 34 months in one or two weekly doses of 8 to 14 IU/kg (range, 500 to 1500 IU). Low-dose T [Sustanon® (Aspen); Nebido® (Bayer)] treatment (range, 50 to 250 mg per month) was administered for patients 3, 4, and 5 in parallel with r-hFSH for 3 to 29 months. In patient 3, spermatogenesis was induced with hCG in two weekly doses of 15 to 22 IU/kg (Pregnyl®, range, 1000 to 1500 IU). After induction of puberty, three patients (patients 1, 2, and 3) switched to long-acting T (Nebido®) monotherapy (range, 250 mg every 6 weeks to 1000 mg every 3 months). The treatment protocols are described in Fig. 1 and Table 2.

B. Ethics

This retrospective study based on electronic patient data was approved by the local Ethics Committee of the Helsinki University Hospital and the University of Helsinki, and all centers (Helsinki University Hospital, Turku University Hospital, and Kuopio University Hospital) possessed research permits. Informed consent of patients and/or their guardians was obtained.

C. Statistics

Changes in TV and concentration of inhB during r-hFSH pretreatment were assessed with Wilcoxon signed-rank test. The data are presented as mean (SD) unless otherwise stated. $P <$ 0.05 was accepted as indicating statistical significance. Statistical analyses were performed with SPSS statistical software for Windows, version 22.0 (SPSS, Chicago, IL).

2. Results

A. Patient Case Histories

Clinical findings and molecular genetic diagnoses are summarized in Table 1 and 2. Patients 1 and 5 are not related, although they carry the same mutation. The overall impact of r-hFSH pretreatment on AMH, FSH, inhB, and TV is shown in Fig. 2. Figure 3 describes the individual
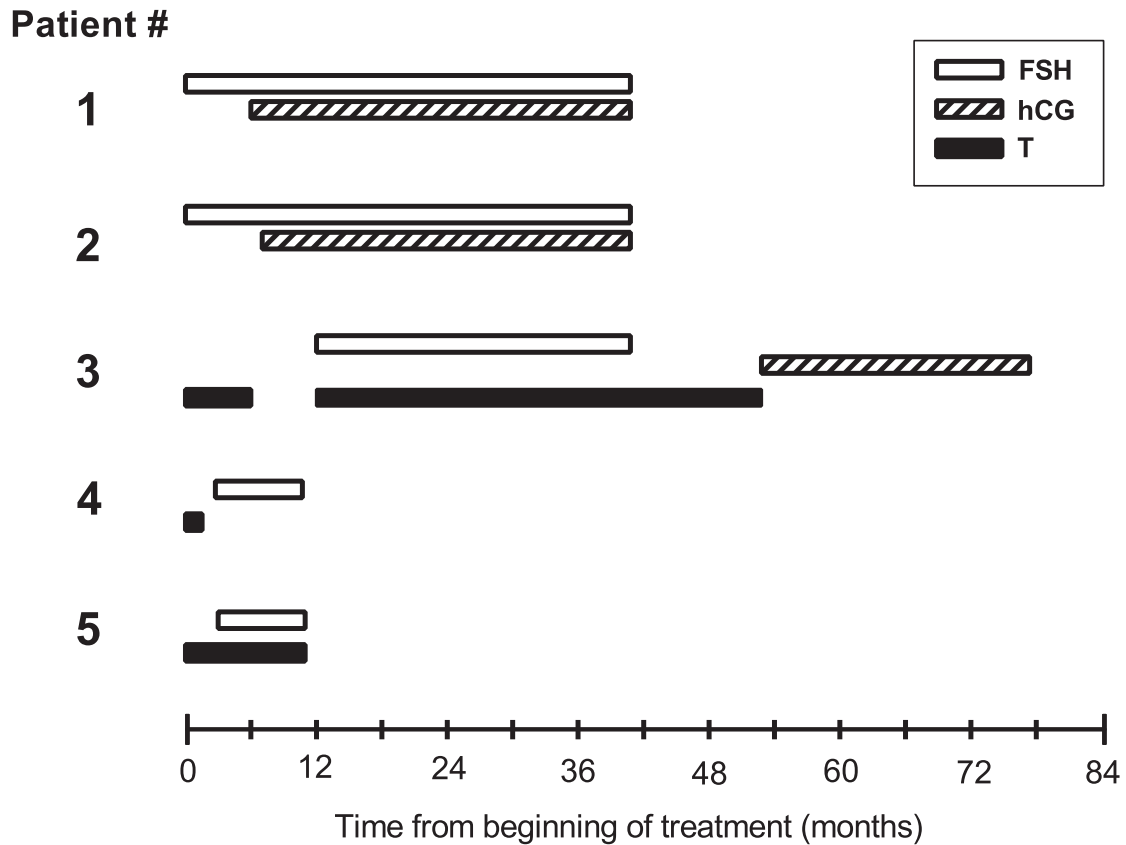
Figure 1. Treatment schemes of five boys with CHH on a timeline starting from each boy's first hormonal therapy. White box represents r-hFSH; dashed box, hCG; and black box, T.

