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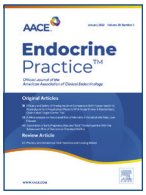

Original Article

Effect of Combined Low Dose Human Gonadotropic Hormone , Follicle Stimulating Hormone, and Testosterone Therapy (LFT Regimen) Versus Conventional High Dose Human Gonadotropic Hormone and Follicle Stimulating Hormone on Spermatogenesis and Biomarkers in Men With Hypogonadotropic Hypogonadism

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ABSTRACT

Objective: In male congenital hypogonadotropic hypogonadism (CHH), it was observed that lower dose human gonadotropic hormone (hCG) can maintain normal intratesticular testosterone levels. We propose this study to compare the low-dose hCG, follicle stimulating hormone (FSH), and Testosterone (T) [LFT Regimen] to conventional treatment to induce virilization and fertility.
Design: This open-label randomized pilot study was conducted from June 2020 to December 2021.
Subjects and outcome measures: CHH were randomly assigned to either the LFT regimen (Group A)-low-dose hCG (500U thrice per week), FSH (150U thrice per week), and T(100 mg biweekly) or conventional therapy(GroupB) with high hCG dose(2000U thrice per week) and the same FSH dose. The hCG dosage was titrated to reduce anti-mullerian hormone (AMH) by 50% and normalization of plasma T in groups A and B, respectively. The primary objective was to compare the percentage of individuals who achieved spermatogenesis between the two groups.
Results: Out of 30 patients, 23 (76·7%) subjects achieved spermatogenesis, and the median time was 12 (9-14·9) months. There was no difference in achieving spermatogenesis between the two groups (64·3% vs 7·5%,$P = 0·204$), and even the median time for spermatogenesis was similar (15months vs 12months,$P = 0·248$). Both groups had nonsignificant median plasma AMH at spermatogenesis, [6·6 ng/ml (3·3-9·76) vs4·41 ng/ml (2·3-6·47), $P = 0·298$]. Similarly, the median plasma Inhibin B at

Abbreviations: AMH, Anti-mullerian hormone; AMS, Aging Males scale; CHH, congenital hypogonadotropic hypogonadism; FG, Ferriman-Gallwey; FSH, follicle stimulating hormone; GnRH, Gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; HH, Hypogonadotropic hypogonadism; HPT, hypothalamic-pituitary-testicular; Inh B, Inhibin B; ITT, Intratesticular testosterone; LH, luteinizing hormone; PDS, Pubertal Development Scale; T, testosterone; TV, testicular volume; QoL, quality of life; ROC, Receiver Operating Characteristics; SD, standard deviation.

Type of article: clinical trial.

Attestation statement: The subjects in this trial have not concomitantly been involved in other randomized trials. Data regarding any of the subjects in the study have not been previously published unless specified. Data will be made available to the editors of the journal for review or query upon request.

Data sharing statement: Data will be shared for further analysis if required.

Ethics approval: The Institutional Ethics Committee of the Postgraduate Institute of Medical Education and Research, Chandigarh (India), approved the study (No. PGI/IEC/2020/000514). It was registered in CTRI on June 5, 2020, bearing a registration number CTRI/2020/06/025622 (www.ctri.nic.in). The first subject was enrolled on June 11, 2020.

Informed consent: Written informed consent for enrolment was obtained from participants or their relatives as applicable.

Consent for publication: All authors have done sufficient work in the field to justify authorship for this article and have provided consent for publication.

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spermatogenesis between groups were comparable [152·4 pg/ml (101·7-198·0) vs49·1 pg/ml (128·7-237·3), $P = 0·488$].

Conclusions: A reasonable approach to induce fertility in male CHH is to initiate combination therapy using FSH, low-dose hCG targeting AMH <6·9 ng/ml, along with T to achieve normal range. Monitoring AMH could serve as a proxy indicator of spermatogenesis.

Introduction

Two primary functions of mature male testis are spermatogenesis and androgen production. Both these functions are under the control of the hypothalamic-pituitary-testicular (HPT) axis. Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus acts on the pituitary to stimulate the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), the 2 main endocrine stimulators of the testis. LH causes the maturation of interstitial Leydig cells, which are the primary source of testosterone (T) in males. FSH causes the multiplication of immature Sertoli cells, which release the anti-mullerian hormone (AMH).[1] Under the influence of intratesticular testosterone (ITT), immature Sertoli cells mature and release growth factors required for germ cell survival. Therefore, an optimal level of ITT is a prerequisite for adequate spermatogenesis. Mature Sertoli cells also produce Inhibin B (Inh B), which regulates FSH secretion via negative feedback on the HPT axis.[2,3] Low AMH and high Inh B levels indicate the presence of mature Sertoli cells and thus are an indirect functional marker of optimal IIT and spermatogenesis.[1,4]

In males with congenital hypogonadotropic hypogonadism (CHH), the HPT axis fails to get activated during adolescence, resulting in low gonadotropin and androgen levels. This results in the absence of secondary sexual characters, loss of libido, infertility etc.[5] Hormonal replacement is the first-line option in the management of hypogonadotropic hypogonadism (HH). The choice of hormone to be administered primarily depends on whether fertility is desired or not. Testosterone replacement therapy is the standard of care for those seeking HH treatment for manifestations other than infertility. On the other hand, if the patient's main treatment objective is to gain fertility, gonadotropin therapy or pulsatile GnRH therapy needs to be started. Due to the cumbersome nature of the GnRH portable pump, gonadotropin therapy seems more feasible.[6,7]

The regime of gonadotropin therapy has evolved over the years, from human chorionic gonadotropin (hCG) monotherapy to initial hCG followed by a combination of hCG and FSH. Currently, FSH and hCG are administrated from the outset of treatment as many studies have shown better results of this regimen in the induction of spermatogenesis.[8] Usually, high-dose hCG is used in standard gonadotropin therapies. The rationale behind using such doses is to achieve normal plasma total T level, which is traditionally used as a surrogate marker of optimal IIT levels required for spermatogenesis. However, recently, it has been observed that adequate ITT levels essential for sperm production could be achieved even at hCG doses lower than that required for normalizing plasma total T levels.[9,10] Thus, administration of high-dose hCG might not be obligatory for fertility induction in HH patients.

