Endocrine Practice 27 (2021) 1119–1127

Contents lists available at ScienceDirect

Endocrine Practice

journal homepage: www.endocrinepractice.org

Original Article

Gonadotropin Therapy Once a Week for Spermatogenesis in Hypogonadotropic Hypogonadism

Wanlu Ma, MD [1], Jiangfeng Mao, MD [1], Min Nie, PhD [1], Xi Wang, MD [1], Junjie Zheng, MD [1], Zhaoxiang Liu, MD [2], Bingqing Yu, MD [1], Shuyu Xiong, MD [1], Ming Hao, MD [1], Yinjie Gao, MD [1], Wen Ji, MD [1], Qibin Huang, MD [1], Rui Zhang, MD [1], Shuying Li, MD [1], Yaling Zhao, MD [1], Bang Sun, MD [1], Xueyan Wu, MD [1,*]

[1] Department of Endocrinology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China
[2] Department of Endocrinology, Beijing Tsinghua Chang Gung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, China

ARTICLE INFO

Article history:
Received 5 January 2021
Accepted 19 April 2021
Available online 26 April 2021

Key words:
gonadotropin treatment
hypogonadotropic hypogonadism
spermatogenesis

ABSTRACT

Objective: Hypogonadotropic hypogonadism (HH) can be caused by congenital HH (CHH), pituitary stalk interruption syndrome (PSIS), and pituitary injury (acquired HH). Gonadotropin therapy, typically administered every other day or twice a week, is commonly used to promote spermatogenesis. The aim of this retrospective study was to evaluate the efficacy of weekly gonadotropin therapy on spermatogenesis in patients with HH ($n = 160$).

Methods: The patients' diagnoses include Kallmann syndrome (KS) ($n = 61$), normosmic CHH (nCHH) ($n = 34$), PSIS ($n = 48$), and acquired HH ($n = 17$). The rate of successful spermatogenesis and median time to achieve spermatogenesis among these 4 subgroups were compared as well as between a weekly group ($n = 95$) and a twice-a-week group ($n = 223$) of CHH patients.

Results: Once-a-week gonadotropin therapy resulted in 74% (119/160) of HH patients achieving spermatogenesis with significantly increased testicular volume and total testosterone levels ($P < .001$). The median period of spermatogenesis was 13 (interquartile range[IQR] 11.4-14.6) months. Larger basal testicular volume ($P = .0142$) was an independent predictor for earlier sperm appearance. Six spontaneous pregnancies occurred. Compared with the twice-a-week regimen for spermatogenesis, the weekly injection group had a similar median time of sperm appearance (14 [IQR, 11.6-16.4] vs 15 [IQR, 13.5-16.5] months), success rate (78% [74/95] vs 64% [143/223]), sperm concentration (20.9 [IQR, 5.0-46.3] vs 11.7 [IQR, 2.1-24.4] million/mL), and progressive sperm motility (40.8 ± 27.3% vs 36.9% ± 20.2%).

Conclusion: Weekly gonadotropin therapy is effective in inducing spermatogenesis, similar to that of twice-a-week therapy. A larger basal testicular size was a favorable indicator for earlier spermatogenesis.

Introduction

Infertility is a global issue that occurs at a prevalence of 15%, of which 50% to 60% of cases are males.[1] Male hypogonadism may result in failure to produce normal physiologic concentrations of testosterone and/or sperm. Hypogonadotropic hypogonadism (HH), one of few types of hypogonadism that could be treated, includes congenital HH (CHH), congenital hypopituitarism, and acquired HH.[2]

CHH is a genetic disorder caused by gonadotropin-releasing hormone (GnRH) deficiency and/or resistance and is divided into normosmic CHH (nCHH) and Kallmann syndrome (KS) based on the presence of olfactory disorders.[3,4] Congenital hypopituitarism predominantly consists of pituitary stalk interruption syndrome (PSIS), in which multiple pituitary hormones are deficient. Acquired HH results from tumors, radiation, infiltrative diseases, apoplexy, surgery, head trauma, and subarachnoid hemorrhage.[2]

Pulsatile GnRH and gonadotropin therapy may promote testicular development, testosterone synthesis, and spermatogenesis.

Abbreviations: BMI, body mass index; CHH, congenital hypogonadotropic hypogonadism; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; HMG, human menopausal gonadotropin; IQR, interquartile range; KS, Kallmann syndrome; LH, luteinizing hormone; MRI, magnetic resonance imaging; nCHH, normosmic CHH; PSIS, pituitary stalk interruption syndrome; $t_{1/2}$, half-life; TT, total testosterone; WHO, World Health Organization.

* Address correspondence to Dr Xueyan Wu, Department of Endocrinology, Peking Union Medical College Hospital, No 1 Shuaifuyuan, Dongcheng District, Beijing 100730, China.
E-mail address: wsheyan@vip.sina.com (X. Wu).

https://doi.org/10.1016/j.eprac.2021.04.009
1530-891X/

Studies have confirmed that the combined therapy of human chorionic gonadotropin (HCG) and human menopausal gonadotropin (HMG), 2 or 3 times a week, had an overall success rate of 75% to 85% in achieving spermatogenesis.[5-7] No guidelines on the regimens of gonadotropin therapy have been agreed upon. Typical doses for HCG vary from 500 to 2500 IU, whereas HMG varies from 75 to 225 IU two to three times a week.[8-10]

Twice-a-week injections may be inconvenient for young adults occupied with school and work on weekdays, especially for students living in school dormitories. Thus, injection of gonadotropin once a week on weekends would be more convenient and manageable, especially for young adults. It may also reduce the psychologic pressure of being noticed by peers. However, it is unclear whether gonadotropin injection once-a-week would be effective in inducing spermatogenesis. Drugs with a long half-life ($t_{1/2}$), such as long-acting recombinant growth hormone ($t_{1/2}$ = 34.0 ± 8.1 h)[11,12] and dulaglutide ($t_{1/2}$=4.7-5.5 d),[13] are administered once a week. After intramuscular injection, the $t_{1/2}$ of serum HCG is 31 ± 3 hours, and that of HMG is 37 h. The serum testosterone peak appears at 72 hours after HCG injection,[14] suggesting gonadotropin treatment could be provided once a week. Therefore, this study evaluated the efficacy of once-a-week HMG/HCG therapy on spermatogenesis of male HH patients.

