



Prior testosterone replacement therapy may impact spermatogenic response to combined gonadotropin therapy in severe congenital hypogonadotropic hypogonadism

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Abstract

Objective To study the effect of prior testosterone replacement therapy (TRT) on the spermatogenic response to combined gonadotropin therapy (CGT) in severe and partial phenotype congenital hypogonadotropic hypogonadism (CHH) patients.

Design Retrospective cohort study.

Setting Tertiary care center.

Patients Patients of CHH without (n = 17) and with prior TRT (n = 18) were subdivided into severe and partial groups, based on mean testicular volume ≤ 3 cc and > 3 cc respectively.

Intervention Participants were treated with hMG at a dose of 75–150 U 3/week and gradually escalating doses of hCG until maximum dose (2000 U 3/week or 5000 U 2/week) or serum total testosterone of ≥ 3.5 ng/ml was reached.

Main outcome measures Final mean TV, trough serum testosterone (T), sperm concentration

Results Thirty-five patients (20 severe, baseline mean TV of 3.6 ± 2.7 ml) were started on CGT at 24.8 ± 6.1 years. The median duration of prior TRT was 38 (IQR 10–63.75) months in the exposed group. After 33 ± 12 months, final mean TV was 8.9 ± 5.5 ml, 86% achieved serum testosterone > 3.5 ng/ml and 70% achieved spermatogenesis [median 5 (0–12.6) million/ml]. Patients without prior TRT had significantly higher peak sperm count than those with prior- TRT (median 9 vs 0.05 million/ml, $p = 0.004$). This effect of prior TRT was more pronounced in severe phenotype patients (median 7 vs 0 million/ml, $p = 0.01$).

Conclusion Prior-TRT may interfere with spermatogenic response to CGT in CHH patients, especially in those with a severe phenotype.

Keywords Combined gonadotropin therapy (CGT) · Congenital hypogonadotropic hypogonadism (CHH) · Pubertal induction · Prior androgen therapy · Testosterone replacement therapy (TRT)

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Introduction

Congenital isolated hypogonadotropic hypogonadism (CHH) in men is one of the few treatable causes of male infertility [1]. 75–80% of CHH patients achieve spermatogenesis, either with combined gonadotropin therapy (CGT) comprising of human chorionic gonadotropin (hCG) and recombinant follicle-stimulating hormone (rFSH)/human menopausal gonadotropin (hMG) or pulsatile gonadotropin-releasing hormone (GnRH) therapy [2]. Degree of spontaneous testicular development and cryptorchidism are the well-known predictors of response [2]. Typically, partial phenotype [single testicular volume (STV) ≥ 4 ml or bi-testicular volume (BTV) ≥ 8 ml] patients have earlier (~ 6 months vs ~ 18 – 24) and better spermatogenic response to CGT than those with severe phenotype (STV < 4 ml or BTV < 8 ml) [1]. Poor spermatogenic response to CGT in the latter has been attributed to the absence of mini-puberty and slender Sertoli cell pool to support future spermatogenesis [1]. Sequential therapy with FSH pre-treatment followed by CGT or GnRH [3, 4], and more recently, gonadotropin therapy during infancy to mimic mini-puberty have been proposed to improve the future fertility potential in such patients [5].

The role of prior testosterone replacement therapy (TRT) on the future spermatogenic response to CGT remains debatable [6]. Two previous studies from the same center have shown the detrimental effects of prior TRT [7, 8]. However, few studies, including a meta-analysis, have refuted a negative impact of prior TRT on spermatogenesis and fertility [2, 9–11]. The majority of studies reporting no effect of prior TRT on spermatogenic response to CGT have included hypogonadotropic hypogonadism (HH) patients of varied etiologies (pre- and post-pubertal onset). Also, the conclusion regarding the effect of prior TRT in the meta-analysis was limited by ecological fallacy [2]. Importantly, two recent, larger studies found no interference from prior TRT; however, there were several potential confounders in these studies which might have affected the interpretations regarding the effect of prior TRT [11, 12].

