



Open Access

ORIGINAL ARTICLE

Male Endocrinology

Testosterone undecanoate supplementation together with human chorionic gonadotropin does not impair spermatogenesis in males with isolated hypogonadotropic hypogonadism: a retrospective study

Yin-Wei Chen^{1,2}, Yong-Hua Niu^{1,2}, Hao Xu^{1,2}, Dao-Qi Wang^{1,2}, Hong-Yang Jiang^{1,2}, Gaurab Pokhrel^{1,2}, Tao Wang^{1,2}, Shao-Gang Wang^{1,2}, Ji-Hong Liu^{1,2}

Gonadotropin therapy is commonly used to induce virilization and spermatogenesis in male isolated hypogonadotropic hypogonadism (IHH) patients. In clinical practice, 5.6%–15.0% of male IHH patients show poor responses to gonadotropin treatment; therefore, testosterone (T) supplementation can serve as an alternative therapy to normalize serum T levels and promote virilization. However, treatment with exogenous T impairs spermatogenesis and suppresses intratesticular T levels. This retrospective study aimed to determine whether oral testosterone undecanoate (TU) supplementation together with human chorionic gonadotropin (hCG) would negatively affect spermatogenesis in IHH patients compared with hCG alone. One hundred and seven IHH patients were included in our study. Fifty-four patients received intramuscular hCG and oral TU, and 53 patients received intramuscular hCG alone. The median follow-up time was 29 (range: 12–72) months in both groups. Compared with the hCG group, the hCG/TU group required a shorter median time to normalize serum T levels ($P < 0.001$) and achieve Tanner stage (III and V) of pubic hair and genital development ($P < 0.05$). However, there were no significant differences in the rate of seminal spermatozoa appearance, sperm concentration, or median time to achieve different sperm concentration thresholds between the groups. In addition, there were no significant differences in side effects, such as acne and gynecomastia, observed in both groups. This study indicates that oral TU supplementation together with hCG does not impair spermatogenesis in treated IHH patients compared with hCG alone, and it shortens the time to normalize serum T levels and promote virilization.

Asian Journal of Andrology (2019) 21, 413–418; doi: 10.4103/aja.aja_107_18; published online: 25 December 2018

Keywords: human chorionic gonadotropin; isolated hypogonadotropic hypogonadism; spermatogenesis; testosterone undecanoate; virilization

INTRODUCTION

Isolated hypogonadotropic hypogonadism (IHH) is a rare congenital disorder characterized by absent or incomplete sexual development and infertility. The incidence of IHH was estimated to be 1:10 000 in males and 1:50 000 in females.¹ The causes of IHH include deficient development and migration of gonadotropin-releasing hormone (GnRH) neurons and deficient GnRH secretion and action.² IHH is classified into two subtypes, Kallmann syndrome (KS) and normosmic isolated hypogonadotropic hypogonadism (nIHH). Kallmann syndrome accounts for approximately 50%–60% of IHH patients and is associated with anosmia or severe hyposmia, whereas nIHH is associated with a normal sense of smell.³

The goals of the treatment in male IHH patients include virilization induction and fertility restoration. Testosterone replacement treatment (TRT) is primarily used to induce genital maturation and promote secondary sexual characteristics.^{4–7} Virilization can also be induced by

pulsatile infusion of GnRH or gonadotropin treatment consisting of human chorionic gonadotropin (hCG) alone or combined with human menopausal gonadotropin (hMG)/recombinant follicle-stimulating hormone (rFSH).^{4,8–10} For the fertility restoration, the pulsatile infusion of GnRH and gonadotropin treatment, but not TRT, can induce spermatogenesis.^{8,9,11,12}

In clinical practice, 5.6%–15.0% of male IHH patients show poor responses (e.g., subnormal serum T levels) to gonadotropin treatment, even at high doses of hCG.^{13,14} For IHH patients exhibiting poor responses, an alternative therapy could combine testosterone (T) with gonadotropin treatment to normalize serum T levels and promote virilization. However, TRT has adverse effects on spermatogenesis, which has been reported extensively. TRT in eugonadal men suppressed intratesticular T (ITT) levels, induced germinal epithelial atrophy, and impaired spermatogenesis.^{15–17} Notably, previous TRT history for patients with hypogonadotropic hypogonadism was recognized

¹Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; ²Institute of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China.

Correspondence: Dr. JH Liu (jhliu@tjh.tjmu.edu.cn)

Received: 11 April 2018; Accepted: 12 October 2018

as an independent factor that could decrease the likelihood of spermatogenesis and conception.¹⁸

Considering the above adverse effects of TRT on spermatogenesis, it is unclear whether testosterone supplementation together with hCG is a suitable choice for IHH patients. The objective of this study was to investigate whether oral T undecanoate (TU) supplementation together with hCG negatively affects spermatogenesis in IHH patients compared with hCG alone.

PATIENTS AND METHODS

Patients

From January 2011 to May 2017, 201 patients were included in this retrospective study. Patients were diagnosed on the basis of the standard criteria¹⁹ and received hormonal treatment at the Urology Clinic of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. However, 94 patients were excluded from data analysis because of change of treatment, poor compliance, or an incomplete medical record. Therefore, only 107 patients were available for retrospective data analysis (Figure 1). The study protocol complied with ethical guidelines and was approved by the Ethics Committee of Huazhong University of Science and Technology. Written informed consent was obtained from each patient before inclusion in the study.