Apart from infertility, eunuchoid body habitus, lack of libido, sexual infantilism, absence of secondary sexual characters, etc, are the major manifestations of HH and can lead to anxiety, depression, and poor quality of life (QoL) in such patients.[11] Low plasma total T level is responsible for these presentations, and hence its normalization is also an essential aspect of HH management. This can be achieved by either high dose hCG or exogenous T therapy. Though high-dose hCG will normalize plasma total T levels, few studies have reported that long-term usage of high-dose hCG can have a detrimental effect on testicular functioning.[12,13] On the other hand, exogenous T will not impair sperm production in HH as the HPT axis of these patients is already nonfunctional.

With this background, we postulated that low-dose hCG with FSH could be an alternative approach to standard high-dose hCG-based therapy for the treatment of infertility in HH patients, while concomitant administration of exogenous T could be given for triggering virilization and improvement of other hypogonadal-related symptoms. Titration of hCG dose could be done based on plasma AMH levels as a proxy marker of ITT, instead of plasma T.

Methodology

This open-labelled prospective randomized pilot study was conducted in the Endocrinology Outpatient Department at a tertiary institute in North India from June 2020 to December 2021. The trial had been registered.

Male patients aged 14-40 years, diagnosed with CHH and willing to participate were included in the study. Diagnosis of HH was made based on history and clinical examination suggestive of sexual infantilism and low hormone levels of plasma FSH, LH, and total T (Reference values: FSH- 1·5-12·4mIU/ml, LH- 1·7-8·6mIU/ml, T- 9·9-27·8 nmol/l). A clinically estimated mean testicular volume (TV) of less than 4 mL was an additional criterion used to ensure the exclusion of partial gonadotropin deficiency. Postpubertal or adult-onset HH, functional hypogonadism, testicular disorders, cryptorchidism, deranged liver function tests and prior gonadotropin therapy were considered as exclusion criteria.

Clinical parameters were recorded, including anthropometry and virilization by tanner staging (Genitalia stage and pubic hair stage) and modified Ferriman-Gallwey (FG) score for males.[14] TVs by orchidometer and ultrasound were done and the mean of Right and Left TVs were noted. Baseline plasma FSH, LH, T, cortisol, thyroxine, prolactin, serum electrolytes, complete hemogram, liver function tests, Inh B, AMH, and Estradiol were measured. Hormonal estimation was done using electrochemiluminescence immunoassay (Elecsys 2010 Analyzer, Roche Diagnostics). The dynamic range of AMH is 0.01-46 ng/ml with a lower limit of detection of 0.01 ng/ml. The inter-assay coefficient of variation was 3.5% and the intra-assay coefficient of variation was 1.7%. Magnetic resonance imaging of the brain focusing on the pituitary, hypothalamus, olfactory placode, and sulcus was done. To assess patient satisfaction with virilization Pubertal Development Scale (PDS) was used and QoL was evaluated using the Aging Males Symptoms (AMS) scale.[15,16]

The sample size was estimated based on the percentage of individuals who achieved spermatogenesis—considering a 20% difference in those who achieved spermatogenesis with high-dose hCG and LFT regimen as non-significant. Our sample size was 12 subjects for each group at a power of 80% and confidence interval of 95%. For possible attrition, it was decided to include 30 subjects in total. A random allocation sequence table with a block size of 4 was used for randomization. Subjects were randomly assigned to either

group A, receiving LFT regimen, a combination of low-dose hCG (500U thrice per week, subcutaneous) plus FSH (150U thrice per week, subcutaneous) plus T (100 mg biweekly, intramuscular) or group B, receiving conventional therapy of hCG (2000U thrice per week, subcutaneous) plus FSH (150U thrice per week, subcutaneous). The hCG and FSH used were highly purified urinary derivatives (INTAS pharmaceuticals) and for T, it was T enanthate (Zydus Cadila).

Subjects were followed up till the achievement of spermatogenesis (sperm count $>1 \times 10^6$/ml) or a maximum of 18 months, whichever was earlier. Follow-up visits were conducted every 6 weeks. At every visit, assessment for virilization (facial hair, voice change, FG Score, PDS, Tanner staging), QoL by AMS scale and clinical examination for potential side effects like acne and gynecomastia was done. Plasma T and AMH levels were measured 6 weekly, and Inh B every 3 months. In group A, to ensure endogenous T (produced by Leydig cells under the effect of hCG treatment) measurement, a sample for plasma T was taken after skipping the third dose of exogenous T injection so that there is a gap of 4 weeks before measuring nadir total T. The dose of hCG in group A was titrated (increment of 1000-2000U per week) according to the 6 weekly AMH values, with a target fall of 50% from the previous value while in group B, titration was based on plasma total T with a target to normalize it. Exogenous T dose was titrated to target nadir total T > 9 nmol/L in group A which was measured after 24 h of hCG injection. TV assessment by orchidometer, sonographic of TV and semen analysis (after 3 to 5 days of abstinence) was done at 3 monthly intervals.

The primary objective of our study was to compare the percentage of individuals who achieved spermatogenesis between the two groups (sperm count $>1 \times 10^6$/ml). The secondary objective was to compare the median time to induce spermatogenesis, TV, endogenous T, AMH, Inh B levels, and weekly hCG dose at spermatogenesis, virilization, and QoL.

Statistical Analysis

Data obtained were analyzed by IBM SPSS version 26·0 (IBM Corp). Variables were analyzed for descriptive data and normality of distribution. Categorical variables were shown as numbers and percentages. Continuous data were reported as mean with standard deviation (SD) for normal distribution or median with interquartile range for skewed distribution. Inter-groups and Intra-groups analysis of baseline and follow-up parameters was done using Chi-Square/Fisher's Exact test for categorical variables, Mann–Whitney U test, and Wilcoxon Signed rank/Friedman ANOVA test for continuous variables without normal distribution. Kaplan–Meier method with Log-rank test was used to compare the median time for spermatogenesis between the groups. The time to initiation of spermatogenesis or censoring of the data was measured from randomization on an intention-to-treat basis. The primary analysis included data from each patient up to the date of spermatogenesis initiation, or last follow-up visit. Correlation between ≥2 continuous variables was done using Spearman's Rho. Receiver Operating Characteristic (ROC) curves were calculated to find cut-off values for various parameters as required. Binary logistic regression analysis was conducted to find predictors of successful Spermatogenesis. Cox Regression analysis was conducted to find predictors for the time for initiation of Spermatogenesis. For all statistical tests, $P < 0·05$ was considered significant.