In patients with partial phenotype, Sertoli cells would have had some exposure to mini-pubertal FSH with partial development prior to TRT. In contrast, patients with a severe phenotype have an immature testis with late fetal characteristics, due to lack of exposure to the perinatal FSH surge of mini-puberty. In the latter group, as demonstrated in histologic studies of primates, TRT may lead to early maturation of Sertoli cells suggesting a larger potential for the detrimental effect of prior TRT on the spermatogenic response to CGT in them [13]. Lack of phenotype-based subgroup analysis might have masked the effect of prior TRT in most of the previous studies reporting no

detrimental effect of prior TRT. Hence, we have analyzed the spermatogenic response to CGT in a cohort of CHH patients, including a phenotype-based subgroup analysis.

Patients

This retrospective study was conducted at a tertiary health care center in Mumbai, India. The institutional review board (Institutional Ethical committee II, Seth G S Medical College and KEM Hospital, Mumbai) approved the study. A waiver for consent was granted because of the retrospective nature of the study. Case records (2010–2019) of CHH patients receiving CGT for ≥ 12 months duration were analyzed. Diagnosis of CHH in a patient ≥ 18 years was based on following criteria: (1) clinical features of hypogonadism; (2) serum testosterone ≤ 1.0 ng/ml, and low/normal gonadotropin levels; (3) normal levels of other pituitary hormones; (4) absence of sellar lesion on magnetic resonance imaging and (5) no apparent systemic illness or stress. One of the following additional criteria was needed for the diagnosis below 18 years: (1) history of micropenis and/or cryptorchidism; (2) extra-reproductive phenotype or genetic variation associated with CHH. A proband was classified as Kallmann syndrome (KS) if the sense of smell was severely reduced on inquiry and/or 12-odor UPSIT (University of Pennsylvania smell identification test, Sensonics Inc, Cross-cultural version) score of ≤ 9 . Else, he was classified as normosmic CHH (nCHH). Severe and partial CHH phenotype was defined as the baseline (measured before giving TRT in the exposed group) mean TV ≤ 3 ml and > 3 ml respectively. To our best knowledge, none of the participants had received prior hCG or GnRH therapy. Based on the dose and duration of androgen exposure, patients were divided into two cohorts: without prior TRT and with prior TRT. Patients without prior TRT never received androgen or had minimal (50–100 mg testosterone enanthate given IM monthly for a total of 3–4 months only) exposure by the referring clinicians. This minimal dose was used in few ($n=4$) patients presenting before 18 years of age to “jumpstart” puberty with an initial provisional diagnosis of constitutional delay in growth and puberty and so was not counted as TRT. Patients with prior TRT had received androgen treatment for pubertal virilization in gradually (three monthly) escalating doses (50 mg incremental) starting from 50 mg once a month, reaching the adult dose (200–250 mg testosterone enanthate given IM every 2–3 weeks) around 9–12 months. From the last testosterone enanthate injection, eight weeks of washout period was considered in all patients before initiating CGT.

Combined gonadotropin therapy regimen included subcutaneous administration of highly purified urinary-derived hCG (FERTIGYN®, Sun Pharma, India) and hMG (GMH-HP®, Sun Pharma, India) available as lyophilized ampoules

with diluent via self-injection using an insulin syringe. All the patients were concurrently initiated on hMG and hCG. All the cases were started with hMG 75 units $3\times/$ week and dose escalation to 150 units $3\times/$ week was done in cases with inadequate inhibin-B and/or spermatogenesis responses. Initial dose of hCG in patients without prior TRT was 500 U twice weekly and 1000–2000 U twice weekly in patients with prior TRT. Dose of hCG was titrated every three months based on the trough (72 hours after the last hCG injection) serum total testosterone levels to achieve and maintain a level of ≥ 3.5 ng/ml, or till maximum hCG dose (2000 units thrice a week or 5000 units twice a week) was reached. Baseline and follow up testicular volumes were measured using a Prader orchidometer by one of the first two authors (RS/VP). Serum inhibin B levels had been documented at baseline and once every 6–12 months. On follow up, spermatogenesis was evaluated with semen analysis at 3–6 months intervals, which was reported in a standard format. Ejaculates were collected by masturbation at least 48 h after sexual abstinence. Response to CGT was recorded in terms of serum total testosterone ≥ 3.5 ng/ml, peak sperm concentration (defined as the highest sperm count achieved with CGT), and proportion of patients achieving various sperm output thresholds (any sperm, ≥ 5 million/ml, ≥ 10 million/ml, and ≥ 15 million/ml).