Clinical measurements

For each patient, body mass index (BMI), Tanner stage, testicular volume (TV), the presence of cryptorchidism, particular phenotypes (*e.g.*, cleft lip and palate), chromosome karyotype, and medical history were documented at the first hospital visit. Pubic hair and genital Tanner stage were evaluated by a senior physician with reference to five Tanner stages of pubertal development.²⁰ The physician recorded which stage most represented the current stage of patients. TV was measured by a Prader orchidometer (Foshan Bifrost Technology Co., Foshan, China), including the scrotum. Serum sex hormone levels, whole blood count, hepatic and renal function, thyroid hormones, and cortisol levels of each patient were measured, and magnetic resonance imaging (MRI) was performed for the head, including pituitary gland and olfactory bulb/tract.

Laboratory analyses

At each visit, blood samples were taken before 10 a.m., 48–72 h after injection of hCG. Serum FSH, luteinizing hormone (LH), and total T and estradiol (E_2) levels were determined by chemiluminescence immunoassay (UniCel DXI 800; Beckman Coulter, Fullerton, CA, USA). The normal reference ranges of FSH, LH, total T, and total E_2 were 1.27–19.26 mIU ml⁻¹, 1.24–8.62 mIU ml⁻¹, 1.75–7.81 ng ml⁻¹, and

20–75 pg ml⁻¹, respectively. Semen samples were initially collected after repeated ejaculations and again after 2–7 days of abstinence, once the patients were capable of masturbation. However, when no spermatozoa were found in these wet preparations, the samples were centrifuged (3–16KL; Sigma, Osterode, Germany) at 3000 g for 15 min to determine if any spermatozoa are present in a larger sample. The semen collection and analysis complied with the WHO laboratory manual for the examination and processing of human semen (fifth version).²¹

Treatment and follow-up

Potential benefits and side effects of the two treatment options were explained to each patient beforehand, and the patients voluntarily selected their own treatment. Fifty-four patients chose to receive the intramuscular injection of hCG (Livzon Pharmaceutical Co., Zhuhai, China) together with oral T undecanoate soft capsules (Catalent France Beinhem S.A., Beinhem, France), while 53 patients chose to receive hCG alone. Initially, patients in both groups had 2000 IU hCG injected twice per week. Oral TU was taken at 40 mg twice daily. To ensure effective absorption, oral TU soft capsules were taken with fatty meals according to the user manual. In the first 6 months, hCG doses were adjusted according to patients' serum T levels. The maximum dose of hCG used in our study was 8000 IU twice per week. Patients were reviewed at intervals of 3–6 months, and their BMI, Tanner stage, TV, serum FSH, LH, T and E_2 , and semen parameters were measured at each visit.

Clinical outcomes

The major study outcomes included Tanner stage, TV, serum sex hormone levels, spermatogenesis, and the potential side effects. The TV was the mean value of bilateral TV. Seminal spermatozoa appearance (sperm concentration >0 ml⁻¹) was defined when at least a single spermatozoon was observed in a semen sample under the microscope (BX43; Olympus, Tokyo, Japan) after centrifugation at 3000 g for 15 min. We recorded the follow-up time required to achieve different thresholds of Tanner stages (III and V), to normalize serum T levels (1.75–7.81 ng ml⁻¹), and to achieve sperm concentrations >0 and $\geq 15 \times 10^6$ ml⁻¹. Side effects (severe acne, gynecomastia, allergy, and other discomfort associated with treatment) and pregnancy during the follow-up were also recorded.

Data analyses

Posttreatment parameters from the most recent visit were used for data analysis. Data analysis was performed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). Normally distributed data were presented as mean \pm standard deviation (s.d.) and analyzed by nonpaired *t*-tests, and nonnormally distributed data were presented as median (range or interquartile range) and analyzed by nonparametric tests. Differences in the binary outcomes between the two groups were compared by the Chi-squared test (χ^2 test). Kaplan–Meier plots were used to analyze the median time to achieve different thresholds of parameter values. Statistical power analysis was performed using NCSS and PASS version 11.0 (NCSS LLC., Kaysville, UT, USA). Subgroup analysis was performed using the basal TV (≥ 4 ml and <4 ml). Difference were considered statistically significant when $P < 0.05$.

RESULTS

Baseline characteristics

Clinical characteristics at baseline are shown in Table 1. The mean age (year) of patients at the beginning of treatment did not differ between groups. The TV of eight patients could not be measured precisely

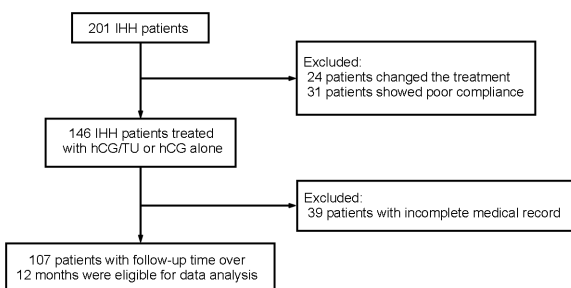


Figure 1: Flowchart of screening patients. IHH: isolated hypogonadotropic hypogonadism; hCG: human chorionic gonadotropin; TU: testosterone undecanoate.

(six patients with cryptorchidism, one with scrotal dysplasia, and the other with anorchia). No significant differences were found in pubic hair and genital Tanner stages, TV, and serum sex hormone levels between the groups (all $P > 0.05$). Laboratory results for whole blood counts, hepatic and renal function, thyroid hormones, and cortisol levels were all within the normal range.