Methods

Patients

Male HH patients were included in this retrospective study. Before treatment, they had not received pulsatile GnRH therapy and were azoospermic. The inclusion criteria were as follows:

1. Criteria for CHH: men aged >18 years without pubertal development, total testosterone (TT) <100 ng/dL (3.5 nmol/L) with low or normal levels of gonadotropins,[5] normal levels of other pituitary hormones, and negative findings in sellar magnetic resonance imaging (MRI). No systemic diseases that could cause hypogonadism were detected.
2. Criteria for PSIS: pituitary MRI suggests pituitary dysplasia, and/or pituitary stalk interruption, and/or ectopic posterior pituitary; no history of trauma, inflammation, or tumors; laboratory tests show multiple pituitary hormone deficiencies, including hypogonadism (TT <100 ng/dL).
3. Criteria for acquired HH: defects of multiple pituitary and/or posterior pituitary hormones, including hypogonadism (TT <100 ng/dL), and history of trauma, inflammation, tumor, surgery, or radiotherapy in the sellar region. The specific diagnosis and initial age at diagnosis and treatment of the 17 patients with acquired HH were included in the analysis (Supplementary Table 1).

From January 2005 to September 2019, a total of 542 male HH patients seeking fertility were treated with HCG/HMG. We excluded 159 cases due to poor compliance or incomplete medical data. We analyzed and compared 4 subgroups (KS, nCHH, PSIS, and acquired HH). The spermatogenesis outcomes were compared between 95 CHH patients treated with HCG/HMG once a week and 223 CHH patients treated with HCG/HMG twice a week (The data of the 223 CHH patients treated with HCG/HMG twice a week were retrospectively extracted from our previous study[15]).

Clinical presentation, cryptorchidism, medical history, and family history were recorded. Serum gonadotropins and testosterone levels were evaluated before and during treatment with HCG/HMG. MRIs of the pituitary gland and olfactory nerve were performed. The testicular volume and the median period of achieving sperm production were recorded. Inhibin B levels could not be assessed in our hospital.

Study Approval and Patient Consent

This study was approved by the Institutional Review Board of Peking Union Medical College Hospital (SK1196). All procedures were performed in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from each patient after the purposes of both studies were fully explained.

Treatment and Follow-up

If used, patients discontinued androgen therapy for at least 3 months before starting gonadotropin therapy. For patients with congenital and acquired hypopituitarism, thyroid and adrenal hormones were returned to normal levels before HCG/HMG treatment. For patients with central diabetes insipidus, desmopressin use was continued during the spermatogenic treatment. HCG 5000 U and HMG 150 U (Livzon Pharmaceutical Co) were intramuscularly injected once a week, and dosing was the same throughout the study. Regular follow-up was conducted at an interval of 3 to 6 months during the therapy. For the twice-a-week week group, twice-weekly intramuscular injections of HCG (2000-3000 U) and HMG (75-150 U) were given for 6 months.

Luteinizing hormone (LH), follicle-stimulating hormone (FSH), testicular size, testosterone, and sperm count were measured at each visit. LH, FSH, and testosterone levels were measured by a chemiluminescent method using a commercial kit on an ACS: 180 Automated Chemiluminescence System (Bayer). Testosterone levels were measured 48 hours after HCG injection. The testicular size was measured using a Prader orchidometer, and the mean value of bilateral testicular volume was used in data analysis. Semen samples were collected by masturbation and analyzed according to the standard World Health Organization (WHO) method.[16] Sperm motility was classified as (A) fast progressive sperm, (B) slow progressive sperm, (C) nonprogressive sperm, or (D) immotile sperm. The proportion of each of the 4 motility categories was assessed.

Outcomes

The primary outcome was successful spermatogenesis, defined as the observation under microcopy of 1 sperm in concentrifuged seminal fluid. The period for spermatogenesis was from the start of HCG/HMG treatment to the time when sperm was first detected during the semen analysis.

Secondary outcomes were specific sperm concentrations and conception. Four sperm thresholds (>0 million/mL, any sperm that was observed under microscopy; >5 million/mL; >10 million/mL; and >15 million/mL) were recorded according to the sperm concentration. Self-reported pregnancy was noted. According to the WHO criteria, a sperm concentration >15 million/mL is above the reference range threshold for adult males.[16]

Statistical Analysis

SPSS v17.0 was used for data analysis (SPSS Inc). Normally distributed data were expressed as the mean ± SD, and nonnormally distributed data were reported as the median (interquartile range [IQR]). The paired *t* test was used to compare the difference in the plasma testosterone and testicular volume before and after the treatment. Kaplan-Meier analyses were used to estimate the median time for achieving different sperm thresholds. Cox

regression models were built to analyze the predictors of successful spermatogenesis. A multivariate linear regression model was built to identify the contributing factors to the time for patients to achieve spermatogenesis. The independent samples *t* test was used to compare the baseline differences between KS, nCHH, PSIS, and acquired HH as well as patients with spermatogenesis groups that were either successful or failed. The rates of cryptorchidism, cryptorchidism surgery, family history, and previous androgen and gonadotropin treatment among groups were compared by the X^2 test. Statistical significance was set at $P < .05$.

The age for starting HCG/HMG treatment, body mass index (BMI), peak LH after GnRH analog (triptorelin, 100 μg) stimulation, family history of delayed puberty (0 = no, 1 = yes), history of cryptorchidism (0 = no, 1 = unilateral, 2 = bilateral), basal testicular volume, and previous androgen exposure (0 = none or <3 months, 1 = >3 months) were considered as variables in the Cox regression and multivariate linear regression models.

Results

Baseline Evaluation of 160 HH Patients with Gonadotropin Treatment Once-a-Week

Out of 160 male HH patients who were retrospectively evaluated, 119 succeeded in achieving spermatogenesis. The mean follow-up period was 30.71 ± 17.29 months. Gonadotropin treatment was initiated at the median age of 22.06 ± 6.07 years. Baseline serum levels of LH, FSH, and testosterone were 0.35 ± 0.59 IU/L, 1.1 ± 1.74 IU/L, and 0.55 ± 1.16 nmol/L, respectively. The mean basal testicular volume was 3.10 ± 3.19 mL.

All patients were generally in good condition with normal routine blood and urine test results as well as normal liver and renal function. Thyroid hormones and adrenal glucocorticoid levels were all in the normal range or had been restored to the normal range. During the gonadotropin therapy, plasma testosterone increased from 0.55 ± 1.16 nmol/L to 4.55 ± 2.51 nmol/L ($P < .001$), and testicular volume increased from 3.10 ± 3.19 mL to 10.85 ± 5.10 mL ($P < .001$) (Table 1).

