

The Pulsatile Gonadorelin Pump Induces Earlier Spermatogenesis Than Cyclical Gonadotropin Therapy in Congenital Hypogonadotropic Hypogonadism Men

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Abstract

The objective of this study was to compare the effect of pulsatile gonadorelin pump (PGP) and cyclical gonadotropin (human chorionic gonadotropin [HCG]/human menopausal gonadotropin [HMG]) therapy (CGT) on spermatogenesis in congenital hypogonadotropic hypogonadism (CHH) men. Twenty-eight azoospermic CHH males were included in this nonrandomized study. Ten received PGP and 18 received CGT. The primary endpoint was the earliest time spermatogenesis occurred during 24 months of treatment. Spermatogenesis time was significant earlier in the PGP group than the CGT group (median of 6 and 14 months, respectively, $\chi^2 = 6.711$, $p = .01$). Spermatogenesis occurred in 90% of the PGP group and 83.3% of the CGT group and showed statistically insignificant difference in the superiority analysis and the no-inferior test. Contributing factors significant for spermatogenesis were previous HCG/ or testosterone treatment and the peak serum luteinizing hormone level of triptorelin stimulation test at baseline. Although testis volume and penile length increased significantly from baseline, the differences between the two therapies were not significant. There was a tendency for high serum testosterone level, associated with more facial acne and breast tenderness in the CGT group. Skin allergic erythema scleroma was a common side effect of the PGP. In summary, PGP resulted in earlier spermatogenesis and more desirable testosterone levels than CGT.

Keywords

congenital hypogonadotropic hypogonadism, pulsatile gonadorelin pump, cyclical gonadotropin therapy, spermatogenesis, serum testosterone

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Congenital hypogonadotropic hypogonadism (CHH) is a very rare inherited endocrine and metabolic disease. It is caused by deficiency of the hypothalamic gonadotropin-releasing hormone (GnRH) beginning at birth or in utero with no secondary causes such as pituitary tumor, radiation therapy, trauma, and et al. GnRH deficiency results in hypothalamic–pituitary–gonadal (HPG) axis hypofunction, low levels of sex steroids, delayed/incomplete or absent puberty, sexual immaturity, and infertility (Dwyer, Raivio, & Pitteloud, 2015). The clinical characteristics vary depending on the severity of the deficiency and deformity or dysfunction of other organs. The main treatments for men include pulsatile GnRH, gonadotropin injections, and testosterone replacement. Androgenization and a normal/subnormal level of serum testosterone can

be achieved with testosterone replacement, but fertility requires treatment with gonadotropins or pulsatile GnRH (King & Hayes, 2012).

There is no consensus regarding the optimal approach for restoring fertility in CHH men. The subcutaneous pulsatile gonadorelin (a GnRH analog) pump (PGP) was first reported at the annual meeting of the Chinese Society

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of Endocrinology in August 2010. Prior reports have examined various medications, treatment methods, treatment periods, and endpoints for gonadotropin therapy or pulsatile GnRH therapy. Dwyer et al. (2015) summarized data from a number of studies and reported that pulsatile GnRH seemed to have comparable outcomes with gonadotropin therapy, but the results were somewhat inconsistent. Schopohl (1993) compared pulsatile GnRH (4–16 μg every 2 hr) with continual human chorionic gonadotropin/human menopausal gonadotropin (HCG/HMG) therapy in 36 CHH patients. In the continual HCG/HMG therapy group, the initial HCG dosage was 1,000–2,500 IU two to three times per week for the first 2–3 months; then 150 IU HMG two to four times per week was added. Similar studies were carried out by a number of other authors (Dwyer et al., 2013; Schopohl, Mehlretter, von Zumbusch, Eversmann, & von Werder, 1991). These studies reported that GnRH led to a more rapid initiation of spermatogenesis than continual gonadotropin therapy, but the overall rates of inducing spermatogenesis were not different. A retrospective study conducted by Huang et al. (2015) in China compared pulsatile GnRH (10 μg of Gonadorelin every 90 min) with a continual HCG/HMG injection method (HCG 3,000 IU plus HMG 75 IU twice a week) in 92 CHH patients. The treatment period was 3–18 months. The study concluded that pulsatile gonadorelin induced higher and earlier spermatogenesis than continual HCG/HMG therapy.

Cyclical HCG/HMG therapy is less painful, less costly, and less cumbersome than the continual HCG/HMG method. Zhang et al. (2015) reported that the spermatogenesis effect of cyclical HCG/HMG therapy was not inferior to the continual gonadotropin regimen. However, there has been no published study comparing the effects of PGP and cyclical gonadotropin therapy (CGT).

The aim of this study was to conduct an open-label prospective investigation to compare the spermatogenesis effect of PGP and CGT in CHH men.