Results

A total of 33 patients with HH were reviewed for inclusion in the study. Out of this, 1 patient had postpubertal HH, 1 refused to consent, and 1 had cryptorchidism. Therefore, 30 patients fulfilling the eligibility criteria were randomized into 2 groups (Group A = 14, Group B = 16) and were analyzed (Fig. 1).

Highlights

- LFT regimen is a rational approach for fertility induction.
- Our study demonstrates the AMH and inh B as proxy indicators of spermatogenesis.
- Longer treatment duration, AMH <6.9 ng/ml and T >6.96 nmol/L predicts spermatogenesis.
- The available data do not indicate that prior T treatment has any adverse effect.

Clinical Relevance

This trial described a novel way of treating congenital hypogonadotropic hypogonadism (LFT regimen) and was shown to be noninferior to the conventional treatment. It also explored newer biomarkers (anti-mullerian hormone and Inhibin B) as a treatment monitoring modality. This information from this study can make a significant contribution to clinical practice.

The overall mean (SD) age of the patients was 22·6 (5·3) years. Mean (SD) weight and body mass index were 63·5 (14·9) kg and 22·4 (4) kg/m2 respectively. Of the 30 patients, 46·7% had some form of pituitary defects, most common was agenesis/hypoplasia (7 patients), 16·7% had Kallman syndrome, while in rest, no identifiable abnormalities could be found. Three (10%) subjects had multiple pituitary hormone deficiencies and required corticosteroids and thyroxine replacement. There were no statistically significant differences in the baseline parameters of the 2 groups, as shown in Table 1.

The median weekly doses of hCG at the time of spermatogenesis in groups A and B were 4000 (4000-8000) U/week and 9500 (6000-15000) U/week, respectively ($P = 0·013$). All patients in both groups achieved the target FSH level (4-8mIU/ml) with a constant FSH dose of 150 U/thrice per week. The plasma level was measured within 24 h of FSH injection.

Between group A [64·3% (9 out of 14)] and group B [87·5% (14 out of 16)] ($P = 0·204$) (Table 2), the overall median (interquartile range) time for spermatogenesis was 12 (9-14·9) months. The median time for initial spermatogenesis in group A [15 (12-17·9) months] was not statistically different from that in group B [12 (9-14·9) months] ($X^2(1) = 3·376$, $P = 0·066$) (Table 2, Fig. 2). Comparison of median sperm concentrations between group A and B [8·5 (2·5-18·0) million/ml vs13·0 (2·5-30·0) million/ml] was statistically not significant ($P = 0·569$). Patients achieving spermatogenesis had higher basal FSH, T and Inh B at follow up. (Supplementary Table 1). The median time to spermatogenesis was shorter in patients receiving prior T therapy (Supplementary Table 2).

Hormonal Concentrations

There was a significant increase in T with both regimens. Group A patients were having significantly lower T compared to Group B till 12 months ($P < 0·05$) (Table 3). Spermatogenesis was initiated at a much lower total T level in Group A compared to Group B (8·42 (5·84-11·25) nmol/l vs16·72 (12·78-24·26) nmol/l, $P < .001$).

A remarkable and statistically significant reduction from baseline to the last follow-up AMH levels was observed, dropping from 18·69 (11·14-29·9) to 3.83 (1.85-5.78) ng/mL ($P = 0·005$). No substantial differences in AMH levels were noted between the two