Hormonal assessment

Serum FSH, LH, and total testosterone were measured by chemiluminescence assay (Advia Centaur CP). Serum inhibin B was measured by enzyme-linked immunosorbent assay (ELISA, Diagnostics Systems Laboratories (DSL)) with a limit of detection at 1.6 pg/ml, and range of 12.7–1390 pg/

ml. Intraassay and interassay coefficients of variation were less than 8 and 10%, respectively, for all hormonal assays.

Statistical analysis

The data were analyzed using IBM SPSS Statistics software for Windows, version 25 (IBM Corp., Armonk, NY, USA). Normally distributed continuous data were expressed as mean \pm SD and were compared using unpaired t-test. Skewed continuous data were expressed as median and interquartile range (IQR) and were compared using the Mann–Whitney U test. Categorical variables were expressed as proportions or percentages (%) and compared using Fischer's exact t-test. Spearman's correlation analysis was performed to identify the correlation between the final hCG and hMG doses used with peak sperm concentration. P-value < 0.05 was considered statistically significant.

Results

Baseline characteristics and response to CGT of the whole cohort

Table 1 shows baseline characteristics in the whole cohort, patients with ($n = 18$) and without ($n = 17$) prior TRT. Individual patient data of these patients subdivided according to severe and partial phenotypes have been tabulated in supplementary Table 1. Thirteen patients had KS, whereas 22 had nCHH. Twenty patients had mean TV ≤ 3 ml (severe phenotype), whereas 15 had mean TV > 3 ml (partial phenotype). The mean age of the whole cohort was 24.8 ± 6.1 years. Three patients had uncorrected cryptorchidism, and none

Table 1 Comparison of baseline characteristics and response to treatment of with and without prior TRT cohorts

Variable	Overall cohort	No prior TRT (N = 17)	Prior TRT (N = 18)	P value*
Age (years) Mean \pm SD	24.8 \pm 6.1	23.4 \pm 5.1	26.1 \pm 6.8	0.19
Diagnosis: KS/nCHH (n/n)	13/22	4/13	9/9	0.16
Phenotype: severe/partial (n/n)	20/15	9/8	11/7	0.73
Baseline serum FSH (mIU/ml) Median (IQR)	1.17 (0.55–1.71)	1 (0.5–1.55)	1.28 (0.77–2.26)	0.28
Baseline serum LH (mIU/ml) Median (IQR)	0.47 (0.09–1.12)	0.37 (0.12–0.94)	0.59 (0.08–1.4)	0.67
Baseline serum testosterone (ng/ml) Median (IQR)	0.28 (0.15–0.42)	0.29 (0.21–0.42)	0.27 (0.14–0.3)	0.44
Baseline mean TV (ml) Mean \pm SD	3.6 \pm 2.7	4.2 \pm 3.2	3.2 \pm 2.3	0.29
Cryptorchidism (%)	8.6	13.6	0	0.10
Duration of prior TRT (months) Median (IQR)	NA	NA	38 (10–63.75)	–
Duration of CGT (months) Mean \pm SD	33 \pm 12.4	35.2 \pm 10.5	31.1 \pm 14	0.33

N number of total observations in that particular cohort, n number of positive observations in that particular cohort. CGT combined gonadotropin therapy, IQR inter-quartile range, KS Kallmann syndrome, NA not applicable, nCHH normosmic congenital hypogonadotropic hypogonadism, SD standard deviation, TRT testosterone replacement therapy, TV testicular volume

*P-value is calculated by comparing the cohorts with/without prior TRT

of them received prior TRT. Baseline mean TV for the whole cohort was 3.6 ± 2.7 ml.

After receiving CGT for 33 ± 12 months, mean TV increased to 8.9 ± 5.5 ml, which corresponds to 114% (IQR 60–366%) increment (Table 2). Thirty (86%) patients achieved adult serum total testosterone levels (≥ 3.5 ng/ml) after 9.6 ± 5.9 months of CGT, and the median (IQR) highest trough serum total testosterone level was 5 (4.2–8.1) ng/ml. Thirty-three patients provided semen samples for the final analysis. Of these, 23 (70%) patients showed evidence of spermatogenesis in the ejaculate. The median (IQR) peak sperm concentration was 5 (0–12.6) million/ml (Fig. 1). Only nine (27%) patients achieved a sperm concentration of > 15 million/ml. Final serum inhibin-B level was available in 22 patients (median 64.3, IQR: 30.8–114 pg/ml). The two patients in whom semen analysis data was not available had partial phenotype and had received prior TRT; both these patients demonstrated inhibin-B response (344 ng/ml and 97 ng/ml) to CGT. There was an inverse correlation between final hMG doses (per week) used and peak sperm concentration ($r_s = -0.51$, $p = 0.002$) incongruence with the dose-titration pattern. There was no such correlation between final hCG doses (per week) used and peak sperm concentration ($r_s = 0.12$, $p = 0.49$).

