HSOA Archives of Urology

Research Article

Effect of Gonadotropin Therapies in Adolescent Males with Hypogonadotropic Hypogonadism: Meta-Analysis

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Abstract

Objective: Gonadotropins are a recent strategy for inducing puberty in adolescent males with Hypogonadotropic Hypogonadism (HH). The benefits of combination gonadotropin therapies (hCG+) over Humanchorionic Gonadotropin (hCG) monotherapy are not clear for the prepubertal adolescent population. We performed a meta-analysis to assess therapeutic outcomes, in terms of virilization and testicular growth, inperipubertal boys with HH treated with hCG vs. hCG+ therapy.

Method: A comprehensive search of the PubMed and Google Scholar databases was conducted. Seven studies met our inclusion criteria. We calculated pooled means for the post-treatment mean testicular volume, testosterone levels, and penile length for hCG monotherapy and for hCG+ therapy. A meta-regression analysis was performed to examine the contribution of various factors to post treatment outcomes, including baseline age, testosterone level, treatment duration, and study quality.

Results: Participants were prepubertal (13.3–25.9 years) with weighted mean treatment durations of 10.95 and 28.2 months for hCG monotherapy and hCG+, respectively. Baseline age and testosterone levels were significantly different between groups. The post-treatment mean testicular volume and penile length were not significantly different between treatment groups. The post-treatment testosterone levels were101.89 ng/dL and 424.10 ng/dL for hCG monotherapy and hCG+, respectively (P<0.0001). Treatment duration explained 3.04% of the difference (P<0.0001). However, the difference remained significant after adjusting for treatment duration.

Conclusion: hCG+ provided potential benefits over hCG monotherapy for pubertal induction in male HH. Large prospective studies are needed to establish guidelines for gonadotropin therapy in the adolescent population.

Keywords: Gonadotropin therapies; Hypogonadotropic hypogonadism; Pubertal induction; Testicular volume; Testosterone

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Citation: Agarwal S, Austin P, Wood AC, Karaviti LP (2020) Effect of Gonadotropin Therapies in Adolescent Males with Hypogonadotropic Hypogonadism: Meta-Analysis. Arch Urol 3: 010.

Received: July 09, 2020; **Accepted:** October 15, 2020; **Published:** October 22, 2020

Introduction

Hypogonadotropic Hypogonadism (HH) is one of the etiologies of delayed puberty. Congenital HH occurs in about 1-10 infants per 100,000 live births [1]. Although patients with HH have normal testiclular tissue, the lack of pituitary gonadotropins causes failure of testicular stimulation and testosterone production, leading to delayed puberty.

For pubertal induction, testosterone is currently the recommended standard of care among pediatric endocrinologists [2,3]. Monthly testosterone injections can successfully induce puberty in patients with HH. However, testosterone therapy does not induce testicular growth [4], and additionally reduces testicular volume in normal, unaffected male patients that receive androgen supplementation [5]. Changes in testicular volume are correlated with sperm count changes in adults [4]. Although the effects of testosterone on testicular volume were thought to be fully reversible [5], recent reports have shown that the duration of androgen use was independently and inversely associated with the likelihood of achieving sperm output thresholds and conception [6-8]. Despite cessation of androgen treatment, patients experienced delays in achieving sperm output thresholds, decreased rates of natural conception, and increased requirements for artificial reproductive technologies [7]. Therefore, testosterone clearly exerts several negative effects that reduce fertility and natural conception rates, when used for extensive time periods. Its use should be minimized when possible, in adolescents with HH that desire to preserve future fertility.

Sertoli cell proliferation occurs during late fetal, early neonatal, and peri-pubertal phases [9,10]. Lack of gonadotropin exposure during the peri-pubertal phase in males with early hypothalamic-pituitary-gonadal axis impairments, causes greater impact on Sertoli cell proliferation, compared to those that acquire HH after puberty [10].This preludes to the benefit of using gonadotropin therapy inperi-pubertal males with HH.

Testicular size has been positively correlated with sperm counts, normal sperm morphology, spermatogenesis induction, and unassisted pregnancy in hypogonadal males [7,11]. Therefore, testicular size could potentially serve as a surrogate marker for future fertility outcomes. Treating hypogonadal adult males with Human Chorionic Gonadotropin (hCG) and testosterone restored and maintained normal sperm counts, motility, and morphology [12,13]. In addition, males that underwent pubertal induction with gonadotropin therapy achieved adequate sperm counts in lesser time than those that received androgen therapy [7,14]. Thus, gonadotropin therapies offer prepubescent males with HH, an alternative treatment that might improve future fertility. Additionally, many males desire to have testicular growth in addition to other virilizing effects.

For inducing pubertal development and preserving fertility in peripubertal boys with HH, the American Association of Clinical Endocrinologists guidelines (2002) recommended hCG therapy or Gonadotropin Releasing Hormone (GnRH) therapy [2]; the European Consensus Statement (2015) recommended hCG monotherapy, or hCG plus recombinant Follicle Stimulating Hormone (rFSH), or GnRH [3]; and the Japanese Society for Pediatric Endocrinology (2015) recommended hCG+Rfsh [15]. The inclusion of rFSH in the latter guidelines were based on studies that showed significant increases in testicular size, inhibin B levels, and spermatogenesis with independent rFSH administration prior to initiating other gonadotropins, in males with HH [16-18].