Therapeutic effects

Posttreatment clinical characteristics are shown in **Table 2**. Compared with pretreatment, posttreatment pubic hair and genital Tanner stages were increased significantly in both groups (both $P < 0.001$). Similarly, TV and serum T levels were increased in posttreatment compared with pretreatment in both groups (both $P < 0.001$).

Tanner stage

Posttreatment pubic hair and genital Tanner stages were significantly higher in the hCG/TU group than those in the hCG group (both $P < 0.05$, **Table 2**). Our analysis excluded patients who achieved Tanner stage (III and V) at the beginning of treatment and/or whose TV could not be measured precisely. The hCG/TU and hCG groups required 7 months ($n = 42$, 95% confidence interval [CI]: 5.4–8.6 months) and 11 months ($n = 50$, 95% CI: 9.3–12.7 months), respectively, to achieve pubic hair Tanner stage III ($P = 0.020$; **Figure 2a**). To achieve pubic hair Tanner stage V, the hCG/TU and hCG groups needed 19 months ($n = 52$, 95% CI: 14.5–23.5 months) and 25 months ($n = 53$, 95% CI: 22.6–27.4 months), respectively ($P = 0.010$; **Figure 2b**). Similarly, the median time to achieve genital Tanner stage III was 6 months for the

hCG/TU group ($n = 39$, 95% CI: 5.3–6.7 months) and 9 months ($n = 45$, 95% CI: 7.4–10.6 months) for the hCG group ($P = 0.012$; **Figure 2c**). To achieve genital Tanner stage V, the hCG/TU and hCG groups required 15 months ($n = 48$, 95% CI: 11.7–18.3 months) and 21 months ($n = 49$, 95% CI: 16.5–25.5 months), respectively ($P = 0.004$; **Figure 2d**).

Testicular volume and serum hormone levels

Posttreatment TV showed no significant difference between groups ($P = 0.830$; **Table 2**). Similarly, no significant difference was seen in serum T levels ($P = 0.411$). However, a significant difference was observed in the median time to normalize serum T levels between groups: 3 (95% CI: 2.3–3.7) months for the hCG/TU group versus 7 (95% CI: 6.0–8.0) months for the hCG group ($P < 0.001$; **Table 2**).

Sperm concentration and conception outcome

There were no significant differences in the rate of seminal spermatozoa appearance ($P = 0.928$) or median sperm concentration ($P = 0.917$) between the two groups (**Table 2**). Kaplan–Meier survival analysis showed that the median time to achieve a sperm concentration $>0 \text{ ml}^{-1}$ was 35 (95% CI: 31.3–38.7) months in the hCG/TU group and 30 months in the hCG group (95% CI: 27.9–32.1 months) ($P = 0.613$; **Figure 3a**). The median time to achieve a sperm concentration $\geq 15 \times 10^6 \text{ ml}^{-1}$ was 57 (95% CI: 48.3–65.7) months in the hCG/TU group and 53 (95% CI: 45.3–60.7) months in the hCG group ($P = 0.282$; **Figure 3b**). Eight partners of IHH patients achieved pregnancy during the follow-up period. Among them, three patients received hCG/TU treatment, while the remaining five received hCG alone. Sperm

Table 1: Clinical characteristics at baseline of isolated hypogonadotropic hypogonadism patients

Characteristics	hCG/TU group ($n=54$)	hCG group ($n=53$)	<i>P</i>
Age at the beginning of treatment (year), mean \pm s.d.	21.8 \pm 3.7	22.7 \pm 4.1	0.230
BMI (kg m^{-2}), mean \pm s.d.	21.8 \pm 2.7	21.8 \pm 3.6	0.921
Kallmann syndrome, <i>n</i> (%)	23 (42.6)	26 (49.1)	0.502
Cryptorchidism or cryptorchidism history, <i>n</i> (%)	7 (13.0)	5 (9.4)	0.563
Previous testosterone treatment, <i>n</i> (%)	9 (16.7)	1 (1.9)	0.009
Previous gonadotropin treatment, <i>n</i> (%)	4 (7.4)	9 (17.0)	0.150
Pubic hair Tanner stage, mean \pm s.d.	1.8 \pm 0.9	1.7 \pm 0.6	0.434
Genital Tanner stage, mean \pm s.d.	1.8 \pm 1.0	1.7 \pm 0.7	0.552
Testicular volume (ml), mean \pm s.d.	4.6 \pm 3.2	3.9 \pm 4.2	0.316
Basal FSH (mIU ml^{-1}), mean \pm s.d.	1.4 \pm 1.4	1.4 \pm 1.0	0.923
Basal LH (mIU ml^{-1}), mean \pm s.d.	0.8 \pm 0.8	0.8 \pm 0.7	0.861
Basal T (ng ml^{-1}), mean \pm s.d.	0.4 \pm 0.4	0.4 \pm 0.3	0.565

Both previous gonadotropin and testosterone treatment excluded previous gonadotropin plus testosterone treatment. Analysis of genital Tanner stage and testicular volume excluded patients whose TV could not be measured precisely. IHH: isolated hypogonadotropic hypogonadism; hCG: human chorionic gonadotropin; TU: testosterone undecanoate; BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; s.d.: standard deviation; TV: testicular volume

Table 2: Clinical characteristics after treatment of isolated hypogonadotropic hypogonadism patients