Baseline Feature Comparisons Between KS, nCHH, PSIS, and Acquired HH

Patients were divided into 4 subgroups according to etiology: KS ($n = 61$), nCHH ($n = 34$), PSIS ($n = 48$), and acquired HH ($n = 17$). KS and nCHH patients had a similar BMI, age at initiating treatment, peak LH level after triptorelin stimulation, basal testicular volume, and rate of cryptorchidism (Table 1).

Spermatogenesis Outcomes in All HH Patients

The success rate of spermatogenesis was 74% (119/160) in HH patients. Kaplan-Meier analysis showed that the median time for the first sperm appearance was 13 (IQR, 11-14) months, with an average testicular volume of 8.91 ± 4.93 mL. The median times to reach the sperm threshold of >5, >10, and >15 million/mL were 20 (IQR, 18-21), 24 (IQR, 17-30), and 29 (IQR 20-37) months, respectively (Fig. 2 and Table 2).

Spermatogenesis Outcome Comparison Among KS, nCHH, PSIS, and Acquired HH

The success rate of spermatogenesis was 74% (45/61), 85% (29/34), 69% (33/48), and 71% (12/17), respectively, in KS, nCHH, PSIS, and acquired HH (Fig.1 and Table 2). Kaplan-Meier analysis showed that the median time for the first sperm appearance was 15 (IQR, 11.4-18.6) months in KS,13 (IQR, 9-17) months in nCHH, 15 (IQR, 9.9-20.1) months in PSIS, and 13 (IQR, 11.5-14.5) months in acquired HH (Fig. 3 and Table 2).

Comparison Between Weekly and Twice-a-Week Gonadotropin Regimens

The baseline data between the weekly HCG/HMG-administered group of CHH patients ($n = 95$, KS/nCHH = 34/61) and the twice-a-week HCG/HMG-administered group of CHH patients ($n = 223$, KS/nCHH = 111/112) were compared in Table 3. The weekly group had a larger basal testicular volume, an earlier age at diagnosis and

Table 1
Comparison of Baseline Features of HH, KS, nCHH, PSIS, and Acquired HH Groups

Variables	HH ($n = 160$)	KS ($n = 61$)	nCHH ($n = 34$)	PSIS ($n = 48$)	Acquired HH ($n = 17$)	P[a]
Age at diagnosis, y	20.10 ± 5.97	19.03 ± 5.88	18.23 ± 5.33	22.67 ± 5.35	20.86 ± 7.23	.90
Age at treatment initiation, y	22.06 ± 6.07	21.18 ± 6.49	19.43 ± 3.58	24.76 ± 5.63	23.64 ± 7.19	.89
BMI, kg/m^2	22.91 ±3.86	23.11 ± 4.29	22.73 ± 3.34	21.74 ± 2.97	24.68 ± 4.38	.99
Basal testicular volume, mL	3.10 ± 3.19	2.59 ± 3.01	3.28 ± 2.52	3.00 ± 2.92	5.28 ± 5.53	.97
Testicular volume during therapy, mL	10.85 ± 5.10	10.00 ± 5.03	11.88 ± 5.42	10.50 ± 4.57	11.91 ± 4.68	.33
Baseline testosterone, nmol/L	0.55 ± 1.16	0.54 ± 1.12	0.78 ± 1.38	0.32 ± 0.64	0.72 ± 1.83	.93
Testosterone during therapy, nmol/L	4.55 ± 2.51	3.91 ± 2.27	4.95 ± 2.38	4.78 ± 2.74	5.60 ± 2.65	.53
Baseline LH, IU/L	0.35 ± 0.59	0.43 ± 0.69	0.43 ± 0.58	0.17 ± 0.28	0.37 ± 0.74	.99
Baseline FSH, IU/L	1.10 ± 1.74	1.02 ± 0.91	1.09 ± 1.23	1.37 ± 2.81	0.7 ± 1.28	.99
Peak LH, IU/L	3.35 ± 6.70	4.21 ± 8.71 ($n = 48$)	4.84 ± 5.89 ($n = 31$)	0.65 ± 1.06 ($n = 25$)	0.82 ± 1.58 ($n = 8$)	.99
LH after treatment, IU/L	0.51 ± 1.48	0.65±1.57	0.53 ± 0.95	0.44 ± 1.90	0.08 ± 0.14	.96
FSH after treatment, IU/L	4.03 ± 6.74	3.38 ± 2.61	3.4 ± 2.00	5.55 ± 12.05	3.84 ± 3.75	.99
Rate of cryptorchidism	8.13% ($n = 13$)	13.11% ($n = 8$)	11.76% ($n = 4$)	2.08% ($n = 1$)	0% ($n = 0$)	.56
Previous androgen	54.61% ($n = 83$)	46.67% ($n = 28$)	54.29% ($n = 19$)	60.47% ($n = 26$)	71.43% ($n = 10$)	.47
Previous gonadotropins	30.34% ($n = 44$)	36.84% ($n = 21$)	45.45% ($n = 15$)	17.07% ($n = 7$)	7.14% ($n = 1$)	.42
Rate of delayed pubertal family history	2.50% ($n = 4$)	6.56% ($n = 4$)	0% ($n = 0$)	0% ($n = 0$)	0% ($n = 0$)	.12
Follow-up, mo	30.71 ± 17.29	35.73 ± 17.38	27.73 ± 18.02	29.90 ± 15.95	19.64 ± 12.49	.06

Abbreviations: BMI = body mass index; FSH = follicle-stimulating hormone; HH = hypogonadotropic hypogonadism; KS = Kallmann syndrome; LH = luteinizing hormone; nCHH = normosmic congenital HH, PSIS, pituitary stalk interruption syndrome.
$P < .05$ was defined statistically significant.
[a] P value obtained from the comparison of KS with nCHH.

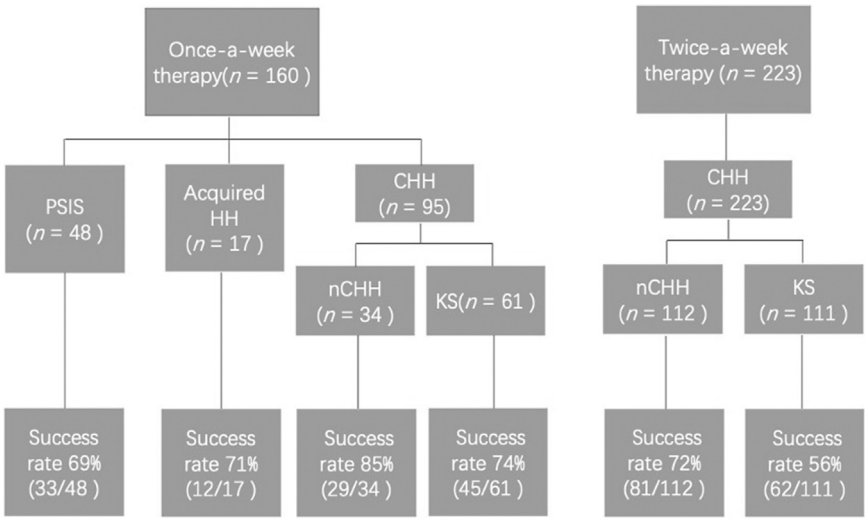

Fig. 1. Flowchart of spermatogenesis rate between the weekly and twice-a-week group. Success rate: rate of successful spermatogenesis. *CHH* = congenital HH; *HH* = hypogonadotropic hypogonadism; *KS* = Kallmann syndrome; *nCHH* = normosmic congenital hypogonadotropic hypogonadism; *PSIS* = pituitary stalk interruption syndrome.

