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Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotrophic hypogonadism?

A multicentre prospective study of hCG/rFSH treatment outcomes during adolescence

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ABSTRACT

Context/objective: Testosterone treatment for pubertal induction in boys with hypogonadotrophic hypogonadism (HH) provides virilisation, but does not induce testicular growth or fertility. Larger studies evaluating the outcomes of gonadotrophin replacement during adolescence have not been reported to date; whether previous testosterone substitution affects testicular responses is unresolved.
We aimed to assess the effects of human chorionic gonadotrophin (hCG) and recombinant FSH (rFSH) in boys and adolescents with HH with respect to a) testicular growth, b) spermatogenesis, c) quality of life (QoL) and to identify factors influencing therapeutic success.
Design/setting: A prospective case study was conducted in 26 paediatric endocrine centres
Patients/interventions: HCG and rFSH were administered until cessation of testicular growth and plateauing of spermatogenesis to (1) pre-pubertal HH boys with absent or early arrested puberty (group A) and to (2) HH adolescents who had previously received full testosterone replacement (group B). **Outcome measures:** bi-testicular volumes (BTVs), sperm concentrations and QoL.
Results: Sixty (34A/26B) HH patients aged 14-22years were enrolled. BTVs rose from 5±5 to 34±3ml in group A vs. 5±3 to 32±3ml in group B, with normal final BTVs (≥24ml) attained in 74%/70% after 25/23months in A/B respectively. Sperm in the ejaculate were found in 21/23(91%)/18/19(95%), with plateauing concentrations after 31/30 months of hCG and 25/25 months of combined treatment. Sperm concentrations were normal (≥15mill/ml) in 61%/32%, with mean concentrations of 40±73 vs. 19±38mill/ml in A/B (n.s.). Outcomes were better in patients without bilateral cryptorchidism, with non-congenital HH causes, higher baseline BTVs, and higher inhibin B and AMH levels. QoL increased in both groups.
Conclusions: HCG/rFSH replacement during adolescence successfully induces testicular growth and spermatogenesis, irrespective of previous testosterone replacement, and enhances QoL.

Introduction

In boys ≥14 years with hypogonadotrophic hypogonadism (HH) and absent or arrested puberty, pubertal induction is performed by administering increasing doses of testosterone-enanthate i.m every 3-4 weeks. This regimen, established as a therapeutic standard in paediatric endocrinology, stimulates normal linear growth, pubertal virilisation and psycho-sexual maturation, but neglects testicular growth and the acquisition of fertility as components of normal puberty; the testes remain in an immature pre-pubertal state (i.e.< 4 ml), and spermatogenesis is not initiated. Replacement of gonadotrophins in adulthood has repeatedly been proven to be safe and effective in initiating testicular growth and spermatogenesis, sufficient for fertility [1, 2, 3, 4, 5]. Small adolescent case studies have demonstrated the "proof of principle" that, along with pubertal virilisation, pubertal testicular maturation with increase in testis sizes and initiation of spermatogenesis can be achieved by combined hCG/FSH replacement [6, 7, 8, 9, 10,11]. However, prospective studies in HH adolescents large enough for evaluation of outcomes of hCG and FSH have not been reported to date. Although a recent paper[12] specifically addressed quality of life (QoL) in relation to gonadotrophin treatment for adult HH, the impact of gonadotrophin substitution on QoL during adolescence is largely unknown. Whether preceding testosterone replacement may adversely affect therapeutic responses of HH adolescents also remains unresolved to date.

In this prospective multicentre study we aimed to assess the effects of human chorionic gonadotrophin (hCG) and recombinant FSH (rFSH) treatment in young patients with HH of various origins with respect to testicular growth and induction of spermatogenesis. We compared the outcomes of pre-pubertal HH boys with those of HH adolescents who had previously received full testosterone replacement for pubertal virilisation. Furthermore, we evaluated pre- and post-treatment QoL in each patient using validated questionnaires. Finally, we assessed the dependence of therapeutic response on variables at baseline.

Patients and methods

The study was performed over 4 years, between 3/2011 and 3/2015, in 26 centres for paediatric endocrinology throughout Germany and coordinated by the first author at the Department for Clinical Andrology, Centre for Reproductive Medicine, University of Münster/Germany.

Ethics

Informed written consent by majors, and assent by minors with consent of their parents was obtained for all procedures. The study was approved by the Ethics Committee of the State Medical Board of Westfalen-Lippe (approval number: 2010-427-f-S).