Subjects and Methods

Subjects

Subjects for this study were recruited from the outpatient clinic of the First Affiliated Hospital of Sun Yat-sen University, China, from March 2013 to February 2017. The enrollment criterion was azoospermic CHH men more than 16 years of age who desired spermatogenesis. CHH was confirmed according to the Chinese Consensus Statement on idiopathic hypogonadotropic hypogonadism (Dou, 2015). The main criteria included (a) micropenis or absent or delayed puberty development; (b) decreased serum testosterone level (<1 nmol/L); (c) decreased or normal follicle-stimulating hormone (FSH)

and luteinizing hormone (LH) levels; (d) normal prolactin level and thyroid and adrenal function; (e) exclusion of the acquired causes, systemic causes, and functional causes of HH, for example, pituitary adenomas or brain tumors, systemic diseases, hemochromatosis, and malnutrition. CHH is divided into two categories, anosmia or hyposmia (Kallmann syndrome [KS]) and normosmia (n-CHH). Associated nonreproductive phenotypes include cleft lip/palate, skeletal abnormalities, hearing impairment, renal agenesis, and mirror movement.

The exclusion criteria were (a) testicular insufficiency (testosterone level <1 ng/ml after HCG stimulation test); (b) cryptorchidism; (c) inability to provide informed consent or undergo follow-up tests.

WeChat communication was used for increasing the study compliance.

Study Design

This open-label study was approved by the Institutional Review Board and Ethics Committee of Sun Yat-sen University (approval number: [2013]C-112). Written informed consent was obtained from all participants. After a clear explanation of the two therapies, the participants were allowed to choose PGP or CGT. All participants underwent a 1-month washout period of no administration of sex hormones.

All participants underwent a complete physical examination and laboratory testing at enrollment and at follow-up visits of the first month and the third month, and then every 3 months until 24 months. Participants were allowed to discontinue follow-up visits earlier than 24 months if spermatogenesis occurred.

Treatments

Pulsatile GnRH therapy. In the PGP group, gonadorelin (a GnRH analog) was initially administered 10 μg every 90 min subcutaneously using a Hypophyseal Hormonal Infusion Pump (Microport, China). The needle was placed subcutaneously in the abdominal wall. The needle, reservoir, and connecting tube were changed every 3 days at home by the participants after having been given clear instructions. The dosage of gonadorelin was adjusted to maintain the normal serum levels of LH, FSH, and testosterone, which were monitored at each visit. Blood samples for the measurement of serum LH, FSH, and testosterone were taken 30 min after a pulsatile injection.

Cyclical gonadotropin (HCG/HMG) therapy. For CGT, HCG was initially injected intramuscularly for the first 3 to 6 months (2,000 IU three times per week) until the serum testosterone level was >3 ng/ml or

testicular volume reached 3 ml as determined by a Prader orchidometer. Then, HMG (75 IU three times per week) was added for 3 months, followed by HCG alone again for another 3 months, and then HCG + HMG for an additional 3 months cyclically. At each visit, blood samples for the measurement of serum testosterone were taken 2 days after an HCG injection. The initial dosage of HCG (2,000 IU three times a week) was decreased to twice or once a week if the serum testosterone level exceeded the upper normal range. The dosage of HMG (75 IU three times a week) was not adjusted. HCG and HMG injections were given at medical centers near the participants' residence.

Assessments

Participants were seen 1 month and 3 months after beginning therapy, and then every 3 months until either spermatogenesis was documented, their sexual partner became pregnant, or the 24-month time point was reached. Testicular volumes were measured at each visit using a Prader orchidometer. Testicular ultrasound examinations were done by a single sonographer to decrease bias (the testicular volume = $0.71 \times \text{length} \times \text{width} \times \text{height}$). Penis length was measured along the dorsum from the base to the end of the tip by a single investigator. The length of foreskin was not included.

At baseline, every subject underwent a triptorelin stimulation test. Serum LH levels were measured every 30 min for 2 hr after a subcutaneous injection of 100- μ g triptorelin. Peak LH values were recorded. All participants underwent an HCG stimulation test at baseline to rule out testicular insufficiency. A testosterone level <1 ng/ml at 72 hr after an intramuscular injection of 5,000 IU HCG was considered to indicate testicular insufficiency. In the HCG/HMG group, blood samples for serum testosterone levels were collected 2 days after an HCG injection. In the PGP group, blood samples for serum LH/FSH/testosterone measurement were collected 30 min after a gonadorelin pulsatile infusion.

Serum LH/FSH/testosterone levels were measured at the central laboratory of the hospital using a chemiluminescent microparticle immunoassay (ARCHITECT, Abbott). The normal reference range of serum testosterone was 1.58–8.77 ng/ml. Semen samples were collected at each visit after successful ejaculation via masturbation after about 7 days of abstinence. Semen samples were analyzed according to the World Health Organization (WHO) guidelines (Cooper et al., 2010).

Outcomes

The primary outcome was the time of qualified spermatogenesis (sperm density ≥ 0.1 million/ml). Other outcomes

included the testicular volume, penis length, serum testosterone level, and adverse events.