initial treatment, a lower percentage of prior androgen treatment, and a higher percentage of previous gonadotropin treatment compared with the twice-a-week group (Table 3). The testosterone level after treatment was similar between the weekly and twice-a-week groups (4.54 ± 2.51 vs 4.86 ± 2.67, $P = .2622$).

The spermatogenesis rate of CHH was 78% (74/95) vs 64% (143/223) in the weekly and twice-a-week group, respectively ($P = .01$). In the weekly group, the median time for first sperm appearance was 14 (IQR, 11.6-16.4) months. The median time for sperm to reach >5, >10, and >15 million/mL was 24 (IQR, 13.8-34.2), 31 (IQR, 20.3-41.7), and 35 (IQR, 16-54) months, respectively. The proportion of CHH patients who achieved sperm concentrations >0, >5, >10, and >15 million/mL was 78% (74/95), 59% (56/95), 51% (48/95), and 43% (41/95), respectively. In the twice-a-week group, the median time of first sperm appearance was 15 (IQR, 13.5-16.5) months. The median time to reach a sperm concentration of >5, >10, and >15 million/mL

Table 2
Spermatogenesis Outcomes Among HH, KS, nCHH, PSIS, and Acquired HH Groups

Variable	HH ($n = 119$)	KS ($n = 45$)	nCHH ($n = 29$)	PSIS ($n = 33$)	Acquired HH ($n = 12$)	P1[a]	P2[b]	P3[c]	P4[d]	P5[e]	P6[f]
Sperm concentration, million/mL	18.1 (5, 46.2)	25.83 (5, 48.2)	14.36 (5.9, 34.8)	18.1 (9.3, 38.1)	6.86 (1.0, 51.8)	.96	.86	.94	.99	.99	.99
A+B% of sperm	0.41 ± 0.27	0.42 ± 0.27	0.38 ± 0.28	0.45 ± 0.23	0.33 ± 0.37	.96	.96	.80	.54	.73	.97
A+B+C% of sperm	0.45 ± 0.28	0.47 ± 0.29	0.41 ± 0.29	0.51 ± 0.23	0.34 ± 0.38	.90	.95	.65	.39	.59	.96
Patient proportion (sperm >0 million/mL)	74% (119/160)	74% (45/61)	85% (29/34)	69% (33/48)	71% (12/17)	…	…	…	…	…	…
Patient proportion (sperm >5 million/mL)	56% (90/160)	56% (34/61)	65% (22/34)	58% (28/48)	35% (6/17)	…	…	…	…	…	…
Patient proportion (sperm >10 million/mL)	48% (77/160)	46% (28/61)	59% (20/34)	48% (23/48)	35% (6/17)	…	…	…	…	…	…
Patient proportion (sperm >15 million/mL)	41% (65/160)	44% (27/61)	41% (14/34)	40% (19/48)	29% (5/17)	…	…	…	…	…	…
Testicular volume at first sperm appearance, mL	8.91 ± 4.93	7.90 ± 4.74	9.04 ± 5.09	10.14 ± 3.63	11.57 ± 5.94	.90	.81	.39	.98	.98	.74
Testicular volume at sperm >5 million/mL, mL	11.38 ± 4.84	11.44 ± 4.68	10.94 ± 5.34	10.60 ± 2.61	13.40 ± 6.15	.99	.99	.93	.99	.86	.89
Testicular volume at sperm>10 million/mL, mL	12.57 ± 5.05	12.07 ± 4.65	12.46 ± 5.94	13.33 ± 2.89	13.80 ± 5.76	.99	.99	.96	.99	.98	.99
Testicular volume at sperm >15 million/mL, mL	13.12 ± 4.45	12.83 ± 4.61	13.25 ± 4.29	13.33 ± 2.89	13.33 ± 5.99	.99	.99	.99	.99	.99	.99
Age at first sperm, y	21.74 ± 5.11	20.84 ± 4.67	20.36 ± 3.23	26.22 ± 5.07	24.67 ± 7.94	.99	.03	.24	.96	.01	.15
Pregnancy	5.04% ($n = 6$)	4.44% ($n = 2$)	0% ($n = 0$)	9.09% ($n = 3$)	5.88% ($n = 1$)	.40	.88	.88	.99	.99	.72

Abbreviations: HH = hypogonadotropic hypogonadism; KS = Kallmann syndrome; nCHH = normosmic congenital HH; PSIS = pituitary stalk interruption syndrome.
A: fast progressive sperm, B: slow progressive sperm, C: nonprogressive sperm.
A+B%: Proportion of sperm moving straight forward and rapidly among all the sperm, which represents sperm motility.
A+B+C%: Sperm activity rate.
$P < .05$ was defined statistically significant.
[a] *P* value obtained from the comparison of KS with nCHH.
[b] *P* value obtained from the comparison of KS with PSIS.
[c] *P* value obtained from the comparison of KS with acquired HH.
[d] *P* value obtained from the comparison of nCHH with PSIS.
[e] *P* value obtained from the comparison of nCHH with acquired HH.
[f] *P* value obtained from the comparison of PSIS and acquired HH.