Inclusion criteria

Males aged 14-22 years with hypogonadotrophic hypogonadism (HH) were enrolled in the study. Subsets of participants were as follows: Boys/adolescents with:

-absent puberty by age 14, confirmed by testicular volumes <4 ml each side, pre-pubertal levels of LH, FSH and testosterone and failure of GnRH agonist (buserelin, 10 µg/kg s.c) to stimulate LH >4 U/l after 4 hours and/or absent pubertal response to "priming" with low-dose (50-100mg) testosterone-enanthate i.m. over 3-6 months.

-early pubertal arrest after age 14, confirmed by arrested testicular growth (with volumes >4≤8 ml each side) and pre-pubertal levels of LH, FSH and testosterone.

-Kallmann syndrome, confirmed by presence of anosmia or severe hyposmia (by "Sniffin-sticks", Burghart Messtechnik GmbH, Wedel, Germany) or *KAL1* mutation.

-congenital or acquired multiple pituitary hormone deficiencies (MPHDs)

-CHARGE syndrome.

Exclusion criteria

Patients with constitutional delay of growth and puberty (CDGP), testicular disorders (primary hypogonadism), Prader-Willi syndrome, functional hypogonadism (due to eating disorders or chronic diseases) or with HH due to untreated abnormalities of the hypothalamic-pituitary region were excluded.

Primary study end points

-Final bi-testicular volumes (BTVs= the sum of both testicular volumes) by Prader orchidometry

-Final sperm concentrations (SCs), according to WHO 2010 criteria [13]

Secondary study endpoints

-QoL post-gonadotrophin treatment, compared to pre-treatment. Assessed by three standardised questionnaires: "Inventory for assessment of quality of life in children and adolescents" ("ILK")[14], "Inventory for assessment of depression in children and adolescents" ("DIKJ") [15], and "Questionnaire for assessment of emotion regulation strategies in children and adolescents" ("FEEL-KJ") [16], and by two questions on self-perceived satisfaction with testis size and masculinity

Treatment protocols

Patients were divided into two groups:

Group A included pre- pubertal HH boys (Tanner stage G1 with testicular volumes (TVs) <4 ml each side or HH boys with early pubertal arrest (Tanner G2-3 with TVs >4≤8 ml).

Group B comprised fully virilised (Tanner G4-5, TVs <4ml each side) HH adolescents who had received at least 1.5 years of full (250 mg i.m. every 3-4 weeks) testosterone-enanthate replacement.

Highly purified urinary-derived hCG (Brevactid®), followed by combined hCG/rFSH (Gonal f®) was self-administered with or without parental help via subcutaneous injections, two injections/week (on

Mondays and Fridays) for hCG and three injections/week for rFSH (on Mondays, Wednesdays and Fridays).

The end of gonadotrophin replacement was defined by cessation of testicular growth and plateauing of sperm concentrations in the ejaculate over at least two observations at 3 monthly intervals.

Protocol group A: For testosterone-naïve pre-pubertal boys (or early pubertal boys with arrested puberty), the following gonadotrophin replacement protocol was recommended: As pre-pubertal boys have not yet attained their adult height, a relatively low starting dose of (250-) 500 IU hCG was injected subcutaneously on Mondays and Fridays, and incremental increases of 250-500 IU hCG per injection every 6 months to a maximum of 3 x 2500 IU hCG s.c./week were recommended. The aim was to achieve pubertal levels (serum testosterone ≥1.5 ng/ml, [5.2 nmol/l]) after around 6 months, and levels in the mid-normal adult range (testosterone >3.5 ng/ml, [12 nmol/l]) by one year. rFSH (follitropin alpha) 3 x (75-)150 IUs.c/week (injected Mondays, Wednesdays and Fridays) was added when pubertal serum testosterone levels (>5.2 nmol/l) were reached, without subsequent rFSH dosage modifications above 150 IU per injection, aiming at physiologic serum FSH target levels between 1-7 U/L.

Protocol group B:

In those adolescents previously treated with testosterone who had completed pubertal virilisation and linear growth (documented by left hand digital epiphyseal fusion), the following gonadotrophin replacement protocol was used: A full (adult) hCG starting dose of 1.500 IU s.c. was initially applied twice weekly (injected subcutaneously on Mondays and Fridays). HCG dose reduction was recommended if erythrocytosis, gynaecomastia or excessive acne occurred. If testosterone levels remained below the normal adult range (<12 nmol/l) after 6-9 months, the hCG dose was increased by increments of 500(-1000) IU per injection every 6 months until achievement of serum testosterone levels ≥ 12 nmol/l, but not above a maximum of 3 x 2500 IU hCG s.c./week. In all patients rFSH (follitropin alpha) 150 IU was additionally injected thrice weekly (on Mondays, Wednesdays and Fridays) after 3 months of hCG, without subsequent dose modifications, aiming at physiologic serum FSH target levels between 1-7 U/L.