responses of patients 1, 2, and 3 to r-hFSH pretreatment followed by induction of puberty with hCG.

Patient 1 was referred to a pediatric endocrinologist at age 14.6 years because of delayed puberty. The patient had undergone surgery for bilateral retractile testis at age 4 years. Clinical examination revealed hyposmia, synkinesia, Tanner stage G1P1, small testes (TV of 0.3 mL), and prepubertal penis. Brain MRI showed absent olfactory bulbs; his gonadotropin and testosterone levels were low for his age; and he was found to carry a hemizygous *ANOS1* nonsense mutation c.571C>T p.(Arg191*) consistent with KS [25]. r-hFSH treatment was started at 14.8 years of age in three weekly doses (66.7 IU subcutaneously), which he received for 6 months. Pretreatment with r-hFSH increased AMH (from 2.5 to 28.1 µg/L) and inhB (from 11 to 47 ng/L) levels and TV (from 0.3 to 0.7 mL) (Fig. 2). Subsequently, puberty was induced with hCG (500 to 1500 IU per week in one or two subcutaneous doses). During hCG therapy, TV increased to 6.7 mL, AMH decreased to baseline values, and inhB peaked to 108 ng/L. After 21 months of hCG treatment (at Tanner stage G4P4 and TV of 6.2 mL), he provided four semen samples with a maximum sperm count of 2.8 million/mL (oligozoospermia) (Fig. 3). Sperm was cryopreserved for possible future use. Thereafter, he switched to T monotherapy (T undecanoate, 500 mg every 6 weeks to 750 mg every 3 months intramuscularly). Adult height at age 18.5 years was 178.7 cm.