Statistical Analysis

The study sample size was the maximum number of participants who could be recruited from March 1, 2013, to March 1, 2015. Data were expressed as mean \pm standard deviation. Kaplan–Meier survival curves were used to analyze the times of spermatogenesis, and the two groups were compared with the log-rank test. Other variables between the two groups were compared with Student's *t*-test or Wilcoxon rank sum test if the data were not normally distributed. χ^2 analysis and no-inferior analysis were used in the comparison of the spermatogenesis rate between the two groups. Multivariate Cox proportional hazards regression analysis was used to examine potential factors influencing spermatogenesis. A *p* value $<.05$ was considered to indicate statistical significance. Missing data were not substituted with the estimated values.

Results

The first participant was recruited on March 2, 2013, and the trial ended on February 28, 2017. Twenty-eight males, 16 to 34 years old (11 with n-CHH, 17 with KS) met the criteria and were enrolled in the study. Ten participants selected PGP, and 18 selected CGT. All participants completed the 24-month follow-up or discontinued participation when spermatogenesis occurred.

Demographic Characteristics

The demographic and baseline clinical characteristics of the participants are shown in Table 1. There were no statistical differences between the two groups at baseline, except for prior treatment history. A positive prior treatment was defined as the use of HCG or testosterone for at least 3 months.

Therapy and Induction of Spermatogenesis

In the CGT group, the initial HCG-only injection was given for 3 months to nine subjects and 6 months to the other nine subjects. Before the first combination HCG/HMG administration, all of them had a serum testosterone level >3 ng/ml, or a testicular volume ≥ 3 ml. The HCG or HCG/HMG was then administered in the cyclical pattern described previously. Because the testosterone level exceeded the upper normal limit, the frequency of HCG was reduced to twice per week for 10 subjects, and for 2 of them it was further reduced to once weekly. For no subject was the dose increased.

Table 1. Baseline Characteristics of the Two Groups Treated With Cyclical HCG/HMG or Pulsatile GnRH.

	Cyclical HCG/HMG (n = 18)	Pulsatile gonadorelin (n = 10)	p
Age (years)	24 ± 5 (16–34)	27 ± 3 (23–32)	.194
Numbers of normosmic/Kallmann patients (cases)	8/10	4/6	.82
Nonreproductive system abnormalities (cases) ^a	7	2	.417
Prior treatment (cases) ^b	2	6	.011
Mean testis size (ml)			
Measured with Prader orchidometer	3.1 ± 1.9	4.9 ± 2.7	.064
Measured by ultrasound examination ^c	1.16 ± 0.80	2.37 ± 1.91	.204
Penile length (cm)	3.84 ± 1.03	4.48 ± 0.99	.196
Genital development, Tanner's stage (I/II/III/IV/V)	9/5/4/0/0	2/5/2/1/0	.243
Pubic hair development, Tanner's stage (I/II/III/IV/V)	9/5/4/0/0	5/3/2/0/0	.987
Serum total T (ng/ml)	0.45 ± 0.21	0.38 ± 0.26	.633
Peak LH level (IU/L)	3.09 ± 2.32	2.83 ± 2.01	.332

Note. GnRH = gonadotropin-releasing hormone; HCG = human chorionic gonadotropin; HMG = human menopausal gonadotropin; T = testosterone; LH = luteinizing hormone.

^aIncluded cleft lip and palate, color blindness, hearing impairment, skeletal deformity, and so forth. ^bIt was defined if the patient had prior received HCG or testosterone treatment for at least 3 months. ^cVolume = length × width × height × 0.71.

In the PGP group, gonadorelin was increased to 15 µg every 90 min because of a low testosterone level for one subject. The gonadorelin dose was also increased for another two subjects when the testosterone level dropped below the lower limit of normal. For one subject, gonadorelin was decreased to 3 µg every 90 min based on his LH, FSH, and testosterone levels.

Spermatogenesis was successfully induced in 9 subjects in the PGP group (90%) and in 15 subjects in the CGT group (83.3%), with the median spermatogenesis times of 6 months and 14 months, respectively. The Kaplan–Meier curves of time to spermatogenesis of the two groups are shown in Figure 1. The log-rank test revealed a significant difference between the two survival curves ($\chi^2 = 6.711$, $p = .01$). First spermatozoa were found 11 ± 8 months after starting HCG and 9 ± 7 months after starting HMG. χ^2 analysis indicated the two rates (90% PGP, 83.3% CGT) were not significantly different ($p = .548$). Power analysis of the noninferior test of the two independent proportions (90% of PGP, 83.3% of CGT) showed a very low power at 0.125. The power is much lower than 0.8, which means the statistic hypothesis that CGT (83.3%) was noninferior to PGP (90%) is insignificant.

One subject in each treatment group had a peak LH level <1 IU/L after triptorelin stimulation. Both had successful spermatogenesis. Five subjects successfully impregnated their female partners, and eventually all had healthy babies. The sperm concentrations were 5.18 (CGT), 5.5 (PGP), 10.1 (CGT), 25.7 (PGP), and 56.1 (PGP) million/ml just prior to conception. The partner of one of the subjects experienced two spontaneous abortions in subsequent pregnancies. The reasons are unknown.