Baseline, follow-up, and outcome measurements

All boys were examined at baseline and during three-monthly follow-up visits while undergoing gonadotrophin substitution; annual bone age estimations were performed in group A.

Individual clinical details at baseline (including underlying causes of HH, and presence or absence of previous cryptorchidism) and outcome data (primary study endpoints) are described in Table 1. Testicular volumes were measured clinically using a Prader orchidometer; summated left plus right

testicular volumes (BTVs) were calculated. To confirm testicular growth and to rule out intra-testicular pathologies, ultrasound investigations were additionally performed (using the formula: length x width x depth/2 for baseline and follow-up volumes and the ellipsoid method [17] for final volumes). AMH and inhibin B levels were assessed at baseline, at initiation of rFSH replacement and on final assessment. LH levels were measured to rule out spontaneous activation of the hypothalamic-pituitary-gonadal (HPG) axis on gonadotrophin substitution; serum testosterone levels were measured every three months to monitor Leydig cell response and, along with FSH levels, adherence to treatment. Once psycho-sexual maturity was attained, ejaculates were collected (by masturbation) after at least 48h of sexual abstinence, and thereafter repeated every three months until plateauing of sperm concentrations in the ejaculate was documented in two follow-up visits. All samples were analysed for volume, sperm concentrations, progressive motility and morphology[13]; total sperm counts were calculated. By the end of gonadotrophin substitution, all patients able to provide a semen sample had a final centralised assessment by one experienced physician (JR), comprising primary and secondary endpoints and final height.

Laboratory methods

Inhibin B and AMH levels were analysed in frozen blood serum samples in the central study centre:
Inhibin B (solid phase sandwich assays, Beckman-Coulter; intra-assay coefficient of variation (CV): 3.3%; (high control: 4.9%); detection limit (DL): 10 pg/ml. Cross-reactivity with inhibin A: 1%). AMH (ELISA, DRG Instruments GmbH; CV: 5.7% (high control: 9.0%); DL: 0.14 pg/ml).
The other standard hormone investigations (LH, FSH, testosterone) were performed by the participating centres during hCG/rFSH substitution and again in the study centre at final assessment.

QoL assessment

All patients were asked to fill in four questionnaires at baseline and by the end of gonadotrophin replacement. The ILK questionnaire included 9 rating items on the adolescent´s perception of his situation in life, involving school, family, personal interests and leisure activities, physical fitness, mental fitness, disease burden and burden by therapeutic interventions. Evaluation was perfomed by calculation of health-related QoL scores (normal: 70-100%) and problem scores (on severity scale from 1-7). The DIKJ questionnaire included 26 items on the adolescent´s emotional and somatic state, including negative feelings and consequences of depressive mood. Evaluation was performed by calculation of t-scores (with significant depression defined as a score >60%). The FEEL-KJ questionnaire included 24 items assessing adaptive and maladaptive strategies for emotional regulation. Two additional questions evaluated “satisfaction with testis size” and “satisfaction with masculinity” on a scale from -2 to+2.

Statistics

Analysis and drafting of figures was performed using Graph Pad Prism 5.0 (GraphPad Software Inc. La Jolla, USA). All results are expressed as the mean ± SD and additional median (range) for QoL data. Where normality of distribution was determined, t-tests for independent samples were conducted; otherwise the Mann-Whitney-U test was used. Dependence between two variables was assessed using Spearman's rank correlation coefficient. Significance was defined as p-value <0.05.

Results

A total of sixty patients aged 14-22 years with HH were enrolled. Group A boys (n=34), mean age 15.5 years, were pre-pubertal or had early arrested puberty. Group B adolescents (n=26), mean age 18.8 years, had received previous full testosterone-enanthate replacement for 1.5-5.7 years (mean: 2.5 years).

Following monotherapy with low doses of hCG replacement, two (additional) previously pre-pubertal patients were recognised as having CDGP and not HH: rising LH serum levels and pubertal testicular growth were observed in these subjects. These patients were not included in the study and hCG replacement was ceased.