Patient 2 presented at age 14.9 years with Tanner stage G1P1 and small testis size (TV 0.3 mL). His gonadotropin and testosterone levels were low, a GnRH stimulation test revealed absent LH response, and his brain MRI and sense of smell were normal. He was diagnosed with normosmic HH because of homozygous *GNRHR* mutation c.416G>A p.(Arg139His) [17, 26–30], and r-hFSH pretreatment was initiated at age 15.1 years (112.5 IU subcutaneously in three weekly doses). AMH increased (from 20.3 to 32.5 µg/L), inhB showed a markedly strong response (from 14 to 118 ng/L), and TV nearly tripled (from 0.3 to
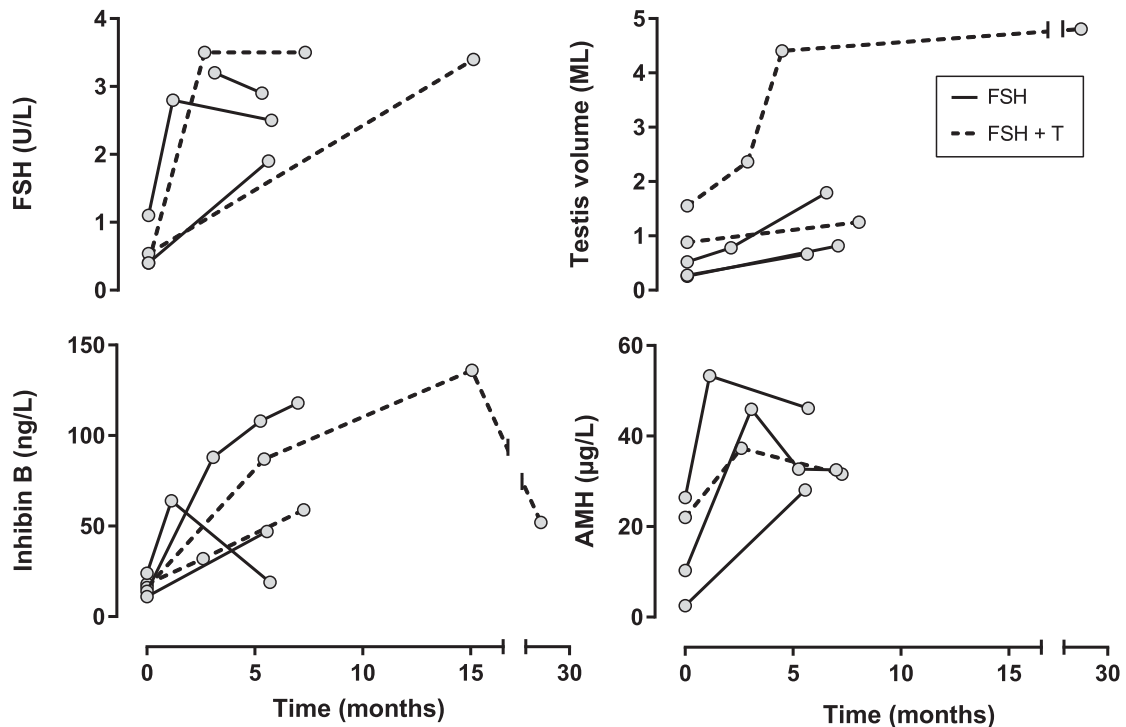
Figure 2. Markers of puberty and testicular function during r-hFSH treatment or combination of r-hFSH and T treatment in five adolescents with CHH: inhB, FSH, AMH, and TV. Patients 1 and 2, marked with a solid line, were treated with r-hFSH; patients 3, 4, and 5, marked with a dashed line, were treated with r-hFSH plus T.

0.8 mL) (Fig. 2). Seven months later, hCG (500 to 1500 IU per week in one or two sub-cutaneous doses) was added to induce spermatogenesis and androgenic puberty. As in patient 1, AMH decreased, T levels increased, and TV increased up to 7.2 mL. He provided four semen samples, the first of which was analyzed after 23 months of hCG + r-hFSH combination therapy (at a Tanner stage of G4P4 and TV of 7.1 mL); maximum sperm count was 13.8 million/mL (oligozoospermia) (Fig. 3). Sperm was cryopreserved for possible future use. Subsequently, gonadotropin treatments were replaced with T monotherapy (T undecanoate, 1000 mg every 3 months intramuscularly). His adult height at age 18.3 years was 175.5 cm.

Patient 3 has been previously described by Tommiska *et al.* [31]. In short, he was referred to a pediatric endocrinologist at 15.5 years of age and was followed up in anticipation of spontaneous activation of puberty until 16.7 years of age. He had small testes (2.3 mL), micropenis, and pubertal stage of G1P1. There was no history of cryptorchidism. Because his puberty did not proceed, he was put on T, 50 mg intramuscularly every 3 weeks. At age 17.7 years, KS was diagnosed; he carried a homozygous *PROKR2* mutation c.701G>A p.(Gly234Asp) [32]. The patient had anosmia and normal brain MRI. T treatment (50 to 125 mg intramuscular doses every 3 weeks) was accompanied with r-hFSH, 100 IU subcutaneously three times a week for 29 months. During that time, inhB increased (from 16 to 136 ng/L) and TV doubled (from 2.3 to 4.8 mL) (Fig. 2). Because of questionable adherence, he was transferred back to T monotherapy (250 mg every 6 weeks to 750 mg every 3 months intramuscularly), during which time TV did not change. At age 21.2 years, the patient desired fertility and spermatogenesis was induced with hCG (1000 to 3000 IU per week in two subcutaneous doses for 25 months). He provided five sperm samples after 18 months of hCG treatment (at Tanner stage of G5P6 and TV of 12.1 mL), maximum sperm count being 9.3 million/mL (oligozoospermia) (Fig. 3), and he was able to conceive a child. Adult height, measured at age 20.6 years, was 173.9 cm.