Recently, therapeutic strategies have shifted towards an increase in the use of gonadotropins, particularly hCG monotherapy, for pubertal induction. hCG primarily targets the Luteinizing Hormone (LH) receptor, which leads to leydig cell stimulation and proliferation. However, both leydig and sertoli cells can be targeted with combination gonadotropin regimens, like hCG+rFSH, GnRH, and hCG + Human Menopausal Gonadotropin (HMG). Although these combination therapies are recommended in several guidelines, they are not extensively used in clinical practice for inducing puberty, largely due to the lack of evidence in the pediatric population. Consequently, pediatric endocrinologists have begun to participate in research focused on how various gonadotropin regimens affect pubertal induction and fertility parameters. One study conducted inpre-pubescent adolescent males showed that hCG+Rfsh treatment maintained larger testicular volumes than hCG Monotherapy [19]. Another study found no significant difference in testicular volumes between the two treatment groups [20]. Currently, convincing data have shown that gonadotropin therapies could induce puberty in males with HH without hampering future fertility. Never the less, no study has systematically compared hCG monotherapy to combination gonadotropin therapies and evaluated pubertal outcomes in prepubescent males with HH.

The present meta-analysis was aimed to investigate whether combination gonadotropin therapies, targeting LH and FSH receptors (with complex administration regimens), were superior to LH receptor targeted hCG monotherapy, in terms of pubertal induction and testicular growth.

Methods

This meta-analysis followed the Preferred Reporting Items for Systemic Reviews and Meta-Analyses (PRISMA) guidelines for design, implementation, and reporting [21].

Search strategy and study eligibility

The PubMed and Google Scholar databases were searched through November 2018 with the following search terms: [("Hypogonadotropic Hypogonadism") AND ("Males") AND ("Treatment")]; [("Pre-pubertal adolescent males") AND ("Hypogonadotropic Hypogonadism")]; and [("Puberty Induction") AND ("Gonadotropin therapy")]. We also manually searched article references to identify other relevant articles.

We considered all prospective or retrospective studies that involved males with HH, with/without other pituitary deficiencies, that received gonadotropin treatment. We included all studies that assessed gonadotropin treatment outcomes, including testicular volume, testosterone, and/or virilization. The search was not restricted by country of origin. We excluded case reports, articles in languages other than English, studies involving adult males, and studies that reported mixed data on HH acquired after puberty. We also excluded studies that included patients with prior androgen or other alternative therapies, and studies within adequate data on outcomes relevant to our review.

Study selection and data extraction

Two reviewers independently evaluated study eligibility, based on the title and abstract. Potentially eligible articles were retrieved, and both reviewers assessed the full-text version for eligibility. Conflicts between reviewers were resolved through discussion. Data were extracted on the study method, sample selection, and relevant outcomes. Data were then tabulated for analysis by a statistician.

Risk of bias assessment and study grading

Two reviewers conducted independent critical appraisals of the studies, based on guidance and suggestions from the PRISMA statement, the Newcastle-Ottawa Scale for assessing the quality of non-randomized studies in meta-analyses and the reviewers' knowledge of this research area (e.g., the quality of measurements for testicular volume) [22].

Study grading (Table 1) was based on three overarching topics:(1) Study population (3 criteria):tests used for confirmation of HH; significant number of post-pubertal males in sample; and attrition ≤20% vs.>20%; (2) study design (3 criteria): outcome of interest was/was not included inthe hypothesis; retrospective/prospective study; and <15vs.>15 patients per treatment group; and (3) quality of the outcome assessment (6 criteria): blinded/non-blinded investigators; testicular volume measured with Prader orchidometer/sonogram; follow-up period>6 months vs. <6 months; radioimmunoassay/chemiluminescence for measuring testosterone; selective reporting of outcomes; and single/multiple investigator study.

Statistical analysis

Analyses were performed with R v.3.5.1, using the 'meta' package, as described by Harrer, et al. [23,24]. We compared three outcomes (testosterone levels, Mean Testicular Volume [MTV], and penile length) between patients treated with hCG monotherapy and patients treated with either a combination of gonadotropin therapies or GnRH (hCG+). We calculated the overall pooled means separately for the two treatment groups.

RISK OF BIAS	STUDIES						
	Aydogdu 2013	Balducci 1997	Bistritzer 1989	Gong 2015	Rohayem 2016	Shiraishi 2014	Bouvattier 1999
STUDY POPULATION							
a. Standard tests for diagnosing HH in pre-pubertal males	1	1	0	0	1	1	1
b. Reported baseline/outcome data separately for pre-pubertal males	1	1	1	1	1	0	1
c. Attrition bias (<20% vs. <20%)	1	1	0	1	1	1	1
STUDY DESIGN							
a. Outcome of interest included in hypothesis	1	1	1	1	1	0	1
b. Retrospective/Prospective design	0	1	0	1	1	1	1
c. Adequate sample size (>15 vs. <15 patients per group)	1	0	0	1	1	1	1
QUALITY OF THE OUTCOME ASSESSMENT							
a. Investigator blinding for clinical outcome assessment	0	0	0	0	0	0	0
b. Method of measuring TV	1	0	0	0	1	1	0
c. Adequate follow-up time	0	1	1	1	1	1	1
d. Type of testosterone assay	1	0	0	1	NE	NE	NE
e. Selective reporting and publication bias	1	1	0	1	0	0	1
f. Single or multiple investigators	0	0	0	1	0	0	0
TOTAL	**8**	**7**	**3**	**9**	**8**	**6**	**8**
GRADE[a]	**Moderate**	**Moderate**	**Very Low**	**Moderate**	**Moderate**	**Low**	**Moderate**

Table 1: Study grading based on risk of bias.