In group A, three patients discontinued hCG/rFSH replacement; one patient with congenital multiple pituitary hormone deficiencies (MPHD), and 2 patients with congenital normosmic HH (CHH). Four patients had not yet reached the therapeutic endpoints at the time of evaluation of the study, leaving 27 participants in group A. Twenty-three group A boys provided semen samples for final assessment. In group B, three patients withdrew from the study: one patient with MPHD after tumour surgery, one patient with congenital MPHD and one patient with CHH, leaving 23 group B participants. Nineteen group B adolescents provided semen samples for final assessment.

Puberty induction

In all group A boys, pubertal virilisation to Tanner stage V occurred without major adverse side-effects. Mild gynaecomastia (Tanner B2-3) was observed in four subjects, severe acne did not occur. Pubertal growth from a mean pre-treatment height of 168±10 cm to a mean final height of 181±8 cm, appropriate for mid-parental target height (180±6 cm) was documented. Bone age matured from 14±1.4 to 17 years. Adolescents in group B grew from 176±9 cm (bone age pre-treatment: 16.7±0.7 years) to 178±8cm (parental target: 177±4 cm). Pubertal T levels were reached after 6±3 months of hCG treatment in group A and after 4±3 months in group B.

Endpoints

Final bi-testicular volumes (BTVs) (Figure 1)

Gonadotrophins were administered for 24±7 / 22±6 months in group A/B, respectively until cessation of testicular growth.

BTVs rose from 5±5 ml at baseline to 10±8 ml on hCG alone and to 34±3 ml after combined treatment with hCG and rFSH in group A and from 5±3 to 8±5 to 32±3 ml in group B (Figure 1). Changes in

testicular sizes in response to gonadotrophin treatment in the different HH patient subsets of both groups (A/B) are detailed in Table 1.

Sperm concentrations (SCs) and other semen parameters

Sperm were found in 91% (21/23) of group A vs. 95% (18/19) of group B patients. Two group A patients (one with KS and one with CHH, both with initial BTVs of 6ml) and one group B patient (with CHH, with initial BTVs of 2 ml) remained azoospermic. Only one of them had a history of bilateral cryptorchidism. Successful microscopic testicular sperm extraction (mTESE) was performed in the latter patient with KS, and mTESE samples were cryostored for potential future use in assisted reproduction. The other two azoospermic patients did not wish to undergo surgery for sperm retrieval.

SCs plateaued after 31±6 / 30±7 months from start with hCG and after 25±9 / 25±9 months of combined hCH / rFSH treatment, in A/B, respectively (Figure 2). Final SCs were normal (≥15mill/ml) in 61% (14/23) in group A and 32% (6/19) in group B, and mean SCs were non-significantly higher in A (40±73 mill/ml) than in B (19±38 mill/ml; p=0.07).

In group B first sperm were found 15±7 months after start of hCG administration and 11±6 months after initiation of FSH treatment. The previously pre-pubertal boys required 2.0±1 years of gonadotrophin replacement before "feeling mature enough" to provide a semen sample for laboratory analysis. In this group, first sperm were documented after 21±10 months of hCG and 17±7 months of combined hCG/rFSH administration.

Mean final ejaculate volume was slightly lower than the WHO normal value in group A (A: 1.3±0.2 ml; B: 3.8±0.8 ml; normal: ≥1.5 ml). Final total sperm counts were not significantly different (A: 60±160 mill; B: 42±55 mill; normal: ≥39 mill; p=0.43), neither was progressive motility (A: 43±18%; B: 42±14%; normal: ≥32%), nor sperm morphology (A: 4±3%; B: 3±2%; normal: ≥4%).

Quality of life (QoL) before and after gonadotrophin replacement

At baseline, health-related QoL scores (ILK) (Figure 4) were at the lower limit of the normal range in both groups (median: A: 74%; B: 75%). Health-related problem scores (ILK) (on a scale from 1-7) were comparable in A and B, but showed large intra-individual variations (median (range): A: 2.0 (0-7); B: 1.0 (0-5)). Group B adolescents had significantly higher baseline depression scores (DIKJ) than group A boys, with less variation (median (range): A: 34 (0-100); B: 50 (35-73); A/B pre-treatment p=0.03).

After gonadotrophin treatment, QoL scores were significantly higher than pre-treatment in both groups (A: 86%; pre/post p=0.03; B: 82%; pre/post p=0.03), accompanied by significantly lower post-treatment problem scores (A: 0; pre/post p=0.04; B: 0; pre/post p=0.01) and lower depression scores in group A (A: 19 (3-94); pre/post p=0.05), while depression scores in group B had not significantly changed (B: 43 (35-66). When comparing post-treatment scores between the two groups, depression

scores of group B were significantly higher than those of A (post-treatment A/B $p<0.01$), while post-treatment QOL and problem scores were not different.