Patient 4 had surgery for unilateral cryptorchidism at age 13.5 years and was followed up by a pediatric surgeon for contralateral retractile testis as well as multiple syndactyly of the
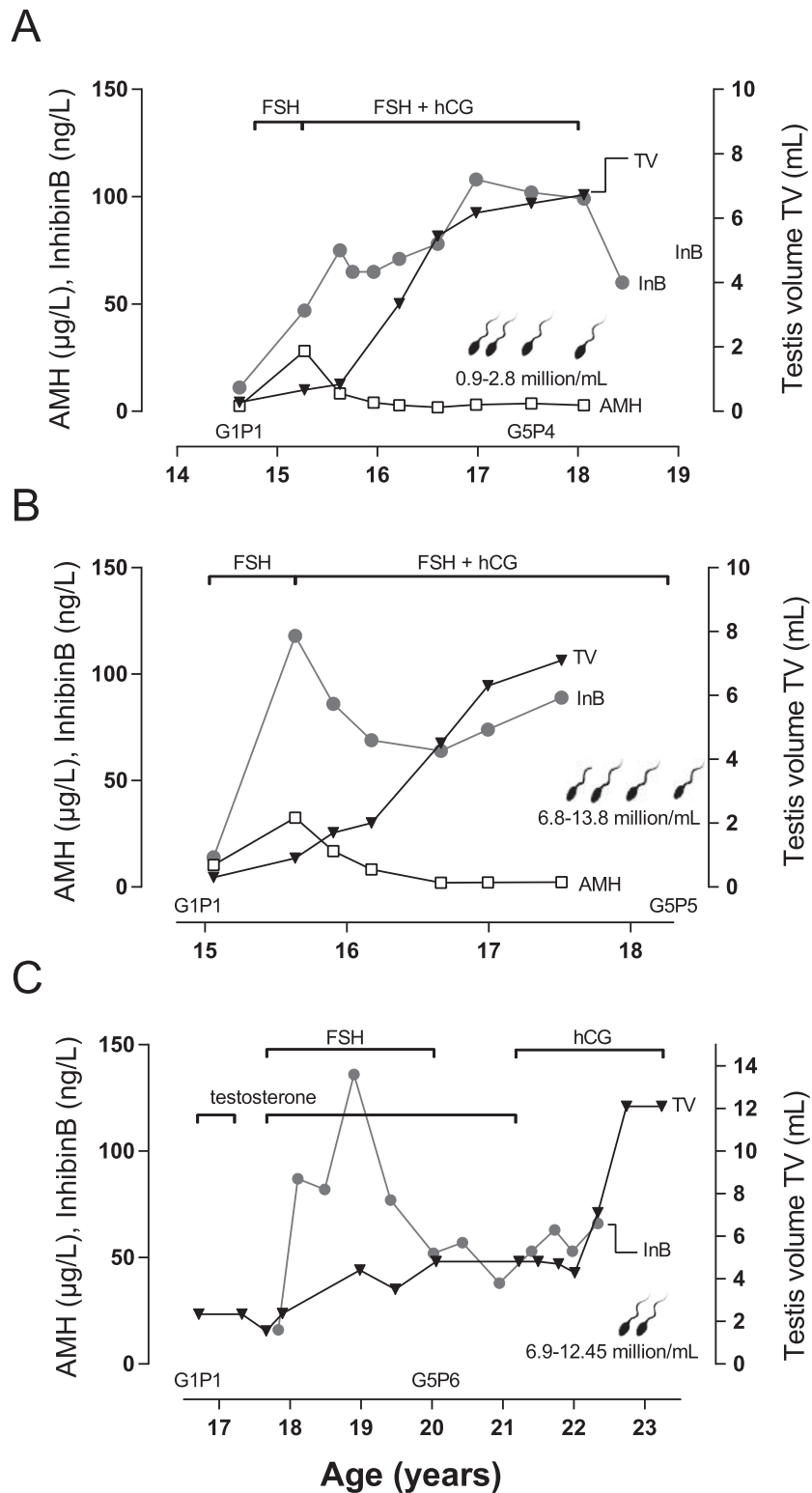
Figure 3. Response to r-hFSH and hCG therapy in three boys with CHH. Schematics and individual responses for treatment in TV, progression of puberty (Tanner G and P stages), AMH, and inhB levels in (A) patient 1 (carrying *ANOS1* mutation), (B) patient 2 (carrying homozygous *GNRHR* mutation), and (C) patient 3 (carrying homozygous *PROKR2* mutation). Maximum sperm count and the time of sperm analyses are indicated by sperm symbols.