HH: Hypogonadotropic Hypogonadism; TV: Testicular volume; NE: Not evaluable
[a]Grades: 1-3: Very low; 4-6: Low; 7-9: Moderate; 10-12: High

We estimated heterogeneity in baseline age, FSH levels, LH levels, testosterone levels, Tanner stages, penile lengths, and MTVs across all included studies and between the pooled studies within each treatment group (hCG monotherapy vs. hCG+). Heterogeneity was presented using the DerSimonian and Laird Q-statistic.

Pooled means for the two treatment groups were calculated using a random effects model due to heterogeneity in baseline factors within each group. The treatment effect was assessed via a significant estimate of heterogeneity in the pooled effects between treatment groups. When a treatment effect was significant (P<0.05), we performed meta-regression to examine whether potential confounders could account for the differences between treatment groups. Confounders included baseline testosterone levels, age, treatment duration, and study quality. We performed this step in univariable analyses (separate models for each potential confounder). When a meta-regression suggested that a given factor significantly influenced the treatment effect (P<0.05), the intercept from a meta-regression using the centered factor was used to estimate the adjusted pooled mean.

Results

Included studies

Of 3227 articles identified, 1474 were duplicates, and 1699 were excluded based on abstract and title screening (Figure 1). We retrieved the full text for54 articles; of these, 17 were pediatric studies. Ten studies were excluded either because the outcome of interest was not clearly reported or because a significant number of patients had history of prior treatments. The final analysis included seven studies [25-31], with five hCG monotherapy and four hCG+ treatment groups.

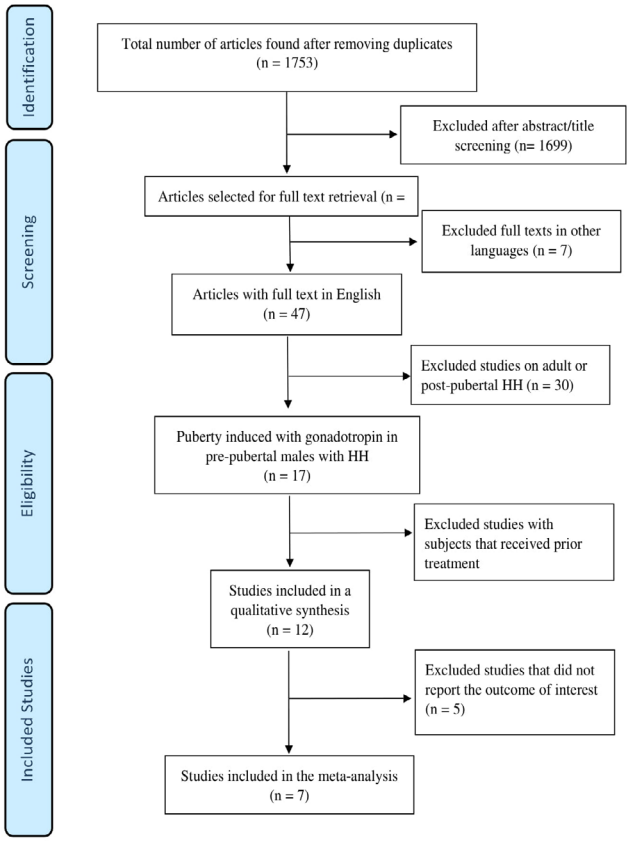

Figure 1: Flow chart of the study selection process.

Study characteristics

In all participants, HH was confirmed with standard diagnostic criteria defined in each study. None of the studies had evidence of including patients with other diagnoses like constitutional delay of puberty. No participant had prior androgen treatment. The mean participant ages ranged from 13.3 to 25.9 years (Table 2). Treatments in the hCG+ group included GnRH alone, hCG+rFSH, or hCG+HMG. The weighted-mean treatment durations were 10.95 months for the hCG monotherapy group and 28.2months for the hCG+ group. Two studies also included participants treated with testosterone [25,27]; these participants were not included in our meta-analysis, but their mean outcome data were included on forest plots as reference. This meta-analysis did not include testosterone treatment, because our search criteria did not capture all studies that employed testosterone treatments.

Outcomes of interest

Our primary outcomes of interest werethe MTV, as a surrogate marker of future fertility, and the total testosterone level. Other outcomes included the penile length and reported adverse events.

Quality of evidence

Of the seven studies included, five had a low risk of bias and a moderate grade of quality (Table 1, Figure 2). Two studies had a high risk of bias with low and very low grades of quality, primarily due to small sample size, attrition bias, lack of detail about investigator blinding, and suboptimal methods for measuring outcomes (Table 1).