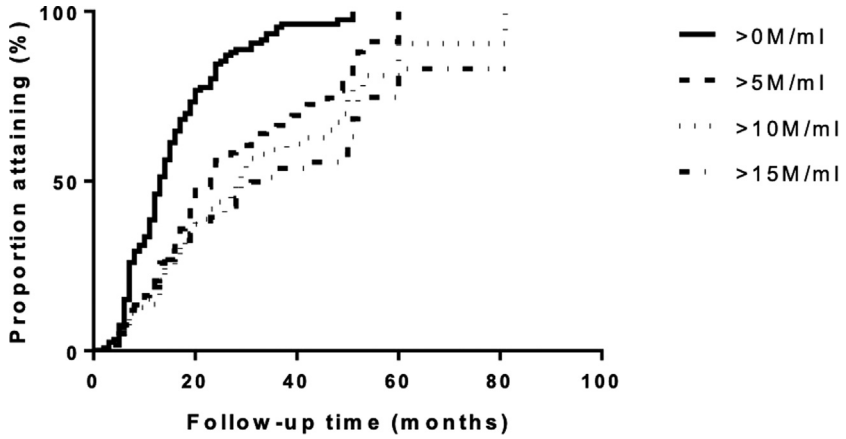

Fig. 2. Median times of achieving a sperm concentration at different thresholds in hypogonadotropic hypogonadism ($n = 119$).

was 27 (IQR, 19.7-4.3), 39 (IQR, 26.7-51.3), and 45 months, respectively. The proportion of CHH patients who achieved a sperm concentration of >0, >5, >10, and >15 million/mL was 64% (143/223), 37% (82/223), 30%(66/223), and 23% (51/223), respectively (Fig. 4).

The Spermatogenesis Outcome in CHH Patients With Cryptorchidism

In our study, out of the 9 patients of CHH with cryptorchidism that have achieved spermatogenesis, the median time for the emergence of sperm was 23.7 months. The average sperm concentration was 17.56 million/mL. In the spermatogenesis group, 7.56% [9/119] had cryptorchidism, while in the non-spermatogenesis group, 9.76% (4/41) had cryptorchidism (Supplementary Table 2).

Predictive Factors for Spermatogenesis in HH Patients

The Cox-related method used the periods of first sperm production as the variable and other factors (including age at initiating treatment, BMI, peak LH, family history,

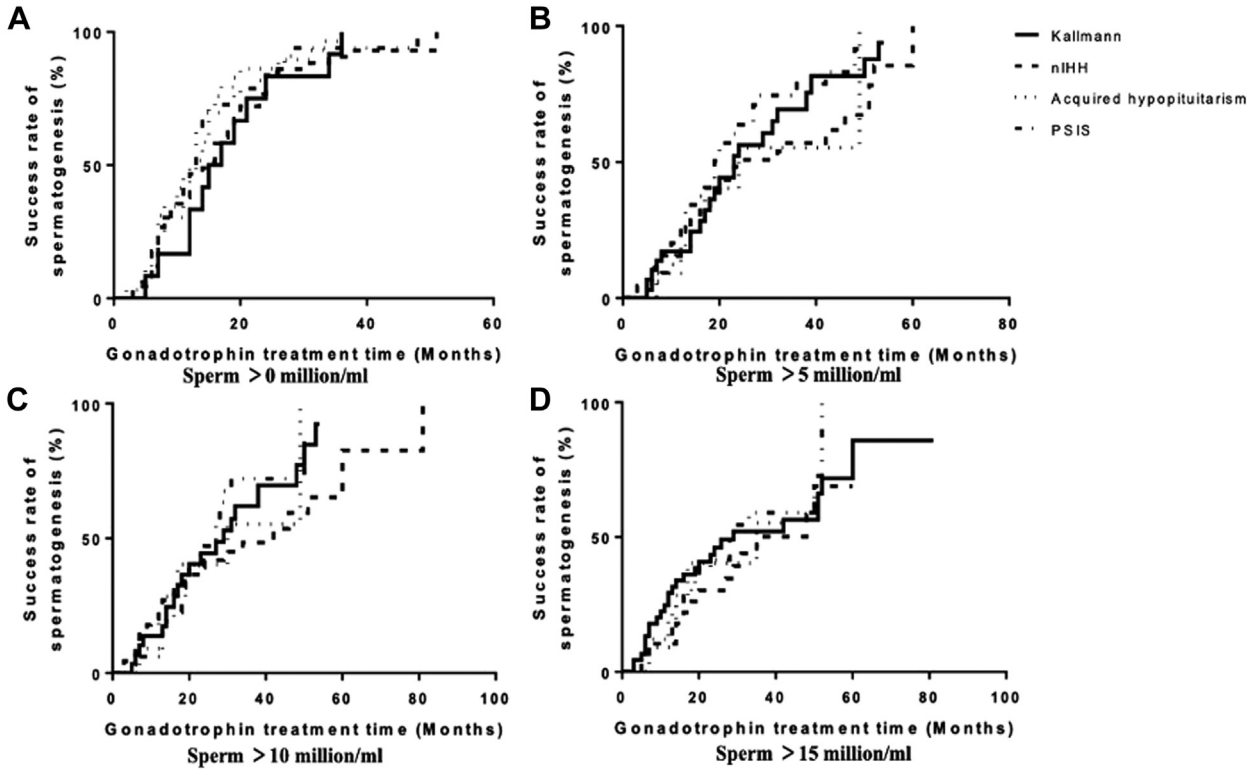

Fig. 3. Median times of achieving a sperm concentration at different thresholds in Kallmann syndrome, normosmic congenital hypogonadotropic hypogonadism, pituitary stalk interruption syndrome, and acquired hypogonadotropic hypogonadism. *A*, Median time to reach sperm >0 million/mL. *B*, Median time to reach sperm >5 million/mL. *C*, Median time to reach sperm >10 million/mL. *D*, Median time to reach sperm 15 million/mL. *B–D*, In the Kallmann syndrome group, the median time to reach a sperm threshold >5, >10, and >15 million/mL was 24 (IQR, 8.5-39.5), 42 (IQR, 20.4-63.6), and 29 (IQR, 6.5-51.5) months, respectively. In the normosmic congenital hypogonadotropic hypogonadism group, the median time to reach a sperm threshold >5, >10, and >15 million/mL, was 23 (IQR, 16.6– 29.4), 29 (IQR 16.9-41.1), and 35 (IQR, 10.3-59.7) months, respectively. In the pituitary stalk interruption syndrome group, the median time to reach a sperm threshold >5, >10, and >15 million/mL was 24 (IQR, 7.8-40.2), 30 (IQR, 0-60.1), and 35 (IQR, 0-76.6) months, respectively. In the acquired hypogonadotropic hypogonadism group, the median time to reach a sperm threshold >5, >10, and >15 million/mL was 19 (IQR, 15.8-22.2), 27 (IQR, 20.7-33.3), and 29 (IQR, 18.2-39.8) months, respectively (see Table 2 for additional information). *IQR* = interquartile range.