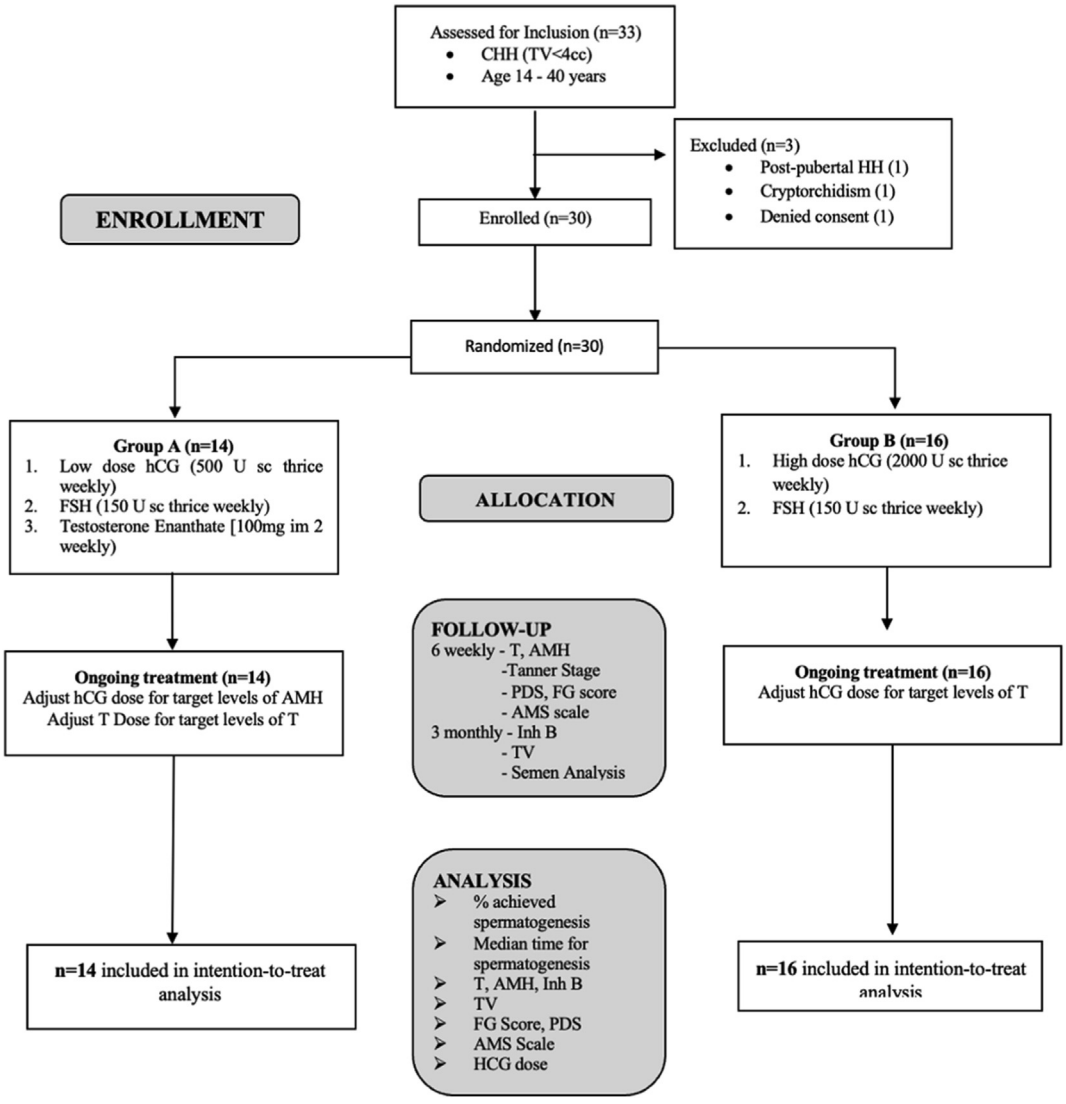

Fig.1. Trial Profile.

groups during serial follow-up assessments (Table 3). Both groups reached similar median plasma AMH levels at the induction of spermatogenesis, (6·6 (3·3-9·76) ng/ml vs4·41 (2·3-6·47) ng/ml, $P = 0·298$).

A notable and statistically significant increase in Inh B levels, rising from 38.88 (10.5-58.63) pg/mL to 161.57 (93.93-161.57) pg/mL ($P = .007$). Interestingly, there were no significant differences in Inh B levels between the groups during the serial follow-up, as highlighted in Table 3. Furthermore, both groups achieved similar Median plasma Inh B at spermatogenesis (152·4 pg/ml (101·7-198·0) vs149·1 pg/ml (128·7-237·3), $P = 0·488$).

Mean Testicular Volume

There was a consistent and noteworthy increase in mean TV with the administered treatment, which was statistically significant ($P = .01$) (Table 3). During the initiation of spermatogenesis, there was no significant difference in mean TV between groups A and B (3·31 (2·6-3·98) cc vs3·67 (3·38-5·06) cc, $P = 0·122$). (Table 2). Although group B exhibited significantly higher TV measurements up to 9 months, the difference was no longer significant from 12 months onward.

Virilization

Subjects in both groups achieved adequate virilization with similar improvement in the Tanner stage, as shown in Table 2. In addition, facial hair growth and voice changes were observed in all subjects in both groups.

A substantial increase in median FG scores from 10 (9-11) to 16 (13-21) ($P < .001$). This change was uniform across both groups, with no statistically significant discrepancies between them ($P = .806$).

A statistically significant increase in PDS from baseline of 8 (7-10) and 9 (7·25-10) to 14 (12·5-16) and 16 (13-18) in Groups A and B, respectively, was achieved ($P < 0·001$). No statistically significant difference was found between the groups in PDS ($P = 0·272$). (Supplementary Table 3).

QoL

There was a statistically significant improvement in the QoL in both groups. After treatment, AMS scores at 9 months in Group A, 32 (24·5-40) and B, 38 (31-40) were significantly lower than pre-treatment (A, 70 (61·2-75·7) and B, 72(65-77)) ($P < 0·001$)

Table 1
Baseline Characteristics of Patients and Their Comparison Between the Groups