Characteristics	hCG/TU group ($n=54$)	hCG group ($n=53$)	<i>P</i>
Follow-up time (month), median (range)	29 (12–72)	29 (12–72)	0.311
hCG dose adjustment, <i>n</i> (%)	15 (27.8)	21 (39.6)	0.195
Height (cm), mean \pm s.d.	176.8 \pm 6.3	177.0 \pm 6.3	0.868
Pubic hair Tanner stage, mean \pm s.d.	4.8 \pm 0.6	4.4 \pm 1.0	0.011
Genital Tanner stage, mean \pm s.d.	4.9 \pm 0.2	4.5 \pm 0.8	0.001
Testicular volume (ml), mean \pm s.d.	12.0 \pm 4.1	12.2 \pm 4.4	0.830
Therapeutic T (ng ml^{-1}), mean \pm s.d.	3.0 \pm 1.2	3.2 \pm 1.8	0.411
Seminal spermatozoa appearance, <i>n</i> (%)	29 (53.7)	28 (52.8)	0.928
Sperm concentration (10^6 ml^{-1}), median (interquartile range)	15.6 (8.3–54.1)	15.7 (9.6–50.1)	0.917

Seminal spermatozoa appearance indicates the rate of seminal spermatozoa appearance (sperm concentration $>0 \text{ ml}^{-1}$) in each group. HCG dose adjustment indicates the rate of patients with hCG dose adjustment in each group. Median sperm concentration was calculated from patients with successful spermatogenesis. Analysis of genital Tanner stage and testicular volume excluded patients whose TV could not be measured. IHH: isolated hypogonadotropic hypogonadism; hCG: human chorionic gonadotropin; T: testosterone; s.d.: standard deviation; TV: testicular volume; TU: testosterone undecanoate



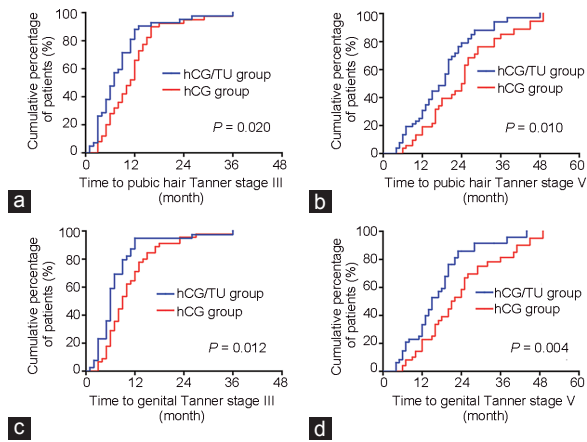


Figure 2: Follow-up time required to achieve different thresholds of Tanner stage (Kaplan–Meier analysis). (a) Cumulative percentage of patients in the hCG/TU group ($n = 42$) and the hCG group ($n = 50$) to achieve pubic hair Tanner stage III ($P = 0.020$). (b) Cumulative percentage of patients in the hCG/TU group ($n = 52$) and the hCG group ($n = 53$) to achieve pubic hair Tanner stage V ($P = 0.010$). (c) Cumulative percentage of patients in the hCG/TU group ($n = 39$) and the hCG group ($n = 45$) to achieve genital Tanner stage III ($P = 0.012$). (d) Cumulative percentage of patients in the hCG/TU group ($n = 48$) and the hCG group ($n = 49$) to achieve genital Tanner stage V ($P = 0.004$). hCG: human chorionic gonadotropin; TU: testosterone undecanoate.

concentrations for the eight fertile patients were above $7 \times 10^6 \text{ ml}^{-1}$, and the lowest value of sperm motility was 6.8%.

Sperm concentration for patients with different basal testicular volumes

In the hCG/TU group, comparisons between subgroups with a basal testicular volume (BTV) $< 4 \text{ ml}$ and $\geq 4 \text{ ml}$ found that the later subgroup had a shorter median time to achieve sperm concentrations $> 0 \text{ ml}^{-1}$ (26 vs 38 months, $P = 0.004$; **Figure 4a**) and $\geq 15 \times 10^6 \text{ ml}^{-1}$ (38 vs 57 months, $P = 0.007$; **Figure 4b**) and a higher median sperm concentration (28.0×10^6 [interquartile range: 12.0×10^6 – 77.6×10^6] ml^{-1} vs 11.6×10^6 [interquartile range: 4.3×10^6 – 31.2×10^6] ml^{-1} , $P = 0.047$; **Figure 4c**). Consistently, there were no significant differences in the rate of seminal spermatozoa appearance or the median time to achieve a sperm concentration $> 0 \text{ ml}^{-1}$ ($P = 0.098$; **Figure 4d**) between the hCG subgroups with BTV $\geq 4 \text{ ml}$ and $< 4 \text{ ml}$. However, the BTV $\geq 4 \text{ ml}$ subgroup in the hCG group had a shorter median time to achieve sperm concentrations $\geq 15 \times 10^6 \text{ ml}^{-1}$ (34 vs 56 months, $P = 0.049$; **Figure 4e**) compared with the BTV $< 4 \text{ ml}$ subgroup. In addition, no significant difference was observed in the median sperm concentration ($P = 1.000$; **Figure 4f**) between the hCG subgroups with BTV $\geq 4 \text{ ml}$ and $< 4 \text{ ml}$.