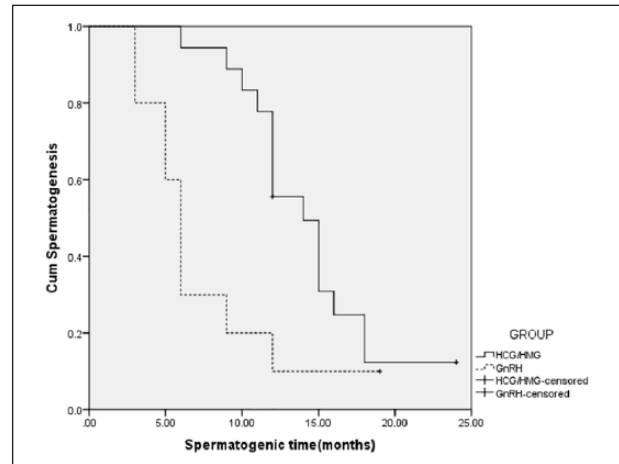


Figure 1. Survival curves of spermatogenesis for cyclical HCG/HMG and pulsatile GnRH therapies (log-rank test, $\chi^2 = 6.711$, $p = .01$).

GnRH = gonadotropin-releasing hormone; HCG = human chorionic gonadotropin; HMG = human menopausal gonadotropin.

Because some subjects stopped treatment when their partner became pregnant, or sperm was present in their ejaculate before 24 months, data of normal or peak sperm concentration were unavailable.

Contributing Factors for Spermatogenesis

In Cox proportional hazards regression analysis, the potential contributors for spermatogenesis were (a) prior treatment history, (b) baseline testosterone, (c) baseline testis volumes, (d) stimulated peak LH level at baseline, (e) nonreproductive system abnormalities, and (f) treatment method (PGP assigned a value of “1”; CGT assigned a value of “0”).

Table 2. Clinical Measurements of Male CHH Treated With Cyclical HCG/HMG or Pulsatile GnRH.

	Testicular volume (ml)		Penile lengths (cm)		Testosterone levels (ng/ml)	
	Cyclical HCG/ HMG	Pulsatile GnRH	Cyclical HCG/ HMG	Pulsatile GnRH	Cyclical HCG/ HMG	Pulsatile GnRH
Number (n)	18	10	18	10	18	10
Baseline	3.1 ± 1.9 (18) ^b [1.16 ± 0.80]	4.9 ± 2.7 (10) [2.37 ± 1.91]	3.84 ± 1.03 (18)	4.48 ± 0.99 (10)	0.45 ± 0.21 (18)	0.38 ± 0.26 (10)
1 month	4.3 ± 2.2 (18)	5.9 ± 2.4 (10)	4.50 ± 1.29 (18)	4.73 ± 1.09 (10)	4.28 ± 2.09 (18)*	2.92 ± 2.41 (10)*
3 months	5.7 ± 2.5 (18)* [2.03 ± 1.19]	7.9 ± 2.04 (10)* [4.26 ± 2.77]	5.17 ± 1.27 (18)*	5.44 ± 1.92 (10)	4.47 ± 2.54 (18)*	4.61 ± 3.56 (10)*
6 months	6.8 ± 2.6 (18)* [2.47 ± 1.21]*	8.8 ± 2.2 (10)* [4.91 ± 2.23]*	5.64 ± 1.54 (18)*	6.29 ± 2.07 (10)*	6.37 ± 4.08 (18)*	4.75 ± 2.67 (10)*
9 months	7.5 ± 2.5 (18)* [3.57 ± 1.62]*	9.5 ± 1.3 (7)* [5.38 ± 1.32]*	5.99 ± 1.67 (18)*	6.78 ± 2.37 (7)*	6.04 ± 3.87 (18)*	5.13 ± 3.18 (7)*
12 months	8.3 ± 2.6 (16)* [3.87 ± 1.92]*	10.6 ± 1.2 (5)* [5.98 ± 1.11]*	6.32 ± 1.63 (16)*	7.08 ± 2.79 (5)*	7.54 ± 4.78 (16)*	2.67 ± 2.15 (5)*
15 months	8.9 ± 2.8 (14)* [4.64 ± 2.43]*	10.8 ± 1.1 (4)* [6.03 ± 1.70]*	6.83 ± 1.66 (14)*	7.50 ± 3.377 (4)*	7.57 ± 5.31 (14)*	2.09 ± 1.02 (4)*
18 months	10.6 ± 2.1 (9)* [4.73 ± 1.79]*	10.8 ± 1.7 (2)* [6.61 ± 2.58]*	7.70 ± 1.24 (9)*	8.75 ± 5.30 (2)*	7.59 ± 4.03(9)*	0.76 ± 0.63 (2)
21 months	10.3 ± 2.3 (7)* [5.07 ± 2.35]*	11.7 ± 2.5 (2)* [6.84 ± 2.23]*	7.76 ± 1.06 (7)*	9.00 ± 4.95 (2)*	8.44 ± 4.02 (7)*	4.97 ± 2.21 (2)*
24months	10.8 ± 2.4 (6)* [5.44 ± 2.48]*	12.3 ± 1.7 (2)* [6.92 ± 2.46]*	7.93 ± 0.92 (6)*	9.00 ± 4.95 (2)*	8.79 ± 5.22 (6)*	4.71 ± 2.10 (2)*

Note. Values are mean ± SD. CHH = congenital hypogonadotropic hypogonadism; GnRH = gonadotropin-releasing hormone; HCG = human chorionic gonadotropin; HMG = human menopausal gonadotropin.