Parameters	Overall ($n = 30$)	Group A ($n = 14$)	Group B ($n = 16$)	*P*-value
Age (y)	22·6 ± 5·37	23·57 ± 5·98	21·75 ± 4·81	0·363
Weight (kg)	63·58 ± 14·94	60 ± 17·77	66·71 ± 11·63	0·226
Height (cm)	167·64 ± 9·41	163·19 ± 10·48	171·53 ± 6·44	0·053
BMI (kg/m^2)	22·42 ± 4	22·09 ± 4·16	22·7 ± 3·97	0·683
US:LS ratio	0·84 ± 0·07	0·85 ± 0·08	0·84 ± 0·07	0·958
Arm span (cm)	172·78 ± 11·52	167·82 ± 13·01	177·13 ± 8·17	0·054
FSH (mU/ml)	0·55 (0·3-1·06)	0·4 (0·3-0·64)	0·89 (0·29-1·16)	0·124
LH (mU/ml)	0·28 (0·1-0·48)	0·27 (0·1-0·45)	0·33 (0·1-0·56)	0·471
DHEAS (ug/dl)	175·32 ± 74·22	175·1 ± 73·45	176·18 ± 108·65	0·987
Thyroxine (ug/dl)	8·24 ± 2·19	8·8 ± 2·11	7·76 ± 2·2	0·198
Cortisol (nmol/L)	244 (195·25-355·15)	279 (191-420·73)	228·68 (195·5-324)	0·485
Prolactin (ng/ml)	7·17 (5·38-9·12)	8·05 (5·22-12·92)	6·65 (5·27-8·91)	0·485
Hemoglobin (g/dl)	12·45 (11·83-14·18)	12·3 (11·1-14·23)	12·45 (11·95-14·18)	0·515
Creatinine (mg/dl)	0·7 (0·63-0·82)	0·66 (0·62-0·8)	0·8 (0·65-0·88)	0·203
Bilirubin (mg/dl)	0·6 (0·43-0·69)	0·6 (0·42-0·8)	0·6 (0·5-0·65)	0·739
AST (U/L)	28·5 (23·75-39)	28 (24·75-42·75)	29·5 (23-38·25)	0·851
ALT (U/L)	29 (20·5-41·75)	29·5 (18·75-45·75)	27 (21-43·25)	0·884
ALP (U/L)	206 (137-277·5)	165 (114-310)	213 (157·5-270·75)	0·614
T (nmol/L)	0·34 (0·09-0·55)	0·4 (0·09-0·51)	0·31 (0·11-0·6)	0·818
AMH (ng/ml)	18·69 (11·14-29·9)	16·59 (11·08-36·18)	20·71 (11·42-28·43)	0·647
Inh B (pg/ml)	38·88 (10·5-58·63)	36·45 (10·39-52·62)	46·27 (18·25-67·83)	0·487
E2 (pg/ml)	5 (5-5)	5 (5-5)	5 (5-6·03)	0·513
Mean TV (cc)	0·79 (0·49-1·26)	0·77 (0·45-1·05)	1·01 (0·54-1·78)	0·170
Tanner (Genitalia) Stage	1 (1-1)	1 (1-1)	1 (1-1)	1·000
Tanner (Pubic Hair) Stage	2 (1-2)	1·5 (1-2)	2 (2-2)	0·095
FG Score	10 (9-11)	10 (9-11·25)	10 (9-11)	0·794
PDS	8·5 (7-10)	8 (7-10)	9 (7·25-10)	0·594
Sperm Count (million/ml)	0 (0-0)	0 (0-0)	0 (0-0)	1·000
Sperm Motility (%)	0 (0-0)	0 (0-0)	0 (0-0)	1·000
AMS Score	72 (63·5-75·75)	70 (61·25-75·75)	72 (65-77)	0·754
Gynecomastia	9 (30%)	3 (21·4%)	6 (37·5%)	0·440
DEXA (T-score)	−2·8 (−3·5–1·35)	−2·8 (−3·55–2)	−2·8 (−3·15–0·95)	0·368
Microlithiasis	3 (10%)	2 (14·3%)	1 (6·3%)	0·343
Panhypopituitarium	3 (10%)	2 (14·3%)	1 (6·3%)	0·586
Synkinesia	3 (10%)	2 (14·3%)	1 (6·3%)	0·586
Kallman Syndrome	5 (16·7%)	1 (7·1%)	4 (25%)	0·336
Prior T Therapy	21 (70%)	8 (57·1%)	13 (81·3%)	0·236
MRI Abnormality	14 (46·7%)	7 (50%)	7 (43·8%)	1·000

Abbreviations: AMS = Aging Males Symptoms; AMH = anti-mullerian hormone; FG, Ferriman-Gallwey; FSH = follicle stimulating hormone; Inh B, inhibin B; LH = luteinizing hormone; PDS = Pubertal Developmental Scale; TV, testicular volume.
Data are shown as mean ± SD for continuous variables with normal distribution and median with interquartile range for continuous variables without normal distribution. *P* values were calculated as follows a) Independent *t* test for continuous variables with normal distribution, b) Mann–Whitney U test for continuous variables without normal distribution, c) Chi Square test or Fisher's exact test for categorical variable.

Table 2
Overall Response to Treatment and their Comparison Among the Groups Following Treatment

Parameters	Overall	A	B	*P*-value
Spermatogenesis (n)	23 (76·7%)	9 (64·3%)	14 (87·5%)	0·204
Median time for Spermatogenesis (mo)	12 (9-14·9)	15 (12-17·9)	12 (9-14·9)	0·248
At Spermatogenesis				
T (nmol/l)	12·81 (8·42-19·20)	8·42 (5·84-11·25)	16·72 (12·78-24·26)	<0·001
AMH (ng/ml)	4·69 (2·68-6·9)	6·6 (3·3-9·76)	4·41 (2·3-6·47)	0·298
Inh B (pg/ml)	150 (118-227·4)	152·4 (101·7-198·0)	149·1 (128·7-237·3)	0·488
Mean TV (cc)	3·6 (3·3-4·5)	3·31 (2·6-3·98)	3·67 (3·38-5·06)	0·122
hCG Dose (U/wk))	6000 (6000-12000)	4000 (4000-8000)	9500 (6000-15000)	0·013
Tanner Scale (Genitalia)	3 (2-3)	3 (2-3)	3 (3-3)	0·167
Tanner Scale (Pubic Hair)	4 (3-4)	3 (3-4)	4 (3-4)	0·055
FG Score	16 (13-21)	16 (12·25-22·75)	17 (13-21)	0·806
PDS	16 (13-17)	14 (12·5-16)	16 (13-18)	0·272
AMS Score	33 (28·25-40)	32 (24·5-40)	38 (31-40)	0·132

Abbreviations: AMS = Aging Males Symptoms; AMH = anti-mullerian hormone; FG, Ferriman-Gallwey; hCG, human chorionic gonadotropin; Inh B, inhibin B; PDS = Pubertal Developmental Scale; TV, testicular volume.
P values were calculated as follows a) Mann–Whitney U test for continuous variables without normal distribution, b) Chi Square test or Fisher's exact test for categorical variable, c) Kaplan–Meier analysis with Log-rank test to compare the median time for Spermatogenesis.

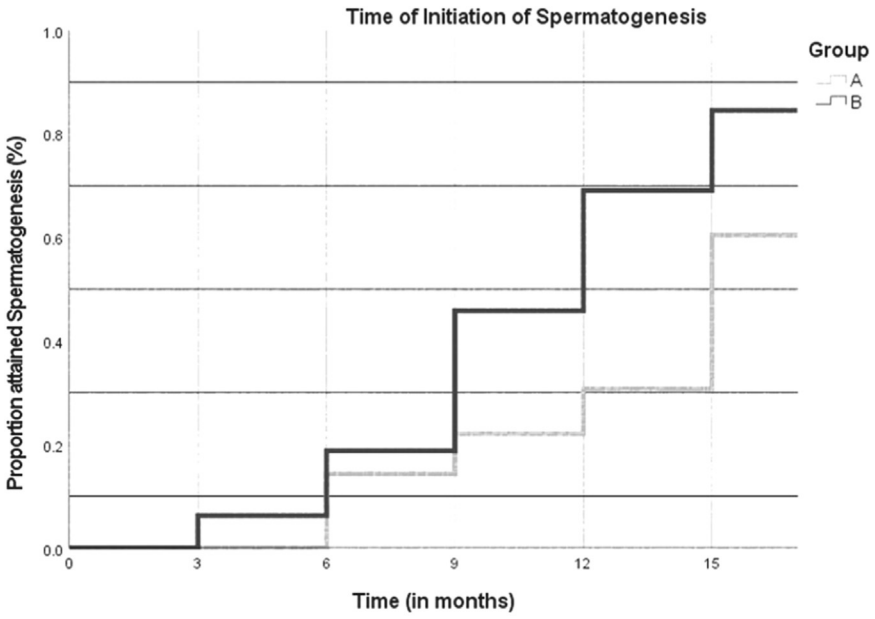

Fig. 2. Kaplan–Meier estimates of the median time of initiation of spermatogenesis (achievement of sperm count of $>1 \times 10^6$ ml) between groups in a time-to-first-event analysis.