Side effects

Severe allergic reactions were not observed in any patients during the follow-up period. Mild acne was recorded in 16.7% (9/54) and 9.4% (5/53) of the hCG/TU and hCG groups, respectively. Gynecomastia was reported in 13.0% (7/54) and 15.1% (8/53) of the hCG/TU and hCG groups, respectively. Among them, 13 patients had mild gynecomastia and two patients had moderate gynecomastia. Their serum E_2 levels were assessed as high, and aromatase inhibitors were therefore administered and gynecomastia was relieved. Frequent erections and injection site infections were not observed in any patients.

Statistical power

In both hCG/TU and hCG group, the statistical power of comparisons between pretreatment and posttreatment were 1.00 in **Supplementary**

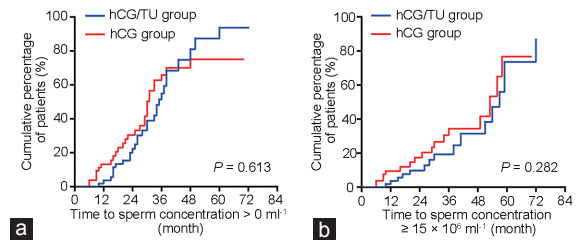


Figure 3: Follow-up time required to achieve different thresholds of semen concentration (Kaplan–Meier analysis). (a) Cumulative percentage of patients in the hCG/TU group ($n = 54$) and the hCG group ($n = 53$) to achieve sperm concentration $> 0 \text{ ml}^{-1}$ ($P = 0.613$). (b) Cumulative percentage of patients in the two groups to achieve sperm concentration $\geq 15 \times 10^6 \text{ ml}^{-1}$ ($P = 0.282$). hCG: human chorionic gonadotropin; TU: testosterone undecanoate.

Table 1 and **2**. However, the statistical power analysis showed that the power of two comparisons in genital tanner stage and median time to normalize serum T were > 0.8 , whereas the remaining comparisons were all < 0.8 (**Supplementary Table 3**). Consistently, the power of comparisons between the hCG/TU subgroups with BTV $\geq 4 \text{ ml}$ and $< 4 \text{ ml}$ were < 0.8 (**Supplementary Table 4**), and same condition was seen in comparisons between the hCG subgroups with BTV $\geq 4 \text{ ml}$ and $< 4 \text{ ml}$ (**Supplementary Table 5**).

DISCUSSION

TRT in male IHH patients aims to induce puberty and secondary sexual characteristics.^{22,23} Even in the era of gonadotropin therapy, T supplementation remains an alternative regimen to maintain normal serum T levels and promote virilization in IHH patients when gonadotropin therapy is ineffective. In our study, we observed that TU supplementation together with hCG had no harmful effects on spermatogenesis compared with hCG alone, and it shortened the time to normalize serum T levels and promote virilization.

Intratesticular T is essential for spermatogenesis and is secreted by Leydig cells under LH stimulation. The ITT levels range from 100 to 1000 fold higher than serum T levels.²⁴ Under physiological conditions, TRT results in a decrease in ITT levels and impairs spermatogenesis. Although serum FSH and LH levels are below the normal range in the majority of IHH patients, their ITT levels can be influenced by the combination of exogenous hCG administration and subnormal serum LH levels during gonadotropin treatment. One study found that TRT plus hCG treatment maintained normal ITT levels during follow-up periods.²⁵ Furthermore, concomitant hCG appeared to maintain semen parameters in hypogonadal men on TRT.²⁶ In concordance with the above observations, the efficacy of both groups in our study was approximately similar in terms of spermatogenesis, such as the rate of seminal spermatozoa appearance, sperm concentration, and the median time to achieve different sperm concentration thresholds.

The median time to achieve sperm concentration $> 0 \text{ ml}^{-1}$ in the hCG group was 30 months, which was much longer than that found in a previous study (11.7 [s.d.: 3.6] months).⁸ Two factors may account for this difference. First, the follow-up interval was longer and inconsistent (3–6 months). Second, following ethical requirements, semen analysis was performed after patients were able to masturbate, which further extended the time recorded.

For male IHH patients, virilization can be induced by either TRT or gonadotropin treatment. To date, few studies have compared the efficacy of TRT and gonadotropin treatment on virilization. One study of IHH patients showed the benefit of T esters or T gel administration over hCG alone, in terms of obtaining higher serum T levels and