Table 3
Comparison of Baseline Features of CHH, KS, and nCHH Between Gonadotropin Treatment Once a Week and Twice a Week

Variable	QW (CHH) ($n = 95$)	BiW (CHH) ($n = 223$)	P^a	QW (KS) ($n = 61$)	BiW (KS) ($n = 111$)	P^b	QW (nCHH) ($n = 34$)	BiW (nCHH) ($n = 112$)	P^c
Age at diagnosis, y	18.28 ± 4.89	20.41 ± 4.61	.00	18.71 ± 5.76	20.81 ± 5.08	.04	17.62 ± 3.1	20.1 ± 4.22	.00
Age at initiating treatment, y	19.95 ± 4.6	22.28 ± 5.17	.00	20.38 ± 5.39	22.37 ± 5.37	.06	19.28 ± 2.97	22.21 ± 5.04	.00
BMI, kg/m^2	22.53 ± 3.32	22.27 ± 3.75	.63	22.74 ± 3.63	21.71 ± 3.45	.17	22.27 ± 2.94	22.7 ± 3.94	.59
Basal testicular volume, mL	3.17 ± 3.15	2.3 ± 1.79	.04	2.92 ± 3.55	2.16 ± 1.61	.24	3.46 ± 2.63	2.42 ± 1.92	.06
Peak LH, IU/L	4.82 ± 8.04	5.25 ± 8.74	.74	4.59 ± 9.26	3.24 ± 3.58	.40	5.15 ± 6.14	6.98 ± 11.21	.33
Rate of family history	2 (2.70%)	10 (6.99%)	.22	2 (4.44%)	9 (14.52%)	.11	0 (0.00%)	1 (1.23%)	.99
Rate of cryptorchidism	7 (9.72%)	20 (13.99%)	.51	6 (13.64%)	9 (14.52%)	1.00	1 (3.57%)	11 (13.58%)	.18
Previous androgen	37 (50.00%)	100 (69.93%)	.00	20 (44.44%)	40 (64.52%)	.03	17 (58.62%)	60 (74.07%)	.11
Previous gonadotropins	29 (40.85%)	17 (11.89%)	<.0001	16 (37.21%)	9 (14.52%)	.00	13 (46.43%)	8 (9.88%)	<.0001
Follow-up, mo	33.86 ± 17.93	27.5 ± 13.28	.00	38.11 ± 16.68	25.94 ± 12.14	.00	27.55 ± 18.15	28.7 ± 14.05	.72

Abbreviations: BiW = gonadotropin treatment twice a week; BMI = body mass index; CHH = congenital HH; HH = hypogonadotropic hypogonadism; KS = Kallmann syndrome; LH = luteinizing hormone; nCHH = normosmic congenital HH; QW = weekly gonadotropin treatment.
Peak LH means highest LH level after stimulation by triptorelin 100 mg.
$P < .05$ was defined statistically significant.
[a] P value obtained from the comparison of gonadotropin injection once a week with gonadotropin injection twice a week in successful spermatogenesis CHH patients.
[b] P value obtained from the comparison of gonadotropin injection once a week and gonadotropin injection twice a week in successful spermatogenesis KS patients
[c] P value obtained from the comparison of gonadotropin injection once a week and gonadotropin injection twice a week in successful spermatogenesis nCHH patients.

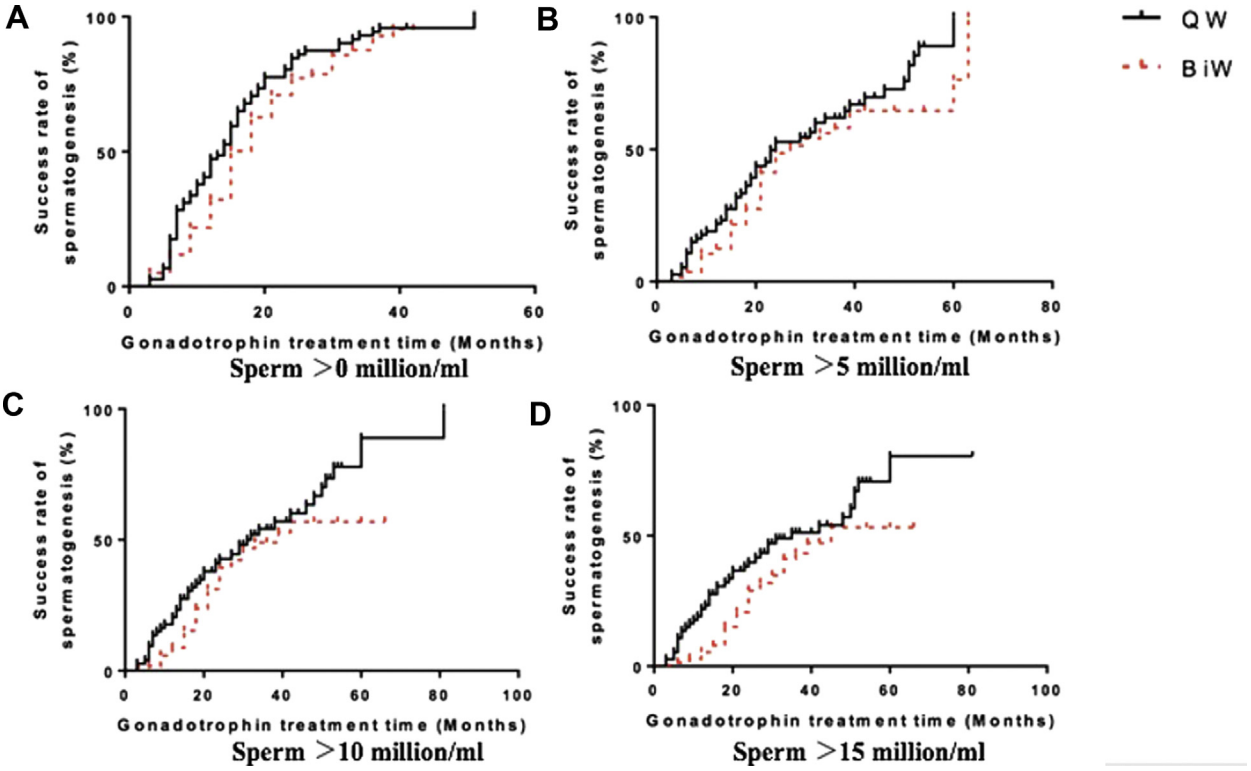

Fig. 4. Median times of achieving sperm concentration at different thresholds with weekly ($n = 74$) and twice-a-week ($n = 143$) gonadotropin treatment. *A*, Median time to reach sperm >0 million/mL. *B*, Median time to reach sperm >5 million/mL. *C*, Median time to reach sperm >10 million/mL. *D*, Median time to reach sperm >15 million/mL. *QW* = weekly, *BiW* = twice a week.