Comparison of baseline characteristics and response to CGT between patients with and without prior TRT

All baseline characters were comparable in patients with and without prior TRT. CHH patients without prior TRT had significantly better spermatogenic responses (any spermatogenesis achieved, sperm count > 5 million/ml, sperm count > 10

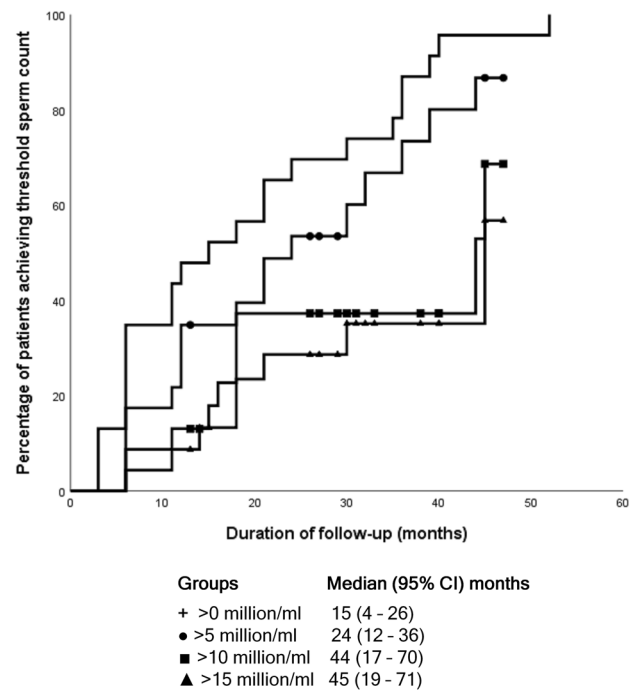


Fig. 1 Median times of achieving sperm concentration at different thresholds. Kaplan–Meier analysis of 23 patients who achieved any sperm in ejaculate was done and median times of achieving sperm concentration more than 0, 5, 10, 15 million/ml were calculated.[CI (confidence interval)]

million/ml, and peak sperm concentration) than those with prior TRT (Table 2). There were no significant differences in serum total testosterone and inhibin-B responses to CGT. Time to achieve adult serum total testosterone level was

Table 2 Comparison of response to treatment of with and without prior TRT cohorts

Variable	Overall cohort	No prior TRT (N = 17)	Prior TRT (N = 18)	P value*
Last mean TV (ml) Mean \pm SD	8.9 ± 5.5	10.4 ± 5.7	7.5 ± 5.1	0.12
TV increment (%) Median (IQR)	114 (60–366)	200 (78.6–366)	100 (50–337)	0.41
Serum testosterone > 3.5 ng/ml (n (%))	30 (86)	16 (94)	14 (78)	0.34
Time to reach serum testosterone > 3.5 ng/ml (months) Mean \pm SD	9.6 ± 5.9	11.5 ± 6.4	7.3 ± 4.3	0.05
Highest trough serum total testosterone level (ng/ml) Median (IQR)	5 (4.2–8.1)	6.5 (4.6–9.8)	4.7 (3.3–7.8)	0.13
Spermatogenesis achieved ^a [n (%)]	23 (70)	15 (88)	8(50) (N = 16)	0.02
Peak sperm concentration ^a (million/ml) Median (IQR)	5 (0–12.6)	9 (4.4–35)	0.05 (0–5.77)	0.004
Sperm count > 5 million/ml ^a [n (%)]	17 (51.5)	12 (71)	5 (31) (N = 16)	0.04
Sperm count > 10 million/ml ^a [n (%)]	11 (33)	9 (53)	2 (12.5) (N = 16)	0.025
Sperm count > 15 million/ml ^a [n (%)]	9 (27)	7 (41)	2 (12.5) (N = 16)	0.12
Serum inhibin-B (ng/ml) Median (IQR) (N = 21)	64.3 (30.8–114)	71 (55–115) (N = 10)	55 (18–114) (N = 11)	0.46