Author (year, country) [sub-group]	Study design (% attrition)	Diagnostic criteria	Cause of HH (N)	Baseline status; prior androgen use	Dosage (Treatment duration)	Other hormone deficiencies
Aydogdu (2013, Turkey) [hCG]	Retrospective (no attrition)	1. Absent pubertyat 18 y 2. T <300 ng/dL 3. SubnormalFSH/LH 4. TS 1-2 5. Brain MRI	IHH (25)	Pre-pubertal; None	5000 units twice weekly (6 months)	None
Balducci (1997, Italy) [hCG]	Prospective (no attrition)	1. Pre-pubertal MTV 2. Subnormal T 3. Subnormalresponse to GnRH	IHH (2) aCraniopharyngio-ma (3) Idiopathic MAPD (1)	Pre-pubertal; None	1500 units every 6 days (12 months)	GH (3) LT4 (4) Cortisone (2)
Bistritzer (1989, Israel) [hCG]	Retrospective (45% attrition)	1. Pre-pubertal MTV 2. T<120 ng/dL 3. Subnormal FSH/LH	IHH (22)	Pre-pubertal; None	5000 units weekly (30 months)	None
Gong (2015, China) [hCG]	Prospective (no attrition)	1. Subnormal T 2. Subnormal FSH/LH 3. Brain MRI	KS (15) Normosmic IHH (7)	Pre-pubertal; None	4-step ramp-up regimen every 3 months. Initial: 1000 units twice weekly; Goal: 2000 units every other day (12 months)	None
Rohayem (2016, Germany) [hCG]	Multi-center prospective (20% attrition)	1. Absent pubertyat 14 y 2. MTV <4 mL 3. SubnormalFSH/LH and T levels 4. Subnormal response to GnRH (peak LH <4 U/L)	KS (11) Normosmic HH (10) Pubertal arrest HH (2) Congenital MAPD (5) a Brain tumor (4) CHARGE Syn. (2)	Pre-pubertal; None	Ramp-up regimen. Initial: 250-500 units twice weekly; Goal: 2500 units three times/week (6 months)	Replacement was provided for other hormone deficiencies
Gong (2015, China) [GnRH] or [hCG+]	Prospective (no attrition)	1. Subnormal T 2. Subnormal FSH/LH 3. Brain MRI	KS (8) Normosmic IHH (4)	Pre-pubertal; None	8-10μg of GnRH SC every 90 min (12 months)	None
Rohayem (2016, Germany) [b][hCG+rFSH] or [hCG+]	Multi-center prospective (20% attrition)	1. Absent pubertyat 14 y 2. MTV <4 mL 3. SubnormalFSH/LH and T levels 4. Subnormalresponse to GnRH (peak LH <4U/L)	KS (11) Normosmic HH (10) Pubertal arrest HH (2) Congenital MAPD (5) a Brain tumor (4) CHARGE Syn. (2)	Pre-pubertal; None	hCG: Ramp-up regimen for 6 months. Added rFSH 75-150 IU, 3 times/week (36 months)	Replacement was provided for other hormone deficiencies
Shiraishi (2014, Japan) [hCG+rFSH] or [hCG+]	Prospectivec (no attrition in population of interest)	1. Subnormal morning LH/FSH and T levels 2. Pre-pubertal MTV 3. hCG stimulation test	IHH (10) Pituitary surgery (4) KS (3) Pituitary ischemia (2)	Pre-pubertal; None	hCG for 6 months. Added rFSH 75 IU, 3 times/week (24 months)	Not reported
Bouvattier (1999, France) [hCG+HMG] or [hCG+]	Prospective (no attrition)	1. Absent puberty 2. Bone age >13 y 3. T<50 ng/dL 4. Subnormal response to GnRH 5. Brain MRI	KS (7) PWS (1) Congenital MAPD (7) aCraniopharyngio-ma (6) PSIS (4) IHH (12)	Pre-pubertal; None	hCG: 1500 units twice weekly for 6 months. Added HMG 75 mg, 3 times/week (30 months)	LT4 Corticosteroids Vasopressin
Gong (2015, China) [hCG]	Prospective (no attrition)	4. Subnormal T 5. Subnormal FSH/LH 6. Brain MRI	KS (15) Normosmic IHH (7)	Pre-pubertal; None	4-step ramp-up regimen every 3 months. Initial: 1000 units twice weekly; Goal: 2000 units every other day (12 months)	None

Table 2: Characteristics of the included treatment groups.

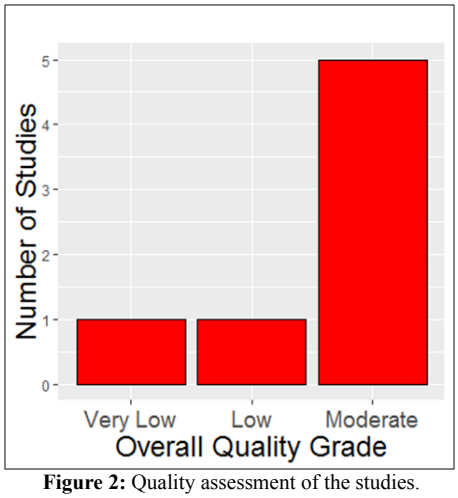

Figure 2: Quality assessment of the studies.