There were no significant changes in response to gonadotrophin treatment in both groups with respect to scores for adaptive and maladaptive strategies of emotional regulation (FEEL-KJ) (data not shown).

Self-reported satisfaction (on a scale from -2 to +2) concerning testis size was considerably higher in both groups after gonadotrophin treatment (mean pre-treatment scores A/B: -1.2/-1.2 vs. +1.3/+1.1 post treatment in A/B, respectively). Satisfaction concerning masculinity increased more in group A (A: from -0.5 to +0.9 vs. B: from -0.2 to +0.2) (supplementary Figure1).

Analysis of baseline variables potentially influencing therapeutic response to gonadotrophins

-Causes of HH

With respect to adherence to treatment, which was better in group A (supplementary Figure 2), there was a trend towards higher final BTVs and higher final sperm concentrations achieved by patients with childhood-acquired causes of HH (MPHD after tumour surgery and CHH with pubertal arrest;) compared to those with congenital causes (Kallmann syndrome, CHH with absent puberty, congenital MPHD (group A: final BTV HH acquired: 50±21ml, vs. HH congenital: 34±14; $p=0.07$; final sperm concentration acquired HH: 94 ± 81mill/ml vs. congenital HH 43 ± 17; $p=0.3$) (Table 1).

-Undescended testes

Final sperm concentrations of patients with bilateral cryptorchidism were lower than for those with unilateral or no undescended testis (Figure 3a). Of the whole cohort of adolescents, 15 subjects (45% of group A and 32 % of group B) (Table 1) had a history of undescended testes, 4 unilateral and 11 bilateral at birth. All had orchidopexy before the age of six, most of them before the age of two.

-Initial testicular size

Initial BTVs (by ultrasound) correlated with final ultrasound BTV on gonadotrophin replacement in both groups (r: A:0.56/B:0.57; $p<0.001$) (Figure 3b). BTVs also correlated with final sperm concentrations (and final total counts) in group A (r: 0.51; $p=0.025$), but not in group B.

-Markers of Sertoli cell maturity (inhibin B, AMH)

Baseline inhibin B levels before gonadotrophin replacement correlated with final BTV in both groups (r: A: 0.51/B: 0.57; $p<0,01$) (Figure 3c). A significant correlation with final SCs (r: 0.64; $p=0.002$) and final total count (Spearman r: 0.73; $p=0.0002$) was found only in group A.

There was a significant correlation of baseline AMH and final SCs and total sperm count (r: A: 0.42/B: 0.41; $p<0.02$) in both groups (Figure 3d), and a significant correlation with final BTVs in group A (r: 0.40; $p=0.047$).

Kinetics of Sertoli cell markers during gonadotrophin replacement

Group A had baseline inhibin B levels of 39±35 pg/ml, rising to 76±61 pg/ml on hCG alone with maximum inhibin B levels on hCG/rFSH of 177±118 pg/ml (normal adult range: 125-330 pg/ml) (Table 1). Mean serum inhibin B levels were lower at baseline in group B with 27±23 pg/ml (p=0.02). HCG-stimulated levels (44±28 pg/ml) and maximum inhibin B levels on hCG+rFSH (122±73 pg/ml) were not significantly different from group A.

Mean baseline AMH levels were not significantly different between groups A/B (respectively 31±32 vs. 20±13 ng/ml), declined to 17±19 / 16±15 ng/ml on hCG, and reaching minimum levels on hCG/rFSH of 5.8±4.3 / 3.7±2.7 ng/ml (normal adult range: 1.3-14.8 ng/ml) (Table 1).

Discussion

Complete pubertal induction

While the standard therapeutic regimen for pubertal induction in boys with HH based on testosterone administration has largely neglected testicular growth and spermatogenesis, this comprehensive prospective study demonstrates that induction of complete puberty including testicular maturation can be achieved by gonadotrophin substitution. In addition, our observations confirm that pubertal virilisation can be induced with gonadotrophins without major adverse effects and with attainment of final heights in the range of mid-parental expectations in boys with HH of various origins.