N number of total observations in that particular cohort, n number of positive observations in that particular cohort, CGT combined gonadotropin therapy, IQR inter-quartile range, KS Kallmann syndrome, NA not applicable, nCHH normosmic congenital hypogonadotropic hypogonadism, SD standard deviation, TRT testosterone replacement therapy, TV testicular volume

*P-value is calculated by comparing the cohorts with/without prior TRT

^aTotal subjects who provided semen samples for analysis were 33

earlier in patients with prior TRT than those without it which is in concordance with the higher initial hCG dosage in the prior TRT group.

Phenotype-based subgroup analysis of patients with and without prior TRT

Baseline characteristics and treatment response of patients with severe and partial phenotypes are compared in Supplementary Table 2. In both severe and partial phenotype subgroups, baseline characteristics (age at CGT initiation, baseline mean TV, proportions of patients with KS, and duration of CGT) of patients with and without prior TRT were similar (Table 3). In patients with severe phenotype, frequency of prepubertal testes (mean TV ≤ 2 ml) and cryptorchidism were not different between those with and without prior TRT.

In the severe phenotype cohort, patients with prior TRT attained significantly lower peak sperm concentration (median: 7 vs 0 million/ml) and tended to less frequently attain any sperm count and sperm count > 5 million/ml than those without prior TRT. In the severe phenotype and prior TRT group four patients failed to achieve adult serum total testosterone concentration compared to none in the severe phenotype and no prior TRT group; the highest trough serum total testosterone level was also lower in the former group than the latter.

In the partial phenotype cohort, there were no differences in spermatogenic, testosterone, and inhibin-B responses between patients with and without prior TRT.

Discussion

We report the spermatogenic and testosterone response to CGT in an exclusive CHH cohort along with phenotype-based subgroup analysis. Our study provides an important insight into the long unanswered question of whether prior TRT affects future spermatogenic potential and unravels the differential effect of prior TRT in patients with partial and severe phenotypes. In the whole cohort, the long term prior TRT adversely affected all spermatogenic response parameters but had no detrimental effects in the partial phenotype subgroup. Interestingly, prior TRT adversely affected peak sperm concentration and tended to affect the attainment of any sperm or sperm count ≥ 5 million/ml in the severe phenotype subgroup. Considering a poorer spermatogenic response to CGT (36–55% vs 71–82%) or GnRH therapy (83% vs 100%) in patients with severe phenotype than those with partial phenotype, identifying a modifiable negative factor in the former group may help to improve the reproductive outcomes in them [1].

The first study to demonstrate a negative effect of prior TRT on spermatogenic response to CGT was published in 2002 [7]. In this study, prior TRT was associated with longer time to achieve sperm count > 20 million/ml by univariate analysis, and the effect was independent of prior gonadotropin therapy. However, prior TRT had no effect in correlated Cox analysis; hence, the negative effect prior TRT in univariate analysis was attributed to selection bias as severe phenotype patients more frequently received prior TRT [7]. Notably, the same group confirmed the detrimental effect of prior TRT later in 2009, in a larger cohort of 75 patients [8]. Patients with prior TRT had slower attainment of all the specified sperm output thresholds than those without TRT [Hazards ratio (HR): 0.439 (0.244–0.791) for any sperm ($p=0.006$), 0.432 (0.227–0.822), sperm count > 5 million/ml ($p=0.011$) and 0.291 (0.12–0.705) for sperm count > 20 million/ml ($p=0.006$)] [8]. On multivariate analysis, the negative effect of prior TRT was consistent on the attainment of all the specified sperm output thresholds and conception.

In contrast, a meta-analysis of 43 studies concluded that prior TRT does not affect spermatogenesis outcomes of CGT (2). The conclusion regarding the effect of prior TRT from this meta-analysis was limited by the inclusion of studies consisting of HH patients with varied etiologies (pre- and post-pubertal onset) and, more importantly, grouping studies into the TRT group even if a single patient in a given study had received prior TRT. Besides, there was a potential misclassification of a large study to no TRT group as evident by the clinical features at baseline (genital stages) and lack of data regarding the use of androgen therapy five weeks before the initiation of gonadotropin therapy [14]. This study had the biggest weight in the no TRT group and chiefly drove the results for the group; hence, the conclusion regarding the effect of prior TRT from the meta-analysis is questionable [2].