Table 4
Predictors for Time of Achieving Spermatogenesis of HH patients treated once a week ($n = 119$) (correlated Cox analysis)

Variable	β	HR	*P*	95% CI lower limit	95% CI upper limit
Age at initiating treatment	−0.02393	0.976	.3716	0.926	1.029
BMI, kg/m^2	−0.00713	0.993	.7996	0.94	1.049
Basal testicular volume	0.14987	1.162	.0142	1.031	1.309
Cryptorchidism	−0.4812	0.618	.2609	0.267	1.43
Prior androgen treatment	−0.00209	0.998	.9929	0.629	1.583
Prior gonadotropin treatment	0.41509	1.515	.1258	0.89	2.577
Peak LH	−0.02505	0.975	.3121	0.929	1.024
Treatment group (BiW) as reference)	0.01886	1.019	.9336	0.654	1.587

Abbreviations: BiW = gonadotropin treatment twice a week; BMI = body mass index, HH = hypogonadotropic hypogonadism; HR = hazard ratio; LH = luteinizing hormone.

cryptorchidism, basal testicular volume, prior androgen therapy, and prior gonadotropins therapy) as influencing factors. We use propensity scores to perform a 1:1 match in patients in the weekly and twice-a-week groups. This showed that a larger basal testicular volume ($\beta = 0.15$, $P = .01$) was a favorable predictor for a shorter time to achieve spermatogenesis in HH patients (Table 4). The multivariate linear regression model was used to evaluate possible influencing factors, as mentioned above, and found no significant predictors for earlier spermatogenesis in HH patients.

Considering the multiple heterogeneous factors affecting HH patients, we used the Cox-related method and multivariate linear regression model to analyze patients with CHH ($n = 95$). Consistent with the findings of HH, larger basal testicular volume ($\beta = 0.22$, $P = .0009$) was a favorable predictor for earlier spermatogenesis (Table 5). Cox regression analysis also indicated that treatment groups (weekly vs twice a week) showed no significant differences in the rate of spermatogenesis and time to achieve spermatogenesis (Table 5).The multivariate linear regression model found no major predictors for earlier spermatogenesis following the treatment (Supplementary Table 1).

Sperm Progressive Mobility and Total Mobility Analysis

At the final follow-up examination, the sperm concentration was 18.1 (IQR, 5-46.2) million/mL in general HH patients treated with gonadotropins once a week. Sperm progressive motility (A+B) was 41% ± 27%, and total mobility (A+B+C) was 45% ± 28%. According to the WHO reference values for human semen,[16] 54.6% (65/119) of these patients attained sperm concentrations >15 million/mL. Of them, 88% (57/65) had sperm progressive motility (A+B) >32%.

Among the 4 subgroups, the average sperm concentration was 25.83 (IQR, 5-48.2) million/mL in KS, 14.36 (IQR, 5.9-34.8) million/mL in nCHH, 18.1 (IQR, 9.3-38.1) million/mL in PSIS, and 6.86 (IQR, 1.0-51.8) million/mL in acquired HH. Sperm progressive motility (A+B) in these 4 subgroups was 42% ± 27%, 38% ± 28%, 45% ± 23%, and 33% ± 37%, respectively. Total mobility (A+B+C) in these 4 subgroups was 47% ± 29%, 41% ± 29%, 51% ± 23%, and 34% ± 38%, respectively (Table 2).

For CHH patients, the sperm concentration was 20.9 (IQR, 5.0-46.3) vs 11.7 (IQR, 2.1-24.4) million/mL, progressive motility (A+B) was 40.8% ± 27.3% vs 36.9% ± 20.2% ($P = .85$), and total mobility (A+B+C) was 44.2% ±28.5% vs 44.4% ± 21.9% ($P = .95$) in the weekly and twice-a-week groups, respectively.

Pregnancy Outcome

Of the patients who had successful spermatogenesis, 6 spontaneous pregnancies occurred (50%, 6/12) in patients who tried to father a child, and 3 infants were delivered with normal external genitalia appearance. Of these 6 patients, 2 were diagnosed with KS, 3 with PSIS, and 1 with acquired HH after transsphenoidectomy for pituitary growth hormone adenoma. The sperm concentrations were >20 million/mL in 4 patients, >15 million/mL in 1 patient, and >1 million/mL in 1 patient. Sperm progressive motility (A+B) of >50% and >40% was observed in 3 and 2 patients, respectively. In the twice-a-week group, 34 patients were married, and 20 pregnancies occurred (59%, 20/34), of which 14 infants were delivered.

Safety Evaluation

During the period of study, gynecomastia developed in 5% (8/160) of the subjects. Acne occurred in 2% (3/160) of the patients. No hepatorenal impairment or allergic reactions were reported.

Discussion

This is the first study to investigate the efficacy of weekly gonadotropin therapy on spermatogenesis in HH patients. We found that the general success rate was 74% (119/160), the median time for achieving the first sperm was 13 months, and basal testicular size was a favorable predictor for earlier spermatogenesis.

Various regimens of gonadotropin treatment have been used in clinical practice. Typically, HCG/HMG is administered 2 or 3 times a week.[8-10] In clinical practice, many patients are high school or college students, and therefore, injections 2 or 3 times a week are often inconvenient, time-consuming, and difficult to adhere to. Earlier studies have confirmed that patients administered medication once a day have better compliance than patients administered medication 2 to 3 times a day.[17] Similarly, weekly injection of long-acting human recombinant growth hormones also significantly improved patient adherence and compliance compared with daily injection.[11] Therefore, it is believed that weekly gonadotropin treatment may promote therapy adherence and compliance.

Among CHH patients, 78% (74/95) succeeded in spermatogenesis with gonadotropin treatment once a week. This result was similar to the twice-a-week regimen, which had a success rate of 75% to 85%.[5-7] Compared with the 2 times a week strategy, the weekly injection group had a similar rate of spermatogenesis, median time of sperm appearance, sperm concentration, and sperm progressive motility. These results seem inconsistent with previous studies suggesting multiple low-dose HCG administration, in contrast to a single high dose, enhances Leydig cell steroidogenesis.[18] However, patients in the weekly group had a larger basal testicular volume, an earlier age at diagnosis and initial treatment, a lower percentage of cryptorchidism, prior androgen treatment, and a higher percentage of previous gonadotropin treatment compared with the twice-a-week regimen, which may have predisposed this group to greater success in the weekly treatment. Further cohorts with similar baselines are needed to better illustrate this question.