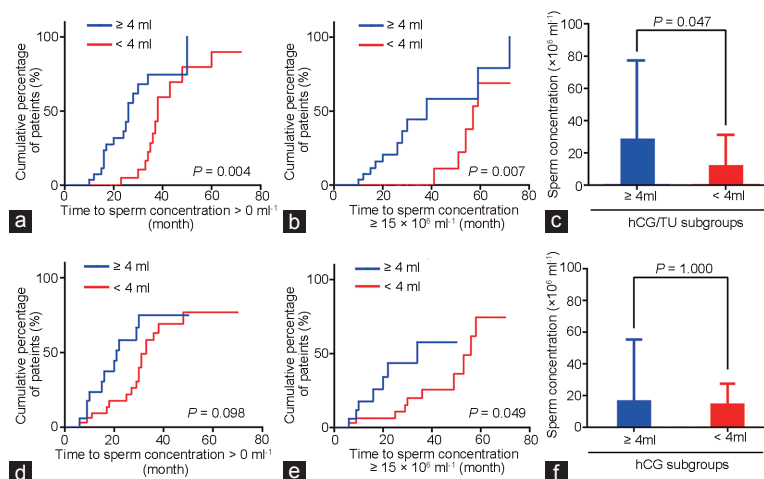


Figure 4: Follow-up time required to achieve different thresholds of sperm concentration between subgroups with BTV ≥ 4 ml and < 4 ml. (Kaplan–Meier analysis). (a) Cumulative percentage of patients in the hCG/TU subgroups with BTV ≥ 4 ml ($n = 27$) and < 4 ml ($n = 23$) to achieve sperm concentration > 0 ml $^{-1}$ ($P = 0.004$). (b) Cumulative percentage of patients in the hCG/TU subgroups to achieve sperm concentration $\geq 15 \times 10^6$ ml $^{-1}$ ($P = 0.007$). (c) Median sperm concentration with interquartile range in the hCG/TU subgroups with BTV ≥ 4 ml and < 4 ml ($P = 0.047$). (d) Cumulative percentage of patients in the hCG subgroups with BTV ≥ 4 ml ($n = 17$) and < 4 ml ($n = 32$) to achieve sperm concentration > 0 ml $^{-1}$ ($P = 0.098$). (e) Cumulative percentage of patients in the hCG subgroups with BTV ≥ 4 ml and < 4 ml to achieve sperm concentration $\geq 15 \times 10^6$ ml $^{-1}$ ($P = 0.049$). (f) Median sperm concentration with interquartile range in the hCG subgroups ($P = 1.000$). hCG: human chorionic gonadotropin; BTV: basal testicular volume; TU: testosterone undecanoate.

more advanced Tanner stage after 6 months of follow-up.⁴ A similar study found that IHH patients achieved Tanner stage V after receiving intramuscular TU for 15 months.⁵ Our results echo these studies; TU supplementation together with hCG required a shorter time to achieve different thresholds of Tanner stage (III and V) compared with hCG alone. The earlier virilization may be attributable to earlier normalization of serum T in the hCG/TU group. However, this efficacy needs to be confirmed in future investigations.

In the analysis of different BTV, our current findings suggested that the BTV ≥ 4 ml subgroup required a shorter time to achieve sperm concentrations > 0 or $\geq 15 \times 10^6$ ml $^{-1}$ compared with the BTV < 4 ml subgroup. Consistent with our findings, it was reported that the rate of seminal spermatozoa appearance and sperm concentrations were higher in IHH patients with BTV ≥ 4 than BTV < 4 ml after gonadotropin treatment.²⁷ Moreover, gonadotropin-deficient males with larger TV required a shorter time to achieve different sperm concentration thresholds (> 0 , 5×10^6 , and 20×10^6 ml $^{-1}$).²⁸ Thus, a larger BTV (≥ 4 ml) may be a positive prognostic factor associated with successful and earlier spermatogenesis.

Acne and gynecomastia were the most frequent side effects recorded in both groups. However, there were no significant differences in the rates of acne and gynecomastia, indicating that TU supplementation did not promote acne and gynecomastia more than hCG alone.

Owing to its safety profile on spermatogenesis and improved virilization, we suggest that TU supplementation together with hCG provides a suitable choice of therapy for IHH patients who show poor responses to gonadotropin, even at high doses of hCG. However, in IHH patients exhibiting good responses to gonadotropin treatment alone, although TU supplementation may result in earlier normalization of serum T and virilization, the increased medical costs and limited improvement in virilization should be taken into consideration.

Our study has several limitations. First, owing to its retrospective nature and limited sample size, the statistical power of some comparisons is relatively low. The power analysis of many comparisons

indicated that a large number of IHH patients (*e.g.*, 19 360 patients in one comparison) would be required to detect differences at 80% power. However, it is difficult to recruit many patients because of the low incidence of this rare disease (1:10 000 in males). Second, variation in geographical distances has led to inconsistent intervals for follow-up reviews. A shorter and more regular follow-up period is required. Third, the formulation of oral TU has low bioavailability and is highly dependent on the lipid content of food intake. Fourth, bone density is an important outcome in the treatment of IHH patients, but was not measured in our study. Bone analysis should be included as a necessary follow-up outcome in future investigations.

CONCLUSIONS

Our results indicate that oral TU supplementation together with hCG does not impair spermatogenesis in the treatment of IHH patients compared with hCG alone, and it shortens the time to normalize serum T levels and promotes virilization. However, prospective, multicenter, randomized studies with larger sample size are needed to validate our conclusions.

AUTHOR CONTRIBUTIONS

JHL, SGW, and TW designed the study. YWC, YHN, HX, DQW, HYJ, and GP carried out the follow-up reviews and participated in clinical data collection. YWC and YHN performed the statistical analysis, and YWC drafted the article. JHL and YHN revised the article. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

The work was supported by the grant from the National Natural Science Foundation of China (Project No. 81671443, 81601270).

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.