Two recent, larger studies by Rohayem et al. and Liu et al. have identified severe phenotype (smaller baseline TV and/or cryptorchidism) as an unfavorable prognostic factor for spermatogenic response to CGT but negated such a role of prior TRT [11, 12]. Although most of the participants had severe phenotype in Rohayem et al. study, the response in terms of any sperm appearance was almost universal (91–95%) irrespective of prior TRT. However, sperm count > 15 million tended to be less frequent in those with prior TRT (32% vs 61%, $p=0.07$) which suggests a possible interference from prior TRT with higher-level spermatogenic output thresholds. Obtaining such a trend in the cohort of severe phenotype patients, but not in the several other studies including mixed phenotype patients [2, 9, 10], corroborates with our observation of predominant interference from prior TRT in patients with severe phenotype. The discordant observations in the study by Liu et al. might also be due to lack of phenotype-based subgroup analysis; hence,

Table 3 Differential response of reproductive phenotypes in with/without prior TRT cohorts

Parameters	Severe phenotype			Partial phenotype		
	No prior TRT (N=9)	Prior TRT (N=11)	P-value	No prior TRT (N=8)	Prior TRT (N=7)	P-value
Age (years) Mean \pm SD	23.4 \pm 6.7	25.5 \pm 4.8	0.42	23.3 \pm 3	27.1 \pm 9.5	0.3
Diagnosis: KS/nCHH (n/n)	4/5	6/5	1	0/8	3/4	0.08
Baseline serum FSH, (mIU/ml) Median (IQR)	0.74 (0.0–0.94)	0.89 (0.49–1.31)	0.22	1.53 (1.15–3.1)	2.26 (1.5–3.27)	0.42
Baseline serum LH (mIU/ml) Median (IQR)	0.12 (0.07–0.33)	0.09 (0.07–0.56)	0.82	0.97 (0.88–1.14)	1.4 (0.89–2)	0.35
Baseline serum Testosterone (ng/ml) Median (IQR)	0.29 (0.18–0.35)	0.2 (0.14–0.28)	0.17	0.33 (0.26–0.72)	0.52 (0.28–1)	0.52
Baseline mean TV (ml) Mean \pm SD	1.78 \pm 0.51	1.64 \pm 0.71	0.63	6.88 \pm 2.68	5.64 \pm 1.49	0.30
Baseline mean TV \leq 2 ml (n (%))	8 (89)	9 (82)	1	NA	NA	NA
Cryptorchidism (n (%))	3 (33)	0 (0)	0.07	NA	NA	NA
Duration of prior TRT (months) Median (IQR)*	–	51 (10–66)	–	–	12 (11–49.5)	–
Duration of CGT (months) Mean \pm SD	39.8 \pm 10.2	34.3 \pm 13.3	0.32	30.3 \pm 8.9	26.1 \pm 14.5	0.5
Response to therapy						
Last mean TV (ml) Mean \pm SD	7.5 \pm 3.2	5.9 \pm 5	0.42	13.8 \pm 6.3	10.1 \pm 4.4	0.22
TV increment (%) Median (IQR)	366 (250–450)	250 (100–425)	0.38	85.7 (44.5–122)	57 (30.5–119)	0.9
Serum testosterone > 3.5 ng/ml [n (%)]	9 (100)	7 (64)	0.09	7 (87)	7 (100)	1
Highest trough serum total testosterone level (ng/ml) Median (IQR)	5.21 (4.7–10.5)	4.4 (2.7–6)	0.048	6.6 (4.1–7.6)	5.1 (4.8–8.8)	0.95
Spermatogenesis achieved [#] [n (%)]	8 (89)	5 (45)	0.07	7 (87)	3 (60) (N=5)	0.51
Peak sperm concentration [#] (million/ml) Median (IQR)	7 (4–10)	0 (0–1)	0.01	24.6 (6–43)	5.1 (0–8)	0.14
Sperm count > 5 million/ml [#] [n (%)]	6 (67)	2 (18)	0.06	6 (75)	3 (60) (N=5)	1
Sperm count > 10 million/ml [#] [n (%)]	3 (33)	1 (9)	0.28	5 (62)	1 (20) (N=5)	0.27
Sperm count > 15 million/ml [#] [n (%)]	2 (22)	1 (9)	0.57	4 (50)	1 (20) (N=5)	0.56
Serum inhibin-B (ng/ml) Median (IQR) (N=21)	78.6 (58.3–114) (N=7)	46.5 (18.1–91.3) (N=8)	0.35	64.3 (57–89.6) (N=3)	97 (60.5–220) (N=3)	NP