Treatment protocols

We hereby suggest a protocol for hCG/rFSH replacement in pre-pubertal boys and testosterone-virilised adolescents with HH that is effective, irrespective of the underlying aetiology. While protocol B (aiming at testicular maturation after completed virilisation) is comparable to regimens previously described for adults[1, 3, 4], protocol A was established for complete pubertal induction in pre-pubertal boys, allowing for developmental immaturity (including delayed bone age) and aiming to achieve physiologic pubertal increments in serum testosterone levels during the first year of hormone replacement via progressive hCG dose escalation.

Differential diagnosis of CDGP

The suggested protocol A enables activation of the pubertal GnRH pulse generator in cases of unrecognised constitutional delay of puberty (CDGP) by use of low initial hCG doses, exerting only minimal suppressive effects on the hypothalamo-pituitary-gonadal (HPG) axis. We thereby identified two patients wrongly diagnosed with HH. Nevertheless, special attention to LH levels during gonadotrophin replacement seems mandatory in view of this challenging differential diagnosis.

Somatic outcomes (primary study end points) and duration of gonadotrophin replacement

The findings of this study provide evidence that pubertal virilisation, in concert with pubertal testicular growth and initiation of spermatogenesis, can be successfully induced during adolescence, with >72%

normal (adult) final testicular sizes and >92% evidence of full spermatogenesis achieved by combined treatment with hCG and rFSH. Treatment for 6 months with hCG, followed by 25 months with rFSH, i.e. around 2.5 years of gonadotrophin administration seems to be required in adolescents to achieve full individual potential for testicular growth and spermatogenesis.

Previous studies on adolescents

Previous studies, including a small number of pre-pubertal HH boys have demonstrated the "proof of principle" that hCG induces a rise in serum testosterone levels, resulting in virilisation [8, 10, 26, 27], and that hCG, combined with FSH stimulates testicular growth and activates spermatogenesis in adolescents [6, 7, 8, 9, 10,11]. Table 2 provides an overview on these studies, in comparison to our study.

Gonadotrophin preparations

The efficacy and safety of gonadotrophin substitution in adult male HH patients for initiating testicular growth and spermatogenesis, sufficient for fertility, has been reported on several occasions. [1, 2, 3, 4] HCG contains almost exclusively LH-like bioactivity [18], stimulating testosterone production by Leydig cells; FSH is required for spermatid maturation (spermogenesis) during the initiation, and for maintenance of quantitatively normal spermatogenesis at puberty and thereafter [19, 20]. HCG has been used as a source of LH since 1952 [21] and urinary human menopausal gonadotrophin (hMG), applied to substitute for FSH since 1966 [1, 2, 6, 7, 22]. Highly purified urinary FSH has been available since 1997/98 [2, 4], and recombinant FSH (rFSH) since 1995 [5, 23, 24, 25]. In this study, rFSH was used, as it is the only FSH preparation licenced for fertility induction in hypogonadotrophic males in Europe.

Arguments for conventional pubertal induction in HH

An argument that has been raised in favour of the traditional replacement regimen using testosterone enanthate for puberty induction in HH is the practicability of one (or two)-monthly i.m. injections and the low costs of this replacement strategy. While the expenses of urinary-derived hCG-replacement are comparable, rFSH is expensive. Another reason for compliance with testosterone is related to the assumption that fertility is "not yet an issue" at an adolescent age and that the current strategy is satisfactorily addressing the patient´s needs in terms of masculinity.

Impact of gonadotrophin replacement on quality of life

Our results of QoL assessment pre-gonadotrophin treatment demonstrate that the feeling of "being different" in terms of sexual development at a time when normal puberty occurs, has a negative impact on the young HH patient´s well-being. Higher pre- treatment depression scores in boys who had previously received testosterone for puberty induction (group B), compared to testosterone-naïve boys (group A) indicate that replacement of testosterone does not resolve this problem. Boys with HH do have pervasive and persistent concerns with body image and future fertility prospects. In support of this, a recent paper identifying unmet health needs of CHH patients based on a web-based assessment

found that these individuals often struggle with the psychosocial sequelae of CHH [28]. Our study provides evidence for reduced anxiety and improved overall QoL parameters, when physical pubertal normality is achieved, and when the potential for future fatherhood is demonstrated by activated spermatogenesis.

Although satisfaction with testicular size was remarkably higher after gonadotrophin replacement in both treatment groups, we observed lesser final satisfaction with masculinity and persistently higher depression scores, even after treatment, in group B. Previous induction of incomplete puberty by testosterone thus seems to neglect a "window of opportunity" to provide self-assurance and promote confidence for the future. Body image and fertility concerns in the HH patient may therefore best be addressed at a peer-related time. In support of this, overall compliance of study patients with taking five s.c. injections per week was surprisingly good and even better in previously pre-pubertal boys who were still under parental supervision.