Table 5
Predictors for Time of Achieving Spermatogenesis of CHH Patients ($n = 95$) Treated Once a Week (correlated Cox analysis)

Variable	β	HR	*P*	95% CI lower limit	95% CI upper limit
Age at initiating treatment	0.03383	1.034	.2484	0.977	1.096
BMI, kg/m^2	−0.02722	0.973	.4278	0.91	1.041
Basal testicular volume	0.22812	1.256	.0009	1.098	1.437
Cryptorchidism	−0.62839	0.533	.2028	0.203	1.403
Prior androgen treatment	0.5715	1.771	.0478	1.006	3.119
Prior gonadotropins treatment	0.27975	1.323	.2886	0.789	2.218
Peak LH	0.07066	1.073	.0136	1.015	1.135
Treatment Group (BiW as reference)	0.07957	1.083	.748	0.666	1.759

Abbreviations: BiW = gonadotropin treatment twice a week; BMI = body mass index, CHH = congenital hypogonadotropic hypogonadism; HR = hazard ratio; LH = luteinizing hormone.

However, our study pointed out the possibility of gonadotropin therapy with a lower injection frequency.

Among these factors, larger basal testicular size has been associated with earlier spermatogenesis.[10-20]. The success rate of spermatogenesis varies according to the etiology. For instance, acquired HH seemed more prone to successful spermatogenesis due to larger testicular size.[21,22] Our study showed a similar spermatogenesis rate between nCHH, PSIS, and acquired HH, possibly due to the small patient number in the acquired HH group.

Our study showed that serum testosterone gradually increased with weekly injection therapy, similar to the twice-a-week injection.[23-25] After treatment, the KS and nCHH groups had similar testosterone levels. Weekly fluctuation of testosterone levels, caused by HCG weekly injection, may cause periodic erectile function and physical strength. However, our patients did not have symptoms or complaints in this regard.

The median time for sperm appearance in patients treated weekly was 13 months. This result was similar to the median of 6 to 11 months that occurred with gonadotropin therapy administered 2 or 3 times a week.[10,23-25] Comparable with previous studies, acquired HH patients needed the shortest time to achieve spermatogenesis (13 months). Acquired HH patients often experience complete pubertal development and have a relatively good testicular condition, thus providing greater potential for spermatogenesis.[21,22] Consistent with previous studies, there was no significant difference between the KS and nCHH groups.[26] The time to achieve sperm production was similar between the PSIS and CHH groups, indicating that both groups have similar spermatogenesis potential.[21,27]

The testicular volume increased from 3.1 ± 3.19 mL to 10.85 ± 5.1 mL ($P < .001$) with weekly gonadotropin treatment, and there was no significant difference between the 4 subgroups. Basal testicular volume has been consistently predicted as a key indicator for successful spermatogenesis.[5,10,19,28] Favorable factors usually associated with spermatogenesis, such as lower BMI,[5] less previous androgen exposure,[19] and previous gonadotropin use,[19,20] were not confirmed to be key indicators for successful spermatogenesis in our study.

Cryptorchidism is often identified as an adverse factor of spermatogenesis in pulsatile GnRH treatment and gonadotropin treatment.[27-34] In our study, the median time for the emergence of sperm was 23.7 months in CHH patients with cryptorchidism that have achieved spermatogenesis ($n = 9$), which was significantly longer than that of total CHH patients ($n = 74$, 14 months). The average sperm concentration was lower than the average sperm concentration of total CHH patients (17.56 vs 20.9 million/mL). These results indicate that cryptorchidism may be associated with longer periods to achieve spermatogenesis and lower sperm concentration. However, the Cox-related analysis and multivariate linear regression model did not conclude that cryptorchidism was negatively associated with spermatogenesis in our study. The spermatogenesis group and nonspermatogenesis group did not show a significant difference in the proportion of patients with cryptorchidism (7.56% [9/119] vs 9.76% (4/41); $P = 0.74$). However, our model to determine whether cryptorchidism affects spermatogenesis may be underpowered by the small number of patients with cryptorchidism in our cohort.

During this study, 6 spontaneous pregnancies occurred, and 3 healthy children were delivered. Our study showed a large variation in sperm concentration in those patients that successfully impregnated their partners. Sperm concentration is not the dominant factor for pregnancy because spontaneous pregnancy can even occur with a very low sperm concentration.[35] In a study where 22 CHH patients impregnated their partners, 5 of the patients had a sperm count <1 million/mL.[35]

No severe adverse events were observed during gonadotropin therapy. Acne and gynecomastia occurred in ≤5% of the patients. Acne is caused by increased serum testosterone, and gynecomastia is induced by estrogen aromatization from increased testosterone levels. Thus, gonadotropin therapy is considered safe for patients with HH.[24]

There are certain limitations to our study. First, as a retrospective study, patients who had poor response to gonadotropin therapy may have been lost during the follow-up, causing an increased rate of successful spermatogenesis. Second, the success rate of spermatogenesis could have been influenced by the limited number of participants, especially for patients with acquired HH. Third, gene mutations in CHH patients were not investigated, which may have significantly influenced the effect of gonadotropin-induced spermatogenesis. Fourth, the heterogeneous baseline of the weekly and twice-a-week groups may have caused bias in our outcomes, and therefore, prospective clinical studies are required to control for confounding factors. High school students or college students are more likely to choose weekly therapy because they live in the school dormitory and find weekly intramuscular injections at weekends much more convenient, which could possibly cause the patients in the weekly group to be younger at initial treatment and finally achieve a better spermatogenesis rate. Fifth, the increased weekly fluctuation of testosterone levels may cause erectile dysfunction and psychologic pressure, which may need further investigation in the future. It is possible that patients with mild gonadal axis dysfunction may be more suitable for weekly therapy.

Conclusions

In summary, compared with the traditional twice-a-week regime, weekly gonadotropin therapy had similar efficacy in achieving spermatogenesis in male HH patients. Therefore, weekly gonadotropin therapy provides a more convenient therapeutic option for HH patients.

Acknowledgment

We thank Boston Professional Group Editing for their linguistic help. This work was supported by the Project of National Natural Science Foundation of China (81771576 and 81971375), and Beijing Municipal Natural Science Foundation (7202151).

Author Contributions

W.M.: methodology, data collection, analysis, and original draft preparation; J.Z., Z.L., B.Y., Y.G., Q.H., R.Z., M.H., S.X., S.L., Y.Z., W.J., and B.S.: data collection and follow-up; X.W. and M.N.: draft review and editing; J.M. and X.W.: conceptualization and funding acquisition, followup,and draft review and editing. All authors have read and agreed to the published version of the manuscript.

Disclosure

The authors have no multiplicity of interest to disclose.

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