(Supplementary Appendix). AMS scores were not significantly different when comparing post-treatment scores between the groups ($P = 0{\cdot}132$). All patients were successful in giving semen samples at the final follow-up.

Adverse Effects

During the follow-up, gynecomastia occurred in 13·3% (4/30) of subjects overall, and all were in group B (25%, 4/16 of patients in Group B). Acne occurred in 16·7% (5/30) patients overall and 7·1% (1/14) and 25% (4/16) in Groups A and B, respectively. ($P = 0{\cdot}336$) Treatment-related adverse effects were higher in Group B than in Group A; however, none required treatment discontinuation and were managed carefully. No allergic reaction to the highly purified urinary gonadotropins was noted in either Group.

Predictors for Spermatogenesis

We applied logistic regression to ascertain the effect of various baseline parameters on the likelihood of successful spermatogenesis induction. Age ($P = 0{\cdot}005$), baseline FSH ($P = 0{\cdot}012$), and presence of microlithiasis ($P = 0{\cdot}001$) were found to significantly impact the initiation of sperm production, while presence of panhypopituitarism or Kallman syndrome, prior T treatment, baseline T, AMH, Inh B, and TV had no significant effect on likelihood of successful spermatogenesis. However, on multivariate analysis, none of the above baseline parameters were found to be significant. Cox regression showed that a lower FG score (OR = 3·311, 95% confiCI 1·299-8·442, $P = 0{\cdot}012$) was a statistically significant predictor of longer duration of therapy required for spermatogenesis (Supplementary Tables 4 and 5).

During follow-up, T ($P < 0{\cdot}001$), AMH ($P = 0{\cdot}009$), Inh B (p-0·007), TV ($P < 0{\cdot}001$), the dose of hCG ($P = 0{\cdot}004$), duration of therapy($P < 0{\cdot}001$) and percent change in AMH from baseline ($P < 0{\cdot}001$) significantly predicted successful spermatogenesis. A multivariate logistics regression was performed to ascertain the effects of aforesaid variables on the likelihood that participants have successful spermatogenesis. The logistic regression model was statistically significant, x^2 (8) = 40·358, $P < 0{\cdot}001$· The model explained 58·6% (Nagelkerke R^2) of the variance in spermatogenesis outcome and correctly classified 86% of the cases. Higher basal T (OR 1·326, 95% CI 1·02-1·73, $P = 0{\cdot}038$), longer duration of therapy (OR 1·38, 95% CI 1·03-1·85, $P = 0{\cdot}033$), and higher percentage change in AMH from baseline (OR 1·06, 95% CI 1·01-1·12, $P = 0{\cdot}034$), were associated with an increased likelihood of spermatogenesis, while high AMH (OR 0·675, 95% CI 0·474-0·961, $P = 0{\cdot}029$) during follow-up was associated with a decreased likelihood of successful spermatogenesis (Supplementary Tables 6 and 7).

We performed ROC curve analysis (Supplementary Fig. 1) on the follow-up parameters, the cut-off values derived predicting successful spermatogenesis were AMH <6·9 ng/ml [Sensitivity(Sn) 81·8%, specificity (Sp) 75·5%, Area under curve (AUC) 0·837, $P < 0{\cdot}001$], Inh B of 92·4 pg/ml (Sn 94·7%, Sp 67·1%, AUC- 0·794, $P < 0{\cdot}001$) and T of 6·96 nmol/L (Sn 87%, Sp 60·8%, AUC- 0·782, $P < 0{\cdot}001$) respectively. The cumulative hCG dose of 3000 IU/week predicted successful spermatogenesis (Sn 95·7%, Sp 35·5%, AUC- 0·678, $P < 0{\cdot}001$). The median time to spermatogenesis had a cut-off of >4·5 months (Sn 82·4%, Sp 77%, AUC-0·877, $P < 0{\cdot}001$), with an average ultrasonographic mean TV of 3·015cc (Sn 82·4%, Sp 77%, AUC-0·817, $P < 0{\cdot}001$). Age <24 years was found to have a favourable outcome (Sn-78·3%, Sp 85·7%, $P < 0{\cdot}001$, AUC- 0·851, $P < 0{\cdot}001$) (Supplementary Table 8).

The increase in sperm count demonstrated a relatively weak, but positive association with Inh B (r = 0·393, 95% CI 0·209-0·550, $P < 0{\cdot}05$), total T (r = 0.315, 95% CI 0.176-0.442, $P < .05$), the duration of therapy (r = 0.422, 95% CI 0.298-0.533, $P < .05$), and TV (r = 0.392, 95% CI 0.225-0.537, $P < .05$). Conversely, as anticipated, albeit weak, there was a negative correlation with AMH (r = −0.385, 95% CI -0.511 to −0.242, $P < .05$). The correlation between sperm concentration and hCG dose was very weak (r = 0.194, 95% CI 0.053-0.327, $P < .05$).

On the other hand, the increase in TV exhibited a moderate positive correlation with plasma Inh B (r = 0.738, 95% CI 0.625 to 0.821, $P < .05$), total T values (r = 0.600, 95% CI 0.468 to 0.706, $P < .05$), the duration of therapy (r = 0.720, 95% CI 0.618 to 0.798, $P < .05$), and a moderate negative association with AMH (r = −0.505, 95% CI -0.631 to −0.353, $P < .05$) (Supplementary Table 9).