Comparison of adolescent outcomes with those of adults

Treatment outcomes in boys and adolescents in our trial were better than those reported for adults [2, 4, 5, 19, 24] (supplementary Table 1).

However, higher sperm concentrations achieved by adolescents may only be a relative advantage as spermatozoa of HH patients treated with gonadotrophins or GnRH have an excellent fertilising potential, despite subnormal counts [3, 8, 29, 30, 3, 32].

Impact of previous testosterone replacement on somatic outcomes

Our study contributes to the question whether treatment effects of gonadotrophins during adolescence are affected by previous testosterone replacement. In line with a recent meta-analysis of previous adult studies[33], which showed no significant association between prevalence of previous testosterone replacement and sperm concentration, we did not observe differences in outcomes with hCG/FSH replacement in terms of testicular size or sperm count achieved in pre-pubertal boys vs. adolescents with prior full-dose testosterone replacement for up to 5.7 years. Only one out of three patients who remained azoospermic had previously received testosterone. In contrast, in another study on adult patients that were previously treated with androgens, a decreased likelihood of achieving sperm output thresholds and conception [34] was observed.

Factors influencing therapeutic response to gonadotrophins

The results of this study indicate that testicular growth potential and satisfactory sperm concentrations in response to gonadotrophin substitution during adolescence depend on various factors at baseline. Patients without previous bilateral cryptorchidism, with non-congenital HH causes, with higher baseline testicular volumes, and with higher baseline inhibin B and AMH serum levels had more favourable outcomes. These findings are in line with predictors of response to treatment that have been defined in adult studies [3, 24, 29, 31, 32, 35, 36] All the above-mentioned parameters reflect the degree of

seminiferous tubular maturation that may have occurred pre-treatment, which in turn is dependent on the onset and the extent of GnRH and/or gonadotrophin deficiency. However, as responses also depend on adherence to treatment, individual outcomes cannot reliably be predicted. The variability in observed response within certain diagnostic subgroups (Kallmann syndrome, CHH, MPHD congenital) may also be due to genetic heterogeneity and oligogenicity [37] or epigenetic phenomena, resulting in different hormone secretion patterns, varying from complete absence of pulses to disorders of amplitude and/or frequency [38].

Further arguments for early gonadotrophin substitution

Timely completed testicular maturation has further considerable advantages: First, it is likely to significantly reduce the time necessary for re-induction of spermatogenesis by future gonadotrophin cycles in adulthood [3, 32], thereby enabling earlier spontaneous conception of the partner. This seems important in view of increasing female age at first pregnancy in modern societies. Second, poor responders to gonadotrophin replacement during adolescence may respond worse with increasing age. Our adolescents were therefore given the opportunity for sperm cryo-preservation, thereby safeguarding a chance for future biological paternity in case of adverse future events concerning fertility. Third, adolescents with persisting azoospermia may undergo mTESE before switching to permanent testosterone replacement and thereby rescue sperm for potential use in future reproduction. This was successfully performed in one patient in our study.

Future prospects

Questions remain as to the optimal timing of treatment with FSH and whether an attempt should be made to expand Sertoli cell numbers, either during the neonatal period (if HH is recognised by the presence of micropenis and cryptorchidism) [39] or before initiating puberty and whether these actions could improve fertility. In a recent study [40], 7 adult HH patients without cryptorchidism given FSH treatment before GnRH substitution had serial testicular biopsies showing Sertoli and spermatogonial cell proliferation and higher final sperm counts than in the control group. However, final SCs were far below the normal range (5.8±2.3 mill/ml).

Conclusions

The availability of hCG and rFSH for replacement of gonadotrophins provides an alternative option for endocrinologists to safely induce puberty in boys with hypogonadotrophic hypogonadism, leading to normal linear growth and virilisation, testicular enlargement and early induction of spermatogenesis. This method is effective and reassuring for affected individuals and is likely to reduce the expense, duration and anxiety of late fertility induction. Favourable outcomes during adolescence appear not to be compromised by short-term prior testosterone substitution. Clinical parameters, reflecting the onset and severity of GnRH and/or gonadotrophin deficiency may serve as tools to predict response to treatment.

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Figure legends

Figure 1

a) Testicular growth over time in response to gonadotrophin replacement with hCG and rFSH in pre-pubertal (group A) and testosterone-virilised (group B) adolescents with HH.

b) Final bi-testicular volumes (BTV) over time to final BTV from start of hCG therapy in group A and B.