*P-value of difference between TRT exposure between severe and partial subgroups of cohort with prior TRT is 0.49

N number of total observations in that particular cohort, n number of positive observations in that particular cohort. CGT combined gonadotropin therapy, IQR inter-quartile range, KS Kallmann syndrome, nCHH normosmic congenital hypogonadotropic hypogonadism, SD standard deviation, TRT testosterone replacement therapy, TV testicular volume

reanalysing the effect of prior TRT in exclusive severe phenotype patients in Liu et al. study may be useful [12].

Though pathophysiologic studies providing histopathologic evidence are required for convincingly demonstrating

the harmful effects of androgen exposure on prepubertal human testes, such studies are rare due to ethical considerations [15]. The spermatogenic output of mammalian testes is dependent on the number of Sertoli cells in the

testes [16]. A study in humans has demonstrated a fivefold increase in Sertoli cell numbers from the late fetal testis to the age of 3 months to 10 years [17]. Patients with severe phenotype lack this increase due to absent mini-puberty [6]. Histopathological examination of prepubertal testes in CHH patients demonstrated immature Sertoli cells lacking tight junctional complexes [15]. Primate study involving juvenile animals had shown that intramuscular administration of androgen induces morphological differentiation of Sertoli cells [13]. In this study, testosterone enanthate (125 mg/week) for 12 weeks resulted in the transformation of pre-Sertoli cells (ovoid/pear-shaped nuclei concentrated towards the center of the seminiferous tubule) to Sertoli cell with more cytoplasm and Sertoli cell nuclei palisading to the periphery of the tubules. This was further supported by an in-vitro study describing increased expression of cell cycle inhibitor (p27Kip1) along with Sertoli cell differentiation markers (Id2, Id3, DMRT1, and GATA1) halted the proliferative phase and promoted maturation of cultured rat Sertoli cells [18]. Anti-Müllerian hormone (AMH) is produced by immature Sertoli cells until their maturation after androgen exposure during puberty. In HH males treated with exogenous testosterone enanthate (250 mg/3 weeks) for 6 months; serum AMH decline was documented (from 221 ± 107 pmol/l to 114 ± 50 and 66 ± 17 pmol/l, at 3 and 6 months respectively) suggesting maturation of immature Sertoli cells [19]. These suggest a negative effect of prior TRT on the subsequent rFSH/hMG induced Sertoli cell proliferation. So, pubertal induction with gonadotropins, rather than androgens, is likely to enhance the future fertility in CHH patients, especially in those with a severe phenotype.

Small sample size, retrospective study design, absence of baseline sperm count, lack of TV measurements by ultrasonography, and lack of qualitative parameters of semen analysis are some of the limitations of the current study. Additionally, inclusion of patients with cryptorchidism adds to heterogeneity in the cohort but does not affect the conclusions derived from the study as all these patients belonged to no prior TRT group. Though gonadal toxicity with higher hCG doses has been shown in in-vitro and rat studies; this was not seen in our few cases treated with higher hCG doses (10,000 units/week) [20, 21]. Also, the inclusion of a few patients receiving minimal doses of androgens into no prior testosterone group is debatable as the exact duration and dose of androgen exposure leading to Sertoli cell maturation are not known.

However, single-center study consisting of an adult predominant cohort with sufficient duration of CGT, relatively longer androgen exposure in the prior TRT group, and phenotype-based subgroup analysis are the major strengths of our study.

Conclusion and study implication

Prior TRT may negatively affect the spermatogenic response to CGT in CHH patients with severe phenotype. Hence, use of gonadotropins, rather than androgens, may be preferred for pubertal induction in them. Larger studies including exclusive CHH patients with severe phenotype are warranted.

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Data availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Compliance with ethical standards

Conflict of interest The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

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