Table 3
Comparison of Serum Hormone (T, AMH, Inh B) Levels and Mean-TV from Baseline to 18 months of Treatment Between the 2 Groups

Parameters		Group	Basal	3m	6m	9m	12m	15m	18m	Friedman (p)
Testosterone (nmol/l)	Median (IQR)	A	0·4 (0·08-0·51)	3·7 (1·84-5·35)	4·35 (1·67-6·75)	6·59 (2·79-8·79)	6·18 (2·43-12·25)	10·53 (5·55-12·85)	10·6 (5·5-10·6)	0.006
		B	0·31 (0·11-0·59)	9·18 (5·14-20·14)	14·5 (12·29-24)	14·75 (10·1-23·36)	16·65 (12·62-19·2)	20·1 (10·17-20·1)	18·21 (18·21-18·21)	0·048
	Mann–Whitney (p)	A,B	0·818	0·005	<0·001	0·002	0·005	0·180	0·180	
AMH (ng/ml)	Median (IQR)	A	16·59 (11·08-36·18)	12·12 (8·98-20·85)	11·38 (4·91-15·4)	8·38 (3·73-13·95)	6·85 (3·23-11·1)	5·44 (3·38-7·19)	5·09 (2·39-6·48)	<0·001
		B	20·71 (11·42-28·43)	11·35 (5·22-21·48)	5·57 (3·74-8·22)	4·69 (2·56-5·98)	4·1 (2·57-6·69)	3·47 (1·97-9·2)	2·07 (1·28-2·07)	<0·001
	Mann–Whitney (p)	A,B	0·647	0·525	0·071	0·023	0·283	0·558	0·101	
Inhibin B (pg/ml)	Median (IQR)	A	36·45 (10·39-52·62)	55·93 (32·47-105·94)	79·93 (50·3-107·46)	130·65 (70·85-231·02)	120·41 (70·8-262·33)	107·14 (54·83-154·92)	161·58 (93·93-161·58)	0·034
		B	46·27 (18·25-67·83)	87·02 (73·23-93·89)	123·04 (92·44-164·43)	164 (144·64-229·71)	199·5 (191-199·5)	172 (172-172)	178 (178-178)	<0·001
	Mann–Whitney (p)	A,B	0·487	0·155	0·039	0·092	0·505	0·157	1·00	
Mean TV (cc)	Median (IQR)	A	0·77 (0·45-1·05)	1·33 (0·67-1·68)	1·88 (0·87-2·86)	3·16 (1·97-4·02)	3·5 (2·26-3·88)	3·48 (3·26-4·85)	3·77 (3·64-3·8)	<0·001
		B	1·01 (0·54-1·78)	1·98 (1·66-2·8)	2·9 (2·5-4·07)	3·94 (3·4-5·05)	4·05 (2·95-4·1)	4·3 (4·1-4·3)	4·75 (4·7-4·75)	0·026
	Mann–Whitney (p)	A,B	0·170	0·005	0·019	0·036	0·180	0·439	0·121	

Abbreviations: AMH = anti-mullerian hormone; Inh B, inhibin B; IQR, interquartile range; TV, testicular volume.
P values were calculated as follows a) Friedman ANOVA for comparison before and after within the groups, b) Mann Whitney U-teat for inter-group comparison at variables time-interval.

Discussion

To the best of our knowledge, this is the first randomized study comparing LFT regimen to conventional treatment with high dose hCG and FSH to induce virilization and fertility in CHH. Through this study, we tried to fill the lacunae in the literature regarding the ideal starting dose, duration, monitoring cut-offs, and the success rate of gonadotropin therapy in HH management. Normalization of total T with hCG therapy was not imperative for spermatogenesis. Longer treatment duration, higher follow-up total T, lower AMH at follow up, and greater reduction in AMH levels from baseline predicted successful spermatogenesis. Monitoring AMH levels can serve as an indicator of potential spermatogenesis, and 3000 IU per week of hCG was the minimal dose required to achieve spermatogenesis. We found that spermatogenesis can be achieved at a mean ultrasonographic TV of 3.01 ml. Furthermore, the analysis did not indicate that prior T treatment has any adverse effect, and spermatogenesis started before normalization of systemic total T at values more than 6.96 nmol/L.

In our study overall, 76·7% of patients achieved spermatogenesis within a median time of 12 months. In our study, 85% of subjects in group B receiving a high dose of hCG and FSH achieved spermatogenesis in the median time of 12 months. These findings were comparable with previous studies and meta-analyses, which showed an overall success rate in achieving spermatogenesis in 75 (69-81)%.[17,18] Some of the previous studies have shown that the time to achieve spermatogenesis ranges from 3 to 18 months, with an average time of 9-12 months.[19,20] However, early response to therapy in these studies might be attributed to the inclusion of patients with partial HH, as indicated by higher baseline TV. All the patients in the present study had CHH, as noted in a baseline mean TV of less than 4 ml. The percentage of individuals achieving successful spermatogenesis with the LFT regimen was slightly less than those receiving high dose therapy (64·3% vs 87·5%), and even the median time for initiation of spermatogenesis was a little longer (15 months vs 12 months), but this difference was not significant. For spermatogenesis, the median dose of hCG was 4000 IU per week with the LFT regimen, which was significantly lower compared to the 9500 IU per week used in the high dose group. This can be attributable to the very low dose of hCG (1500 IU/week) at the initiation of therapy. This dose was suboptimal as atleast 3000 IU/week of hCG was required for spermatogenesis as per the results of the present study.

From our study, hCG at a dose of more than 3000IU/week is closely related to spermarche. A previous study by Coviello et al showed that relatively low doses of hCG could maintain ITT within the normal range.[9] Other studies have shown that long-term high-dose hCG produces inflammatory changes in the testis, leading to germ cell apoptosis.[12,13] In this context, developing a regimen with an optimal dose of hCG sufficient to cause maturation of supporting Sertoli cells and low enough to prevent germ cell damage is necessary.