Heterogeneity in baseline characteristics

Across the studies, we found significant heterogeneity (Supplementary Table 1) in: baseline age (Q=147.2;df=2, *P*<0.001), FSH (Q=13.46;df=5;*P*=0.02), LH (Q=29.27;df=5;*P*<0.001), testosterone (Q=491.34;df=6; *P*<0.001), penile length (Q=39.34;df=2;*P*<0.001), and MTV (Q=35.52;df=8;*P*<0.001),but not Tanner stage (Q=1.73;df=1; *P*=0.19). Then we pooled baseline data across studies within each treatment group and examined potential confounders of treatment effects **(**Supplementary Table 1). Between the two treatment groups, significant heterogeneity was found only in baseline age (Q=121.71; df=1;*P*<0.001) and baseline testosterone (Q=436.74;df=1;*P*<0.001).

Meta-analysis of outcomes

MTV: We evaluated post-treatment MTVs infiveh CG monotherapy groups (n=92) and fourh CG+ groups (n=95). In addition, three testosterone groups (2 testosterone injections and 1 testosterone gel; n=68) were included in the forest-plots as reference [25,27].

Pooled mean baseline MTV (95% CI) was 2.03 mL (1.83- 2.24) and 1.98 mL (1.66- 2.30) for the hCG monotherapy and hCG+ groups, respectively. The pooled mean post-treatment MTVs (95%CI) for the hCG monotherapy and hCG+ groups were6.60 mL (3.18–10.02) and 10.02 mL (8.30-11.75), respectively (Figure 3), but the difference was not significant (Q= 3.07;df= 1;*P*=0.0799).However, within-group heterogeneity in MTV was significant in both groups (hCG monotherapy: Q=334.49;τ^2=15.10;I^2=98.8%;*P*<0.01; hCG+: Q=15.03; τ^2=2.32;I^2=80.0%;*P*<0.01). It was unclear which factors contributed to heterogeneity in the hCG+ group. However, in the hCG monotherapy group,one study had a 95%CI for MTV that fell outside the 95%CI of the overall pooled mean [27], suggesting a strong source of heterogeneity. After excluding that study, a re-analysis revealed a significant treatment effect (Q=34.17;df=2;*P*<0.001).With this exclusion, the pooled post-treatment MTV means (95%CIs) were 4.79 (3.29-6.28) and 10.02 (8.30-11.75) for the hCG monotherapy and hCG+ groups, respectively. This *post hoc* analysis suggested that hCG+ therapies provided a better MTV outcome than hCG monotherapy. However, there was still no table heterogeneity in the hCG+ group (Q=107.96; τ^2=2.26; I^2=97.2%; *P*<0.01) that precluded drawing any firm inferences from these results.

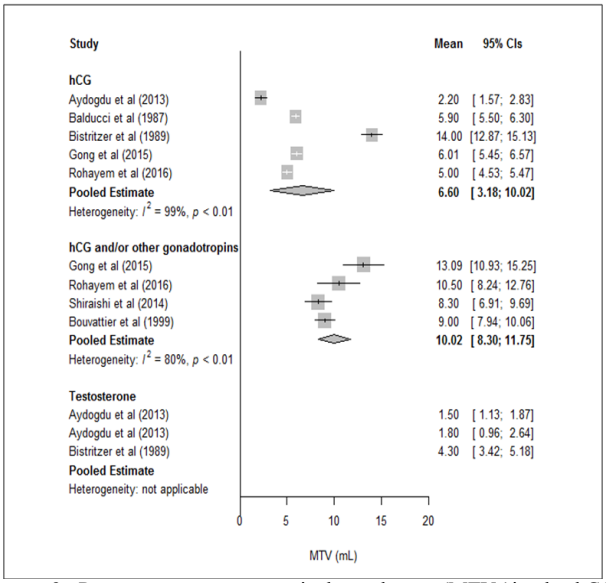

Figure 3: Post-treatment mean testicular volumes (MTVs)in the hCG monotherapygroup versus hCG+ group.

*Bold values are pooled means
Data for testosterone groups were included as a reference.
The 2013 study included two testosterone groups: testosterone gels and injections

Testosterone levels

Post-treatment testosterone levels were available for three hCG monotherapy groups (n=53)and three hCG+groups (n=68). Two testosterone groups (1 testosterone injection and 1 testosterone gel; n=52) were included in the forest-plots as reference [25].

The pooled mean (95%CI) testosterone levels were 101.89 ng/dL (50.7–153.08) and 424.10 ng/dL (304.59-543.62) for the hCG monotherapy and hCG+ groups, respectively. The lack of overlapping 95%CIs indicated that post-treatment testosterone levels were significantly lower with hCG monotherapy than with hCG+, consistent with a significant difference in treatment effect between groups(Q=23.59; df=1; *P*<0.0001; Figure 4).