^aTesticular volume was measured using Prader orchidometer. The figures in the square brackets (mean ± SD) represented the results of ultrasound examinations (volume = 0.71 × length × width × height). ^bThe figures in the brackets represented the number of patients having follow-up visits during the corresponding period.

*Statistically significant difference ($p < .05$) of each follow-up visit compared with the baseline.

Factors that were found to be significant for spermatogenesis were prior treatment history, peak LH level, and treatment method ($p = .04, .022, .038$, respectively).

Serum Testosterone Level, Testicular Size, and Penile Length

The bilateral testicular volume and mean penile length increased significantly from baseline in both groups. However, no significant differences in either measure were found between the two groups (Table 2).

Serum testosterone levels increased and were maintained within the normal range during the treatment in both groups (with dosage adjustments in some subjects). However, the serum testosterone levels in the CGT group fluctuated more and tended to be higher than those in the PGP. Serum testosterone levels seemed to increase more in the CGT group at the times when both hormones were administered, but no statistical significance was observed. Because of high serum testosterone levels, HCG injections were reduced to twice weekly for 10 subjects, and for 2 of them, injections were reduced to once weekly.

Table 3. Adverse Events of the Two Groups.

Cases	Cyclical HCG/ HMG (n = 18)	Pulsatile gonadorelin (n = 10)
Acne*	7	2
Skin red induration*	0	9
Breast tenderness or gynecomastia development	5	2

Note. HCG = human chorionic gonadotropin; HMG = human menopausal gonadotropin.

* $p < .05$.

Safety and Tolerability

Adverse events were reported quite often (Table 3). Facial acne was more common in the CGT group. Red induration around the needle placement point was reported in some subjects in the PGP group. A micro-abscess occurred at the needle site in one subject. The skin side effects gradually weakened and disappeared. At baseline, six subjects in each group reported painless gynecomastia. Two subjects in the PGP group and five in the CGT group complained of breast tenderness or further breast

enlargement after treatment (20% vs. 27.8%), with greater severity in the CGT group. Breast tenderness usually disappeared 3 to 9 months after continuation of treatment. Overall, the treatments were tolerated well by all subjects in both groups.

Discussion

There is no consensus regarding the optimal method to restore fertility in CHH men. Because of the rarity of the condition, only a few studies have compared the effects of pulsatile GnRH with gonadotropin therapy. Dwyer et al. (2015) reported wide variations in the medications, regimes, treatment periods, and endpoints of pulsatile GnRH or gonadotropin therapies between centers. In studies by Christiansen and Skakkebaek (2002) and Delemarre-van de Waal (2004), the initial dosage of GnRH was 5 to 10 μg every 90 or 120 min and was increased until the serum testosterone level was normal. The dose and frequency of HCG/HMG administration also vary between studies; generally, 1,000–2,500 IU HCG was given two to three times a week and 75–150 IU HMG was given two to four times a week. A number of authors (Burgues & Calderon, 1997; Dwyer et al., 2015; King & Hayes, 2012; Rastrelli, Corona, Mannucci, & Maggi, 2014) reported monotherapy with HCG was given for 3–6 months before HMG was added, then continual HCG + HMG, or intermittent HCG \pm HMG was given. Dwyer et al. (2013) described a sequential gonadotropin protocol in which recombinant FSH (rFSH) pretreatment was given prior to rFSH + HCG or prior to pulsatile GnRH. The sequential protocol was more successful in inducing testicular growth and fertility. Another sequential protocol reported was HCG alone (2,000 IU two times a week) for the first 3 months, and urine FSH (uFSH; 75 IU three times a week) added every other 3 months (Zhang et al., 2015). The authors compared the later sequential method with continual HCG + uFSH treatment and found that sequential HCG/uFSH was not inferior to continual treatment with respect to the time of initial spermatogenesis and the overall rate of spermatogenesis. The sequential HCG/uFSH method should be referred to as a cyclical therapy in order to differentiate it from the concept of rFSH pretreatment described by Dwyer et al. (2013).

Why were the enrolled subjects over 16 years old? The main treatment goal for a young CHH male under 16 years old is not spermatogenesis. In this study, all participants were over 16 years old and desired spermatogenesis. All the participants communicated closely with the investigators, and their increased testosterone levels indicated good adherence and compliance in this study.