REFERENCES

- 1 Bhagavath B, Podolsky RH, Ozata M, Bolu E, Bick DP, *et al*. Clinical and molecular characterization of a large sample of patients with hypogonadotropic hypogonadism. *Fertil Steril* 2006; 85: 706–13.
- 2 Bianco S, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2009; 5: 569–76.
- 3 Mitchell AL, Dwyer A, Pitteloud N, Quinton R. Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. *Trends Endocrinol Metab* 2011; 22: 249–58.
- 4 Aydogdu A, Bolu E, Sonmez A, Tasci I, Haymana C, *et al*. Effects of three different medications on metabolic parameters and testicular volume in patients with hypogonadotropic hypogonadism: 3-year experience. *Clin Endocrinol (Oxf)* 2013; 79: 243–51.
- 5 Santhakumar A, Miller M, Quinton R. Pubertal induction in adult males with isolated hypogonadotropic hypogonadism using long-acting intramuscular testosterone undecanoate 1-g depot (Nebido). *Clin Endocrinol (Oxf)* 2014; 80: 155–7.
- 6 Han TS, Bouloux PM. What is the optimal therapy for young males with hypogonadotropic hypogonadism? *Clin Endocrinol (Oxf)* 2010; 72: 731–7.
- 7 Young J. Approach to the male patient with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2012; 97: 707–18.
- 8 Yang L, Zhang SX, Dong Q, Xiong ZB, Li X. Application of hormonal treatment in hypogonadotropic hypogonadism: more than ten years experience. *Int Urol Nephrol* 2012; 44: 393–9.
- 9 Gong C, Liu Y, Qin M, Wu D, Wang X. Pulsatile GnRH is superior to hCG in therapeutic efficacy in adolescent boys with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2015; 100: 2793–9.
- 10 Bouloux PM, Nieschlag E, Burger HG, Skakkebaek NE, Wu FC, *et al*. Induction of spermatogenesis by recombinant follicle-stimulating hormone (Puregon) in hypogonadotropic azoospermic men who failed to respond to human chorionic gonadotropin alone. *J Androl* 2003; 24: 604–11.
- 11 Mao J, Liu Z, Nie M, Wang X, Xu H, *et al*. Pulsatile gonadotropin-releasing hormone therapy is associated with earlier spermatogenesis compared to combined gonadotropin therapy in patients with congenital hypogonadotropic hypogonadism. *Asian J Androl* 2017; 19: 680–5.
- 12 Zhang M, Tong G, Liu Y, Mu Y, Weng J, *et al*. Sequential versus continual purified urinary FSH/hCG in men with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2015; 100: 2449–55.
- 13 Matsumoto AM, Snyder PJ, Bhasin S, Martin K, Weber T, *et al*. Stimulation of spermatogenesis with recombinant human follicle-stimulating hormone (follitropin alfa; GONAL- α): long-term treatment in azoospermic men with hypogonadotropic hypogonadism. *Fertil Steril* 2009; 92: 979–90.
- 14 Warne DW, Decosterd G, Okada H, Yano Y, Koide N, *et al*. A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle-stimulating hormone and human chorionic gonadotropin. *Fertil Steril* 2009; 92: 594–604.
- 15 World Health Organization Task Force on Methods for the Regulation of Male Fertility. Contraceptive efficacy of testosterone-induced azoospermia in normal men. *Lancet* 1990; 336: 955–9.
- 16 Kovac JR, Lipshultz LI. The importance of understanding baseline reproductive function prior to the administration of exogenous testosterone. *Asian J Androl* 2016; 18: 381.
- 17 Khera M. Controversies in testosterone supplementation therapy. *Asian J Androl* 2015; 17: 175–6.
- 18 Liu PY, Baker HW, Jayadev V, Zacharin M, Conway AJ, *et al*. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. *J Clin Endocrinol Metab* 2009; 94: 801–8.
- 19 Liu YL, Zhang MN, Tong GY, Sun SY, Zhu YH, *et al*. The effectiveness of zinc supplementation in men with isolated hypogonadotropic hypogonadism. *Asian J Androl* 2017; 19: 280–5.
- 20 Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970; 45: 13–23.
- 21 World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization; 2010.
- 22 Boehm U, Bouloux PM, Dattani MT, de Roux N, Dode C, *et al*. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 2015; 11: 547–64.
- 23 Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, *et al*. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010; 95: 2536–59.
- 24 Shiraishi K, Matsuyama H. Gonadotropin actions on spermatogenesis and hormonal therapies for spermatogenic disorders. *Endocr J* 2017; 64: 123–31.
- 25 Coviello AD, Matsumoto AM, Bremner WJ, Herbst KL, Amory JK, *et al*. Low-dose human chorionic gonadotropin maintains intratesticular testosterone in normal men with testosterone-induced gonadotropin suppression. *J Clin Endocrinol Metab* 2005; 90: 2595–602.
- 26 Hsieh T, Pastuszak AW, Hwang K, Lipshultz LI. Concomitant Intramuscular human chorionic gonadotropin preserves spermatogenesis in men undergoing testosterone replacement therapy. *J Urol* 2013; 189: 647–50.
- 27 Burris AS, Rodbard HW, Winters SJ, Sherins RJ. Gonadotropin therapy in men with isolated hypogonadotropic hypogonadism: the response to human chorionic gonadotropin is predicted by initial testicular size. *J Clin Endocrinol Metab* 1988; 66: 1144–51.
- 28 Liu PY, Gebiski VJ, Turner L, Conway AJ, Wishart SM, *et al*. Predicting pregnancy and spermatogenesis by survival analysis during gonadotrophin treatment of gonadotrophin-deficient infertile men. *Hum Reprod* 2002; 17: 625–33.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

©The Author(s)(2018)



Supplementary Table 1: Statistical power between pretreatment and posttreatment in the human chorionic gonadotropin/testosterone undecanoate group

	Pretreatment (n=54)	Posttreatment (n=54)	Power	Sample size at 0.8 power
Pubic hair Tanner stage	1.8±0.9	4.8±0.6	1.00	-
Genital Tanner stage	1.8±1.0	4.9±0.2	1.00	-
Testicular volume (ml)	4.6±3.2	12.0±4.1	1.00	-
Therapeutic T (ng ml ⁻¹)	0.4±0.4	3.0±1.2	1.00	-