A meta-regression analysis of testosterone levels showed that the treatment duration significantly contributed tothe between-group difference in treatment effects (β=20.48; Standard Eror [SE]=2.36; *P*<0.0001).Treatment duration explained 3.04% of the difference between groups. After adjusting for treatment duration, the pooled mean (95%CI) testosterone levels were 151.02 ng/dL (118.37-183.67) and 243.53 ng/dL (216.53–270.53) for the hCG monotherapy and hCG+ groups, respectively. The non-overlapping 95%CIs suggested that, after adjusting for treatment durations, post-treatment testosterone levels remained significantly higher in the hCG+ group than in the hCG monotherapy group. The treatment effect was not significantly influenced by the mean baseline participant age (β=9.44; SE=24.97; *P*=0.71), the mean baseline testosterone level (β=3.48; SE=6.82; *P*=0.61),or the study grade (β=32.89; SE=104.27; *P*=0.75).

Both treatment groups had significant within-group heterogeneity in post-treatment testosterone levels (hCG group: Q=25.48; τ^2=1780.50; I^2=92.2%; *P*<0.01; hCG+ group: Q=39.07; τ^2=10069.69; I^2=94.9%;

P<0.01). The 95% CIin the Gonget al. study did not overlap with that of the estimated pooled mean testosterone levels [28]; suggesting that this study might have contributed to the heterogeneity, even though the treatment duration in the Gong, et al. study was not very different from other studies (Table 2). After excluding the Gong, et al. study from the hCG monotherapy group [28], a re-analysis (Supplementary Figure 1) showed pooled mean (95%CI) testosterone levels of 76.45 ng/dL (44.03-108.87) and 424.10 ng/dL (304.59-543.62), for the hCG monotherapy and hCG+ groups, respectively. Thus, post-treatment testosterone levels remained significantly different between the two groups (Q=30.28; df=1; *P*<0.0001).

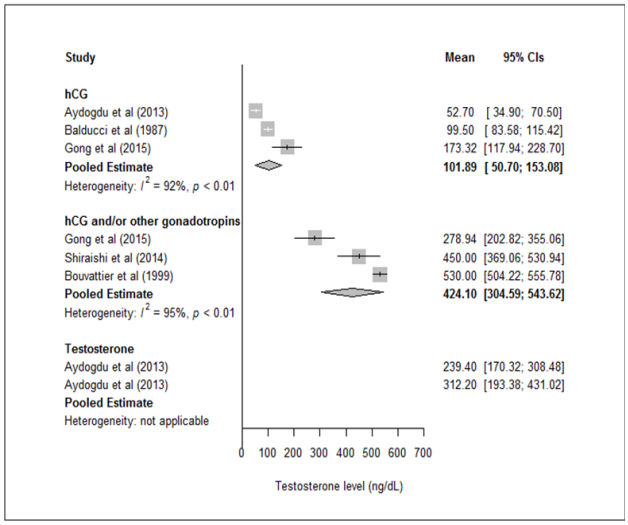

Figure 4: Post-treatment testosterone levelsin hCG monotherapy group versus hCG+group.

*Bold values are pooled means

Data for testosterone groups were included as a reference.

The 2013 study included two testosterone groups: testosterone gels and injections

Even though baseline testosterone levels did not significantly contribute to the estimated treatment effect, we re-ran the analysis after excluding the Gong and the Bouvattier studies that contributed to baseline testosterone heterogeneity (Supplementary Figure 2) [28,31]. However, the difference between the two groups remained significant (Q=20.94;df=1; *P*<0.0001).

Penile length

Penile length was reported in three studies, including two hCG monotherapy groups (n=28) and one hCG+ group (n=12). The pooled mean (95%CI) post-treatment penile lengths were 6.09 cm (5.71–6.46) and 8.03 cm (7.33–8.73) for the hCG monotherapy and hCG+ groups, respectively (Figure 5). The non-overlapping 95% CIs suggested that the hCG+ therapies provided a better post-treatment penile length than hCG monotherapy. However, the small number of studies was insufficient to drawfirm conclusions.

Ameta-regression analysis with penile length as the dependent outcome showed that treatment duration significantly contributed to the estimated treatment effect (β=19.44; SE=2.65; *P*<0.0001). Indeed, treatment duration explained >99% of the treatment effect. However, we had insufficient data points to adjust the pooled means for treatment duration. In contrast, baseline age (β=9.33; SE=25.54; *P*=0.71) and study grade (β=37.97; SE=97.16; *P*=0.70) did not significantly contribute to the estimated treatment effect.

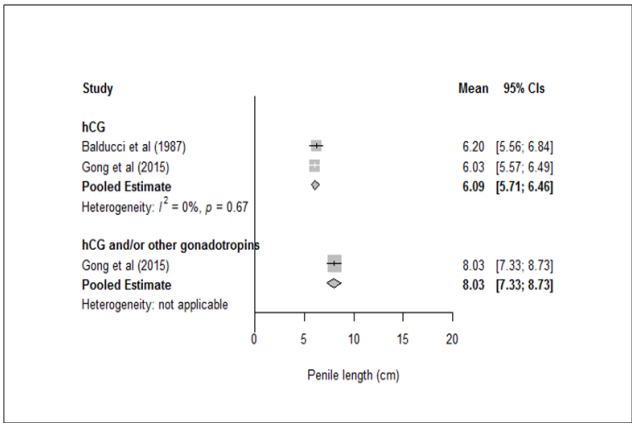

Figure 5: Post-treatment penile length in hCG monotherapy group versus hCG+ group.