Survival analysis showed that PGP was significantly more effective than CGT for inducing spermatogenesis earlier, with a median time to spermatogenesis in the PGP

group of 6 months versus 14 months in the CGT group ($p = .01$). However, the difference of spermatogenesis rates of the two therapies did not provide conclusive evidence that one therapy was better than, or equal to, the other. The reason for the statistic insignificance of the χ^2 analysis and the no-inferior analysis was the small number of participants. For a superiority analysis, the sample size was calculated to be 505 cases in each group, under the conditions of a margin of zero, an effective rate of 83.3% in the CGT group, an effective rate of 90% in the PGP group, and an expulsion rate of 20%. For the no-inferior analysis, the sample size was estimated to be 2,079 cases in each group, under the conditions of a margin of 10%, an effective rate of 83.3% in the CGT group, an effective rate of 90% in the PGP group, and an expulsion rate of 20%. Such a big sample size is hard to achieve for CHH. However, the p value of $<.05$ in the Kaplan–Meier survival analysis suggested the sample size was sufficient for the statistical conclusion of the primary outcome, spermatogenesis time.

Normal or peak sperm concentration data were unavailable because some subjects terminated the study when their partner became pregnant or sperm was present in the ejaculate before 24 months. Although most subjects did not achieve a lower normal sperm concentration of 15 million/ml, a low sperm concentration does not preclude conception. Currently, advanced in vitro fertilization techniques have realized the dream of “one sperm one baby.” As such, the primary outcome of this study was defined as a sperm density ≥ 0.1 million/ml, not ≥ 1 million/ml. Five subjects impregnated their wives naturally. Their sperm concentrations were above 5 million/ml before the pregnancy conceived. All five pregnancies resulted in healthy babies.

King and Hayes (2012) and Liu et al. (2009) reported that factors favoring spermatogenesis included larger pretreatment testicular volume and prior gonadotropin therapy. A number of other authors suggested the unfavorable factors include cryptorchidism and previous androgen therapy (Christiansen & Skakkebaek, 2002; King & Hayes, 2012; Liu et al., 2009; Pitteloud et al., 2002). Waaler, Thorsen, Stoa, and Aarskog (1974) reported that baseline testis volume was an indication of the spectrum of gonadotropin deficiency. They considered a testicular volume <4 ml to indicate complete gonadotropin deficiency, while a testicular volume of ≥ 4 ml was an indication of partial gonadotropin deficiency. Ishikawa, Ooba, Kondo, Yamaguchi, and Fujisawa (2007) reported that a large testis predicted a better outcome for sperm analysis in response to gonadotropin therapy. Flanagan and Lehtihet (2015) concluded that long-term pretreatment with testosterone could suppress regaining normal gonadal function. On the other hand, Ley and Leonard (1985) reported successful stimulation of spermatogenesis was not adversely affected by prior androgen treatment. Based on these prior works, six potential factors

that might influence spermatogenesis were chosen for the Cox proportional hazards regression analysis. The analysis indicated that significant factors predicting spermatogenesis included prior treatment history (HCG or testosterone) and peak LH level at baseline, which also indicates better testes development at baseline. Cryptorchidism, a strong unfavorable predictor of spermatogenesis (Dwyer et al., 2015), was not examined because none of subjects in this study had cryptorchidism.

A few subjects remained azoospermic, although the two therapies provided an overall satisfactory spermatogenesis rate of 85.7%. For subjects who responded poorly, co-treatment with growth hormone (GH) could be given. Shoham et al. (1992) reported that co-treatment with GH promoted testosterone secretion and sperm production. A prolonged treatment may also improve the spermatogenesis rate. CHH is genetically heterozygous, with about 40% of cases attributed to mutations in the identified genes. Studies have indicated that subjects harboring mutations in *KALI* tend to respond poorly to treatment (Maione et al., 2018; Sykiotis et al., 2010). Some authors have suggested that genetic screening could help guide clinical decisions and assess the potential fertility of CHH patients (Maione et al., 2018; Pitteloud, Durrani, Raivio, & Sykiotis, 2010).

Both therapies induced enlargement of the testis, as reported in other studies (Delemarre-van de Waal, 2004; Ley & Leonard, 1985; Schopohl, 1993; Schopohl et al., 1991). The testicular volumes, measured by Prader orchidometer or ultrasound, increased significantly from pre-treatment levels. However, there was no significant difference in the increase between the two methods. Dimensional measurements acquired from ultrasound provide more compelling proof for enlargement of testis, although size measured on ultrasound seemed smaller than the size obtained with the Prader orchidometer. Sakamoto, Saito, Ogawa, and Yoshida (2007) reported similar results regarding size measured by ultrasound and Prader orchidometer.

Testosterone levels were increased and maintained within the normal range in the two treatment groups. PGP provides a simulated physiological secretion pattern of GnRH and has the potential to establish a biochemical feedback loop. In the CGT group, serum testosterone levels fluctuated more than in the PGP group. Serum testosterone levels tended to be higher when HMG was added than when HCG alone was given. The HCG dose was carefully adjusted in order to keep the testosterone level within the normal range. In the PGP, the gonadorelin dose was increased for three subjects and decreased for one in order to maintain a normal testosterone level. The dose-titrating method was based on serum FSH, LH, and testosterone levels. A reduced sensitivity to the pulsatile GnRH was noticed after a period of administration. Delemarre-van de Waal (2004) reported that the secretion of

gonadotropin hormones could be restored after a transient drug withdrawal or increasing the pulsatile GnRH dose.