The percentage increase in systemic total T concentration has no significant effect on the prediction of spermatogenesis. The ROC curve shows that total T exceeding 6·96 nmol/L correlated with spermarche, lower than the normal range of total T in adults. This implied that systemic total T normalization was not necessary for sperm production and spermarche occurs before the T normalizes. Secondly, it is ITT that leads to Sertoli cell maturation and initiates spermatogenesis and is more important than systemic total T. These findings were similar to those in previous studies, showing that systemic total T levels do not correlate with sperm concentration.[21]

However, it is important to note that many HH patients seek medical assistance for absent/minimal virilization, low libido,

erectile dysfunction, and a poorer QoL. To counteract these, optimal levels of T are required.[11,22] As per our regimen, this virilizing androgen dose can be rapidly fulfilled by adding the exogenous T treatment to hCG and FSH, thereby avoiding the usage of high hCG doses to normalize systemic total T, which can cause long-term testicular damage. Without affecting ITT concentration and Sertoli or germ cell proliferation, this exogenous T could improve hypogonadal symptoms and the QoL.[23] Compared to high dose hCG, patients on LFT regimen showed similar improvement in the QoL and virilization, with normalization of T-directed by exogenous T. In a retrospective study, low-dose hCG maintained semen parameters in hypogonadal men undergoing concomitant TRT.[24]

In our study, prior T therapy was not associated with any adverse outcome. This contrasts with the previous report by Liu et al, where prior androgen therapy showed a poor outcome on subsequent fertility.[25] However, that study was not randomized, and the observed association may reflect that the most severe cases of GnRH deficiency might have been identified earlier and thus received androgens. Further subsequent studies and meta-analyses were in accordance with the present study.[17,26]

With the baseline and follow-up data at different time points, we could ascertain the predictors of successful spermatogenesis. In univariate analysis, younger age (<24 years) and basal Inh B levels greater than 26 pg/ml predicted successful spermatogenesis, but these associations did not hold in multivariate analysis. Similar to the current study's findings, a previous study showed that patients with a higher baseline Inh B (>60 pg/ml) had a higher treatment success rate.[27] During follow-up, longer duration of treatment (>4·5months), lower AMH after treatment (<6·9 ng/ml), higher percentage fall in AMH from baseline value, and higher T (6·96 nmol/l) can ascertain spermatogenesis. Using these follow-up variables to predict spermatogenesis has been defined for the first time in the literature.

Inh B is an established predictor of spermatogenesis.[4] Through ROC for Inh B, the value obtained in our study was 92 pg/ml with a sensitivity/specificity of 94·7%/67·1%, suggesting that Inh B > 92 pg/ml were more likely to achieve spermatogenesis during follow-up. Similarly, AMH < 6·9 ng/ml had a sensitivity/specificity of 81·8%/75·5% for achieving spermatogenesis. There is a decline in AMH when Sertoli cells mature under the influence of ITT, produced by Leydig cells due to hCG. Therefore, under the influence of gonadotropins, Sertoli cell maturation is evident as a decline in AMH and a rise in Inh B. It is worth noting that AMH levels began to decline before there was any significant increase in testicular size.[28] Monitoring the concentration of Inh B and AMH can provide information to measure the response to treatment and serve as a proxy indicator for the potential for spermatogenesis.

Regarding TV, we found that larger sonographic TV is a good predictor for early spermarche. The ultrasound mean TV cut-off value of 3·01 ml successfully predicted spermarche with a sensitivity and specificity of 82·1% and 77%, respectively. Therefore, a seminal fluid analysis should be performed when the TV reaches 3·01 ml, and the analysis should be repeated every 2 to 3 months. This is in accordance with previous studies, where TV is a positive predictor of spermatogenesis.[29,30]

The most common adverse reactions were acne and gynecomastia, observed in approximately 16·7% and 13·3% of subjects, and both side effects were more frequent in group B. In a similar study, as many as one-third of patients developed gynecomastia.[6,19] The presumed mechanism is excessive LH-induced aromatase activity, which is best minimized or prevented by using a lower dose of hCG.

A logical approach to induce fertility in HH males is to initiate combination therapy with FSH (targeting FSH in the range of 4-8 mIU/ml), hCG (primarily titrating to achieve target AMH levels), and exogenous T (targeting normal systemic total T). Under these conditions, Sertoli cells will be exposed to the full paracrine ITT levels required for spermatogenesis as evidenced by a decrease in AMH and an increase in Inh B. Exogenous T will provide sufficient virilization and improve the QoL of these subjects. The starting dose of hCG of 3000 IU per week, divided into at least 2 injections reasonable approach to start for induction of spermatogenesis. AMH, Inh B, and T concentrations should be measured every 4-6 weeks, and the dose should be adjusted, if necessary, until the steady target state is reached. This proposed regimen reduces the cost of more hCG doses, prevents the testis from the detrimental effects of high hCG doses, and helps maintain a good QoL.

The strength of our study lies in the fact that it is the first randomized study comparing gonadotropin regimens that followed strict eligibility criteria for only patients with CHH, excluding patients with partial hypogonadism, along with long-term follow-up, and its ability to deliver multiple predictors of successful spermatogenesis, few of which have not been previously reported. Applying the ROC-derived cut-off value and its application during follow-up make the results more robust. Such approaches not only enhance our understanding of normal development but may also be a way to maximize the effectiveness of treatment for patients in the future. The major limitation of the study was its small sample size leading to an underpowered study. Consequently, the significance of the regression analysis was questionable though the trend may remain the same. One factor for the small sample size can be partly attributed to the rarity of the disease. A study with larger sample sizes to evaluate fertility outcomes, with varying dose adjustments of hCG, FSH and T therapy, is required to optimize gonadotropin therapy in individuals with CHH.

Conclusion

The study indicated that initiation of spermatogenesis is both gonadotropin dose and duration-dependent. LFT regimen is noninferior to conventional treatment for CHH. Moreover, systemic total T normalization is not a prerequisite for achieving spermatogenesis, as sperm production can occur much before total T levels normalize. Furthermore, our study demonstrates the potential of AMH and inh B levels as proxy indicators for the likelihood of spermatogenesis.

Disclosure

The authors have no conflicts of interest to disclose.

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