feet. He was referred to a pediatric endocrinologist at age 14.5 years for delayed puberty. Tanner stage was G1P2, and he had micropenis and small testes (0.5 mL). Brain MRI showed hypoplastic olfactory bulbs, and molecular analysis confirmed KS diagnosis [*FGFR1* mutation c.2059 G>A p.(Gly687Arg)] [33]. According to Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/), this mutation is predicted to be probably damaging with highest possible score (score of 1.000). At age 14.7 years, the patient was treated first for micropenis with three monthly T (50 mg intramuscular) injections. R-hFSH pretreatment was subsequently initiated with three weekly 75 IU subcutaneous doses. Despite the prior T treatment, he responded to r-hFSH favorably: After r-hFSH pretreatment for 7 months, his AMH had doubled from 26.4 to 53.3 μg/L, inhB had increased from 24 to 64 ng/L, and TV increased from 0.5 to 1.8 mL (Fig. 2). For patient 4, the treatment is still ongoing.

Patient 5 had a history of unilateral cryptorchidism and congenital unilateral kidney atrophy. At age 13.3 years, clinical examination showed he had small testes (TV 0.9 mL), micropenis, hyposmia, and synkinesis. KS was diagnosed on the basis of hemizygous nonsense mutation in *ANOS1* [c.571C>T p.(Arg191*)] [25, 34, 35]. Treatment was initiated with three monthly 50-mg intramuscular doses of T because of micropenis. T was then combined with r-hFSH in three weekly 75-IU subcutaneous doses for 8 months, during which AMH and inhB increased (from 22 to 37 μg/L and from 11 to 59 ng/L, respectively) and TV increased (from 0.9 mL to 1.3 mL) (Fig. 2). His treatment is ongoing.

B. Summary of Treatment Responses to r-hFSH Pretreatment Followed by hCG

Responses during r-hFSH treatment in inhB, AMH, and FSH levels and TV are summarized in Fig. 2. Hormonal treatment was initiated at the mean age of 14.9 ± 1.2 years (range, 13.3 to 16.7 years). Duration of r-hFSH pretreatment with or without T varied from 6 to 29 months. During r-hFSH treatment, TV doubled from 0.9 ± 0.9 mL to 1.9 ± 1.7 mL ($P < 0.05$) and serum inhB increased from 15 ± 5 ng/L to 85 ± 40 ng/L (maximal value recorded for each patient during treatment; $P < 0.05$). Induction of spermatogenesis and androgenic puberty with hCG treatment was initiated for three of the five patients (Fig. 3): Puberty progressed at a normal rate, TV increased from 2.1 ± 2.3 mL to 8.6 ± 3.0 mL, and patients reached genital maturation at a normal rate in 2.7 to 3.3 years. Each of these three patients provided four to five semen samples with maximum sperm count ranging from 2.8 to 13.8 million/mL; sperm were cryopreserved for future use.

3. Discussion

In patients with CHH, timely diagnosis and treatment to induce puberty are considered beneficial for sexual, bone, and metabolic health and might help minimize some of the negative psychological effects of CHH [2]. These patients are at risk for future infertility; given the overall disease burden, evidence-based treatment should be given to maximize testicular function and keep patients in line with their peers in terms of virilization. They should also be offered psychosocial support. Early intervention, such as neonatal gonadotrophin therapy mimicking minipuberty, may also have beneficial effects on testicular function and genital development. Indeed, because the androgen receptor is only weakly expressed in the infant Sertoli cells, hCG can be administered without maturing the Sertoli cells or inducing spermatogenesis [36–38]. However, the long-term benefits of neonatal gonadotrophin therapy are unknown.