The testosterone level was significantly higher in the CGT group, presumably due to overstimulation (Schopohl et al., 1991). Subjects in the CGT group experienced more facial acne, which may be explained by higher testosterone levels. Pulsatile GnRH therapy induces gonadotropin secretion in a physiological pattern, and subsequently testosterone levels are stable. Skin allergic reactions, such as indurative erythema, was a common problem in the PGP group.

Dwyer et al. (2015) noticed that gynecomastia was a common side effect reported in one third of gonadotropin-treated subjects. The rate was similar in this study. Ley and Leonard (1985) reported no adverse reactions with gonadotropin therapy, and Schopohl (1993) reported no gynecomastia with GnRH therapy. Dwyer et al. (2015) believed gonadotropin treatment results in gynecomastia because it resulted in higher testosterone levels, but also higher estradiol levels. Unfortunately, estradiol levels were not monitored during this study.

None of the subjects in either group had a normal testosterone level or reversal of spermatogenesis after they discontinued treatment (data not shown). The reasons might be the 2-year study period was not long enough or the participants had serious sex organ deformities, for example, very small testis at baseline.

The nonrandom design is a weakness of this study. However, this prospective study certainly collected real-world experience and useful information for clinicians in treating CHH cases. The participants self-selected the treatment method because the treatment expenses were paid by the subjects. The PGP subjects were very motivated, although the pump was inconvenient and the treatment cost was high. The high motivation in this group might have contributed to the favorable PGP results. Serum levels of inhibin B and anti-Müllerian hormone (AMH) were not evaluated because measurements were not available at our laboratory when the study started. Serum levels of inhibin B and AMH are currently used as markers of gonadal function, and their value in CHH should be further studied (Boehm et al., 2015). Another weakness is that the Tanner scale data were not recorded at follow-up visit.

Conclusion

The results of this study showed PGP induced spermatogenesis significantly sooner than CGT did, with median spermatogenesis times of 6 months and 14 months, respectively. Spermatogenesis occurred in 90% of PGP subjects and 83.3% of CGT subjects. Factors that favorably influenced spermatogenesis were prior treatment with sex hormones and the peak LH level after luteinizing hormone-releasing hormone (LHRH) stimulation test at baseline. Both therapies significantly increased testis

volume and penile length. CGT was associated with high and fluctuating testosterone levels, and facial acne and breast tenderness. The skin allergic reaction was a common side effect of PGP.

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Ethical Issues and Disclosures

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Institutional Review Board and Ethical Committee of Sun Yat-sen University. Informed consent was obtained from all individual participants included in the study. The authors declare that they have no conflict of interest.

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References

- Boehm, U., Bouloux, P. M., Dattani, M. T., de Roux, N., Dode, C., Dunkel, L., ... Young, J. (2015). Expert consensus document: European consensus statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. *Nature Reviews Endocrinology*, *11*(9), 547–564. doi:10.1038/nrendo.2015.112
- Burgues, S., & Calderon, M. D. (1997). Subcutaneous self-administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotropic hypogonadism. Spanish collaborative group on male hypogonadotropic hypogonadism. *Human Reproduction*, *12*(5), 980–986.
- Christiansen, P., & Skakkebaek, N. E. (2002). Pulsatile gonadotropin-releasing hormone treatment of men with idiopathic hypogonadotropic hypogonadism. *Hormone Research in Paediatrics*, *57*(1–2), 32–36. doi:10.1159/000057944
- Cooper, T. G., Noonan, E., von Eckardstein, S., Auger, J., Baker, H. W., Behre, H. M., ... Vogelsong, K. M. (2010). World Health Organization reference values for human semen characteristics. *Human Reproduction Update*, *16*(3), 231–245. doi:10.1093/humupd/dmp048
- Delemarre-van de Waal, H. A. (2004). Application of gonadotropin releasing hormone in hypogonadotropic hypogonadism—diagnostic and therapeutic aspects. *European Journal of Endocrinology*, *151*(Suppl. 3), U89–U94.
- Dou, J. (represented the Gonadal experts' team of Chinese Endocrinology Association). (2015). Expert consensus on diagnosis and treatment of idiopathic hypogonadotropic hypogonadism. *Chinese Journal of Chemistry*, *54*(8), 739–744. doi:10.3760/cma.j.issn.0578-1426.2015.08.021
- Dwyer, A. A., Raivio, T., & Pitteloud, N. (2015). Gonadotrophin replacement for induction of fertility in hypogonadal men. *Best Practice & Research Clinical Endocrinology & Metabolism*, *29*(1), 91–103. doi:10.1016/j.beem.2014.10.005
- Dwyer, A. A., Sykiotis, G. P., Hayes, F. J., Boepple, P. A., Lee, H., Loughlin, K. R., ... Pitteloud, N. (2013). Trial of recombinant follicle-stimulating hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. *The Journal of Clinical Endocrinology & Metabolism*, *98*(11), E1790–E1795. doi:10.1210/jc.2013-2518
- Flanagan, J. N., & Lehtihet, M. (2015). The response to Gonadotropin-Releasing Hormone and hCG in men with Prior Chronic Androgen Steroid Abuse and clinical hypogonadism. *Hormone and Metabolic Research*, *47*(9), 668–673. doi:10.1055/s-0034-1398492
- Huang, B., Mao, J., Xu, H., Wang, X., Liu, Z., Nie, M., & Wu, X. (2015). Spermatogenesis of pulsatile gonadotropin-releasing hormone infusion versus gonadotropin therapy in male idiopathic hypogonadotropic hypogonadism. *Zhonghua Yi Xue Za Zhi*, *95*(20), 1568–1571.
- Ishikawa, T., Ooba, T., Kondo, Y., Yamaguchi, K., & Fujisawa, M. (2007). Assessment of gonadotropin therapy in male hypogonadotropic hypogonadism. *Fertility and Sterility*, *88*(6), 1697–1699. doi:10.1016/j.fertnstert.2006.11.022
- King, T. F., & Hayes, F. J. (2012). Long-term outcome of idiopathic hypogonadotropic hypogonadism. *Current Opinion in Endocrinology & Diabetes and Obesity*, *19*(3), 204–210. doi:10.1097/MED.0b013e328353565b
- Ley, S. B., & Leonard, J. M. (1985). Male hypogonadotropic hypogonadism: Factors influencing response to human chorionic gonadotropin and human menopausal gonadotropin, including prior exogenous androgens. *The Journal of Clinical Endocrinology & Metabolism*, *61*(4), 746–752. doi:10.1210/jcem-61-4-746
- Liu, P. Y., Baker, H. W., Jayadev, V., Zacharin, M., Conway, A. J., & Handelsman, D. J. (2009). Induction of spermatogenesis and fertility during gonadotropin treatment