Analysis of genital Tanner stage and testicular volume excluded patients whose TV could not be measured precisely. hCG: human chorionic gonadotropin; TU: testosterone undecanoate; T: testosterone; TV: testicular volume

Supplementary Table 2: Statistical power between pretreatment and posttreatment in the human chorionic gonadotropin group

	Pretreatment (n=53)	Posttreatment (n=53)	Power	Sample size at 0.8 power
Pubic hair Tanner stage	1.7±0.6	4.4±1.0	1.00	-
Genital Tanner stage	1.7±0.7	4.5±0.8	1.00	-
Testicular volume (ml)	3.9±4.2	12.2±4.4	1.00	-
Therapeutic T (ng ml ⁻¹)	0.4±0.3	3.2±1.8	1.00	-

Analysis of genital Tanner stage and testicular volume excluded patients whose TV could not be measured precisely. hCG: human chorionic gonadotropin; TU: testosterone undecanoate; T: testosterone; TV: testicular volume

Supplementary Table 3: Statistical power between the two groups after treatment

	hCG/TU group (n=54)	hCG group (n=53)	Power	Sample size at 0.8 power
Pubic hair Tanner stage	4.8±0.6	4.4±1.0	0.71	127
Genital Tanner stage	4.9±0.2	4.5±0.8	0.92	-
Testicular volume (ml)	12.0±4.1	12.2±4.4	0.06	NA
Therapeutic T (ng ml ⁻¹)	3.0±1.2	3.2±1.8	0.10	NA
Seminal spermatozoa appearance, n (%)	29 (53.7)	28 (52.8)	0.05	NA
Sperm concentration (10 ⁶ ml ⁻¹), median (interquartile range)	15.6 (8.3–54.1)	15.7 (9.6–50.1)	0.05	NA
Median time to achieve pubic hair Tanner stage III (month), (95% CI)	7 (5.4–8.6)	11 (9.3–12.7)	0.53	177
Median time to achieve pubic hair Tanner stage V (month), (95% CI)	19 (14.5–23.5)	25 (22.6–27.4)	0.19	693
Median time to achieve genital Tanner stage III (month), (95% CI)	6 (5.3–6.7)	9 (7.4–10.6)	0.43	212
Median time to achieve genital Tanner stage V (month), (95% CI)	15 (11.7–18.3)	21 (16.5–25.5)	0.28	410
Median time to normalize serum T (months), (95% CI)	3 (2.3–3.7)	7 (6.0–8.0)	0.98	-
Median time to achieve sperm concentration >0 ml ⁻¹ (month), (95% CI)	35 (31.3–38.7)	30 (27.9–32.1)	0.08	2858
Median time to achieve sperm concentration >15 × 10 ⁶ ml ⁻¹ (month), (95% CI)	57 (48.3–65.7)	53 (45.3–60.7)	0.05	19,360

Seminal spermatozoa appearance indicates the rate of seminal spermatozoa appearance (sperm concentration >0 ml⁻¹) in each group. Analysis of genital Tanner stage excluded patients whose TV could not be measured precisely. Median sperm concentration was calculated from patients with successful spermatogenesis. Analysis of median time to achieve Tanner stage (III and V) excluded the patients who achieved Tanner stage (III and V) at the beginning of treatment and whose TV could not be measured precisely. hCG: human chorionic gonadotropin; TU: testosterone undecanoate; T: testosterone; NA: not available; CI: confidence interval; TV: testicular volume

Supplementary Table 4: Statistical power between the human chorionic gonadotropin/testosterone undecanoate subgroups with basal testicular volume ≥4 ml and <4 ml after treatment

	BTV ≥4 ml (n=27)	BTV <4 ml (n=23)	Power	Sample size at 0.8 power
Median time to sperm concentration >0 ml ⁻¹ (months), (95% CI)	26 (24.2–27.8)	38 (39.4–39.6)	0.14	465
Median time to sperm concentration >15 × 10 ⁶ ml ⁻¹ (months), (95% CI)	38 (20.6–55.4)	57 (51.0–63.0)	0.13	548
Sperm concentration (10 ⁶ ml ⁻¹), median (interquartile range)	28.0 (12.0–77.6)	11.6 (4.3–31.2)	0.23	455

Median sperm concentration was calculated from patients with successful spermatogenesis. hCG: human chorionic gonadotropin; TU: testosterone undecanoate; BTV: basal testicular volume; CI: confidence interval

Supplementary Table 5: Statistical power between the human chorionic gonadotropin subgroups with basal testicular volume ≥4 ml and <4 ml after treatment

	BTV ≥4 ml (n=17)	BTV <4 ml (n=32)	Power	Sample size at 0.8 power
Median time to sperm concentration >0 ml ⁻¹ (months), (95% CI)	21 (17.4–24.6)	33 (29.7–36.3)	0.22	315
Median time to sperm concentration >15 × 10 ⁶ ml ⁻¹ (months), (95% CI)	34 (7.2–60.8)	56 (47.4–64.6)	0.20	369
Sperm concentration (10 ⁶ ml ⁻¹), median (interquartile range)	16.3 (8.9–55.4)	14.3 (10.0–27.5)	0.05	NA

Median sperm concentration was calculated from patients with successful spermatogenesis. hCG: human chorionic gonadotropin; TU: testosterone undecanoate; BTV: basal testicular volume; NA: not available