*Bold values are pooled means

Adverse events

No local injection site reactions were reported. Although few adverse events occurred, the hCG+ group reported them more often than the hCG monotherapy group. Pulsatile GnRH therapy (hCG+ group) was associated with increased frequencies of erections and testicular pain in two patients; these events required a reduction in the GnRH dose [28]. Gynecomastia was reported by five patients in the hCG+ group [28,29]. In the hCG monotherapy group, two patients reported gynecomastia, and another patient reported increased acne [28].

Discussion

Our results showed that post-treatment MTVs, in both treatment groups, were higher than the baseline values, but were not significantly different between the two groups. Other pediatric studies that were excluded from our analysis also found significant increases in MTV over baseline with both hCG monotherapy and hCG+ therapies, but there was no comparison between regimens [16,32-34].

Comparison between gonadotropin regimens in adults also found no difference in post-treatment MTVs between hCG monotherapy and hCG+ treatment groups [20,35,36]. Comparison in pediatrics, albeit few, have shown varied testicular growth with these therapies. In a study by Zacharin, et al. the final MTVs, in pre-pubescent males (some received prior androgen treatments), were similar after 9 months of therapy with hCG and hCG+Rfsh [20]. This finding was similar to our findings, but they contrasted with results from Sinisi, et al. who found significantly different final MTVs between these therapeutic groups after 18 months of treatment in prepubertal males [19]. The discrepancy between these studies might be explained by different treatment durations. However, the weighted-mean treatment duration was 19.7 months for all studies analyzed in our meta-analysis, which was comparable to the treatment duration in the Sinisi study.

We found that testosterone levels were significantly lower with hCG monotherapy than with hCG+ therapy suggesting that hCG+ therapies were superior to hCG monotherapy, in terms of androgenic activity. However, there were no reports of decreased libido or fewer erections in the hCG monotherapy group. Our results were not consistent with other pediatric studies [19,20], that found no differences in total testosterone levels between hCG monotherapy and hCG+rFSH groups. This discrepancy might be explained by differences in assays and dose regimens. However, it is difficult to overlook the significant difference that we observed between the treatment groups.

Indeed, hCG+ therapies would have an added stimulative effect on Sertoli cells, but since Leydig cells are primarily responsible for testosterone production, we could not explain the significantly higher levels of testosterone in the hCG+ group. Animal studies have suggested that the addition of FSH improves the androgenic activity of Leydig cells. Previous animal studies had indicated that FSH directly stimulated androgenesis in Leydig cells [37,38]. However, those studies were conducted before the availability of rFSH; therefore, results were likely confounded by contamination with LH components. More recently, the effect of rFSH on testosterone levels were studied *in vivo* (FSH-receptor knockout mice vs. wild-type mice) and *in vitro* (Leydig cells exposed vs. unexposed to FSH anti-serum). Increased androgenesis was not seen in response to rFSH alone. However, androgenesis was increased in Leydig cells treated with both LH and rFSH, compared to those treated with LH alone [39,40]. It was therefore suggested that FSH plays a role in the post-LH receptor signaling promoting steroidogenesis in Leydig cells.

We found significantly higher post-treatment penile lengths in the hCG+ group than in the hCG group. However, it was difficult to draw conclusions, because these findings were based on data from only three studies.

Strengths and limitations

This meta-analysis was the first to compare the effects of different gonadotropin therapies in prepubescent adolescent males with HH. Despite the lack of large pediatric studies on gonadotropin therapies, by pooling study findings, we could evaluate outcomes for more than 100 pediatric patients. We found that hCG monotherapy and hCG+ therapies had similar effects on the final MTV, but significantly different effects on the final testosterone levels. This result was highly novel.

With strict inclusion/exclusion criteria, we avoided intermixing treatment groups with adult or post-pubertal patients or with patients that had received prior androgen therapies. A major limitation of this meta-analysis was the limited number of studies that met our inclusion and exclusion criteria. This small number prevented a full sensitivity analysis within subgroups to reduce the effects of heterogeneity. However, we could evaluate heterogeneity across studies and between treatment groups, and adjusted for it, when there was a significant difference in outcomes. Another limitation was the lack of studies that directly compared outcomes between treatment groups. This limitation made it difficult to draw firm conclusions on the differences in post-treatment outcomes between groups. Finally, we could not fully rule out the possibility that population differences might have occluded some true effects. This possibility remains to be tested in more rigorous study designs.

Clinical implications and future directions

As we shift towards the use of gonadotropin therapy for pubertal induction, it is important to understand the different outcomes and adverse events associated with different therapies. Our approach was an exploratory comparison of the effects of different gonadotropin therapies in pre-pubescent adolescents. Larger prospective pediatric trials are needed to compare different dose regimens and evaluate pubertal outcomes, fertility outcomes, and adverse effects. Ultimately, this knowledge will provide better understanding and a basis for establishing guidelines for gonadotropin therapy adinistration in adolescents with HH.