- of gonadotropin-deficient infertile men: predictors of fertility outcome. *The Journal of Clinical Endocrinology & Metabolism*, 94(3), 801–808. doi:10.1210/jc.2008-1648
- Maione, L., Dwyer, A. A., Francou, B., Guiochon-Mantel, A., Binart, N., Bouligand, J., & Young, J. (2018). GENETICS IN ENDOCRINOLOGY: Genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: New challenges in the era of oligogenism and next-generation sequencing. *European Journal of Endocrinology*, 178(3), R55–R80. doi:10.1530/EJE-17-0749
- Pitteloud, N., Durrani, S., Raivio, T., & Sykiotis, G. P. (2010). Complex genetics in idiopathic hypogonadotropic hypogonadism. *Frontiers of Hormone Research*, 39, 142–153. doi:10.1159/000312700
- Pitteloud, N., Hayes, F. J., Dwyer, A., Boepple, P. A., Lee, H., & Crowley, W. F., Jr. (2002). Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. *The Journal of Clinical Endocrinology & Metabolism*, 87(9), 4128–4136. doi:10.1210/jc.2002-020518
- Rastrelli, G., Corona, G., Mannucci, E., & Maggi, M. (2014). Factors affecting spermatogenesis upon gonadotropin-replacement therapy: A meta-analytic study. *Andrology*, 2(6), 794–808. doi:10.1111/andr.262
- Sakamoto, H., Saito, K., Ogawa, Y., & Yoshida, H. (2007). Testicular volume measurements using Prader orchidometer versus ultrasonography in patients with infertility. *Urology*, 69(1), 158–162. doi:10.1016/j.urology.2006.09.013
- Schopohl, J. (1993). Pulsatile gonadotrophin releasing hormone versus gonadotrophin treatment of hypothalamic hypogonadism in males. *Human Reproduction*, 8(Suppl. 2), 175–179.
- Schopohl, J., Mehlretter, G., von Zumbusch, R., Eversmann, T., & von Werder, K. (1991). Comparison of gonadotropin-releasing hormone and gonadotropin therapy in male patients with idiopathic hypothalamic hypogonadism. *Fertility and Sterility*, 56(6), 1143–1150.
- Shoham, Z., Conway, G. S., Ostergaard, H., Lahlou, N., Bouchard, P., & Jacobs, H. S. (1992). Cotreatment with growth hormone for induction of spermatogenesis in patients with hypogonadotropic hypogonadism. *Fertility and Sterility*, 57(5), 1044–1051.
- Sykiotis, G. P., Hoang, X. H., Avbelj, M., Hayes, F. J., Thambundit, A., Dwyer, A., ... Pitteloud, N. (2010). Congenital idiopathic hypogonadotropic hypogonadism: Evidence of defects in the hypothalamus, pituitary, and testes. *The Journal of Clinical Endocrinology & Metabolism*, 95(6), 3019–3027. doi:10.1210/jc.2009-2582
- Waler, P. E., Thorsen, T., Stoa, K. F., & Aarskog, D. (1974). Studies in normal male puberty. *Acta Paediatrica*, 63(249), 1–36.
- Zhang, M., Tong, G., Liu, Y., Mu, Y., Weng, J., Xue, Y., ... Li, X. (2015). Sequential versus continual purified urinary FSH/hCG in men with idiopathic hypogonadotropic hypogonadism. *The Journal of Clinical Endocrinology & Metabolism*, 100(6), 2449–2455. doi:10.1210/jc.2014-3802