The concept of maximizing sperm production with r-hFSH pretreatment in peripubertal boys is not new and has been applied in Finland during the past 20 years, with favorable outcomes [19]. Herein, we report our experience of this treatment modality in five boys with severe CHH. All patients responded to r-hFSH pretreatment with increased TV, inhB, and AMH concentrations, all surrogates of immature Sertoli cell proliferation [11, 39, 40]. These results agree with previous reports [18–20, 39], although we did not consider testicular

biopsies appropriate as part of clinical treatment of these patients. Therefore, the effect of r-hFSH in this age group must be judged on the basis of the above-mentioned indirect evidence.

In three boys, spermatogenesis was successfully induced with hCG following r-hFSH pretreatment. These patients showed an abrupt decline in AMH levels, consistent with maturation of Sertoli cells due to increased intratesticular T concentrations [15, 16]. This result agrees with those reported by Young *et al.* [39]. Our results thus suggest that the age-dependent upregulation of androgen receptor, described by Rey *et al.* [15], also occurs in patients with CHH. Cryptorchidism and small testicular size are negative prognostic factors for future fertility [10, 21]. In our study, however, two patients with an extremely low initial testicular volume (0.3 mL) responded well to gonadotropin treatment and developed sperm. These results agree with our previous reports [19]. Furthermore, two of the boys (patients 4 and 5) showed good response to r-hFSH despite a phenotype suggesting severe GnRH deficiency in infancy (cryptorchidism and small penile size). These findings support the notion that r-hFSH priming treatment should be targeted especially to patients with CHH who have the poorest prognosis for fertility, as suggested by the recent consensus statement [2].

For patients 3 and 5, the impact of r-hFSH pretreatment was not diminished by previously or simultaneously administered exogenous T, and in patient 4 the treatment of micropenis before r-hFSH did not compromise the response to r-hFSH. These findings suggest that exogenous low-dose T does not disturb immature Sertoli cell proliferation, concordant with the conclusions of a meta-analysis by Rastrelli *et al.* [41]: that previous T therapy does not affect the results of gonadotropin treatment. This notion is further enforced by the finding that patient 3 fathered a child even after 4 years of T treatment when hCG was introduced to his treatment. Of note, we did not observe atypical responses to gonadotropin treatment, such as signs of testicular resistance, of remaining hypogonadal, or of being azoospermic, suggested to occur in as many as 26% of men with CHH [42].

Our patients 1 and 5 had *ANOS1* mutation, which has been previously associated with a severe reproductive phenotype and poor outcome of gonadotrophin treatment [42–44]. In the work of Costa-Barbosa *et al.* [44], 50% of patients harboring *ANOS1* mutation did not respond favorably to GnRH therapy, and Sykiotis *et al.* [42] discovered that in their study population all atypical responders (n = 21) displayed mutations in *ANOS1*. However, both our patients with *ANOS1* mutation had good responses to r-hFSH treatment, and patient 1, for whom puberty was initiated with hCG during the study period, was able to produce sperm. These results suggest that patients carrying an *ANOS1* mutation benefit from peripubertal r-hFSH therapy.

In rare diseases such as CHH, even retrospective studies are limited by the small number of patients, as in this study. Patients in our study presented at different ages, had varying expectations for care, and thus had individually designed treatment protocols. Although treatment protocols were not strictly similar for each boy, we were able to evaluate the effects of r-hFSH treatment in this patient group, in which four of five had known risk factors for poor fertility (low TV or cryptorchidism). While we await the results from prospective multicenter studies to validate the role of r-hFSH pretreatment in the management of boys with CHH, smaller patient case series such as ours add to the existing knowledge of benefits of r-hFSH pretreatment in boys with CHH.

We conclude that spermatogenesis can be induced with gonadotropin treatment in boys with CHH despite extremely low baseline TV and that prior exogenous low-dose androgen treatment does not appear to prevent proliferation of immature Sertoli cells. More important, induction of puberty with gonadotropin treatment in patients with CHH allows preservation of fertility, and this option should be explained to these patients and their families.

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