Conclusion

Our results demonstrated that inducing puberty with either hCG monotherapy or hCG+ therapiesin adolescent males with HH was associated with increases in post-treatment MTVs compared to baseline MTVs. However, post-treatment MTVs were not significantly different between the two treatment groups. Our findings also implied that, compared to hCG monotherapy, hCG+ therapies might result in higher testosterone levels and penile growth, when inducing puberty in males with HH. Our results should increase awareness among pediatric endocrinologists about the use of gonadotropins. This knowledge will aid clinicians in explaining the expected outcomes of different gonadotropin regimens to patients and families.

Acknowledgement

Dr. Wood was funded, in part, by a USDA/ARS cooperative agreement #58-3092-5-001. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does any mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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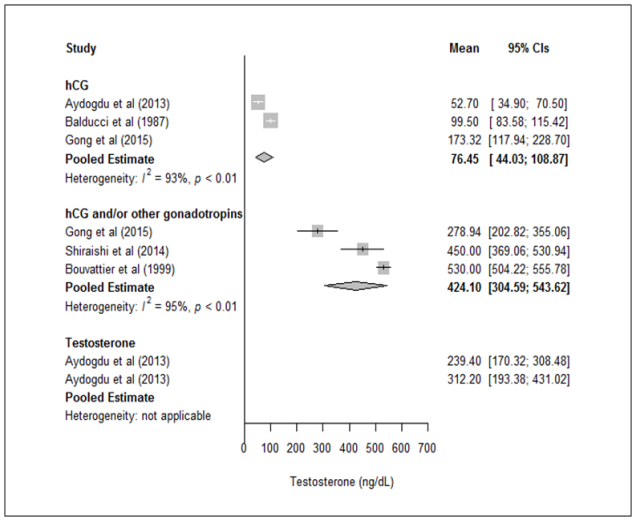
Supplementary Files

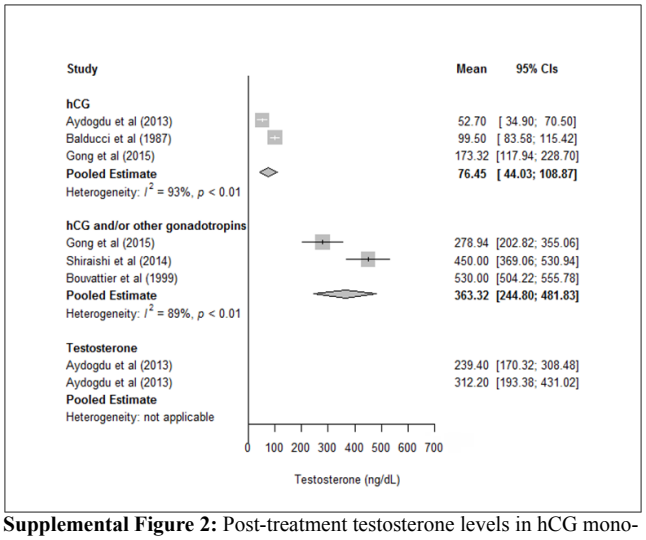
Characteristic	Pooled Estimates				Tests of Heterogeneity					
	hCG		hCG+		Between studies			Between treatment groups		
	N	Mean (95% CI)	N	Mean (95% CI)	Q	df	P	Q	df	P
Age, years	**1**	**21.10 (20.6- 21.7)**	**2**	**16.63 (16.1- 17.2)**	**147.20**	**2**	**<0.001**	**121.71**	**1**	**< 0.001**
FSH, mIU/mL	3	0.79 (0.7- 0.9)	3	0.96 (0.8- 1.1)	13.46	5	0.02	2.47	1	0.12
LH, mIU/mL	3	0.40 (0.3- 0.5)	3	0.49 (0.4- 0.6)	29.27	5	<0.001	1.17	1	0.28
Testosterone, ng/dL	**4**	**7.59 (6.5- 8.7)**	**3**	**24.82 (23.6- 26.0)**	**491.34**	**6**	**<0.001**	**436.74**	**1**	**<0.001**
Subset of studies	2	12.06 (8.4- 15.8)	2	15.66 (12.1- 19.3)	19.37	3	<0.001	1.88	1	0.12
Tanner stage	1	1.30 (1.1- 1.5)	1	1.50 (1.3- 1.7)	1.73	1	0.19	1.73	1	0.19
Penile length, cm	3	5.09 (4.9- 5.3)	2	4.90 (4.8- 5.0)	39.34	2	<0.001	2.24	1	0.13
Mean testicular volume, mL	5	2.03 (1.8- 2.2)	4	1.98 (1.7- 2.3)	35.52	8	<0.001	0.07	1	0.79

Supplemental Table 1: Heterogeneity in baseline characteristics.



Supplemental Figure 1: Post-treatment testosterone levels in hCG mono-therapygroup (excluding the Gong et al. hCG subgroup) versus hCG+ group.

*Bold values are pooled means
Data for testosterone groups were included as a reference.
The 2013 study included two testosterone groups: testosterone gels and injections



Supplemental Figure 2: Post-treatment testosterone levels in hCG mono-therapygroup (excluding the Gong et al. hCG subgroup) versus hCG+ group (excluding the Bouvattier, et al. hCG+ subgroup).

*Bold values are pooled means
Data for testosterone groups were included as a reference.
The 2013 study included two testosterone groups: Testosterone gels and injections

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