The dashed lines indicate the lower limit of normal BTVs (24ml); the black lines indicate mean final BTVs and mean duration from start of hCG until attainment of final BTVs.

A: mean final BTVs: 34±16 ml with 74% (20/27) of patients reaching a normal BTV ≥24ml;
mean duration from start of hCG therapy until final BTV: 24±7 months.

B: mean final BTV: 32±16 ml with 70% (16/23) of patients reaching a normal BTV≥24ml;
mean duration from start of hCG until final TV: 22 ± 6 months; all p>0,05; n.s.

Figure 2

a) Increase in sperm concentration over time in response to gonadotrophin replacement with hCG and rFSH in previously pre-pubertal (group A) and previously testosterone-virilised (group B) adolescents with HH.

Baseline azoospermia was assumed in all boys of group A, as pre-treatment semen analysis was not possible due to psycho-sexual immaturity.

b) Final sperm concentration over time of rFSH treatment, until a plateau in group A and B was reached.

A: mean final sperm conc.: 40±73 mill/ml, with 61% (14/23) of patients reaching a normal sperm concentration ≥15 mill/ml; mean duration from start of rFSH until final sperm concentration: 25±7 months.

B: mean final sperm concentration: 20±9 mill/ml, with 32% (6/19) of patients reaching a normal sperm conc.≥15 mill/ml (A vs. B: p=0.007); mean duration from start of rFSH therapy until final sperm concentration: 25±9 months; (A vs. B: n.s.).

Figure 3

Predictors of response to hCG/rFSH treatment

a) Presence of undescended testes at birth: Mean sperm concentrations for patients with bilateral/unilateral/no cryptorchid testes at birth were: 4±6 / 38±46 / 44±74 mill/ml, respectively.
b) Correlation of initial ultrasound bi-testicular volume (BTV) with final ultrasound BTV in both groups (A+B); Spearman r: 0.56/0.57; p<0.001.
c) Correlation of baseline inhibin B serum levels with final BTV (Prader) in both groups; r: 0.51/0.57; p<0.01.
d) Correlation of baseline AMH serum levels with final sperm quality (sperm concentration and total sperm count) (r: 0.42/0.41; p<0.02) in both groups.

Figure 4

Box and whisker plots showing medians, interquartile ranges (boxes) and ranges (whisters) for results of QoL questionnaires. These were filled in by n= 26 group A boys before gonadotrophin treatment and again (n=15) after puberty induction with gonadotrophins, while n=17 testosterone-virilised adolescents answered all questions prior to gonadotrophin substitution and n=13 of these again following gonadotrophin replacement.

Supplementary Figure 1

Self-reported satisfaction (on a score ranging from -2 to +2) with testis size and masculinity before and after gonadotrophin replacement in previously pre-pubertal boys (group A) and testosterone-virilised adolescents (group B) with hypogonadotrophic hypogonadism.

Supplementary Figure 2

Serum testosterone levels on gonadotrophin replacement with hCG and rFSH in previously pre-pubertal (group A) and previously testosterone-virilised (group B) adolescents with HH in response to Leydig cell stimulation with hCG (subsequently combined with rFSH). Transient drops in levels indicate omission of hCG injections by the adolescent.

References

1. Kliesch S, Behre HM & Nieschlag E. (1994) High efficacy of gonadotropin or pulsatile gonadotropin-releasing hormone treatment in hypogonadotropic hypogonadal men. *Eur J Endocrinol*, **131**, 347-354.

2. Burgués S & Calderón MD. (1997) Subcutaneous self-administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the

559 treatment of male hypogonadotrophic hypogonadism. *Human Reproduction*, **12**, 980-
560 986.
561
562 3. Büchter D, Behre HM, Kliesch S & Nieschlag E. (1998) Pulsatile GnRH or human
563 chorionic gonadotropin/human menopausal gonadotropin as effective treatment for
564 men with hypogonadotropic hypogonadism: A review of 42 cases. *European Journal*
565 *of Endocrinology*, **139**, 298-303.
566
567 4. European Metrodin HP Study Group EMHSG. (1998) Efficacy and safety of highly
568 purified urinary follicle-stimulating hormone with human chorionic gonadotropin for
569 treating men with isolated hypogonadotropic hypogonadism. European Metrodin HP
570 Study Group. *Fertil Steril*, **70**, 256-262.
571
572 5. Bouloux P, Warne DW & Loumaye E. (2002) Efficacy and safety of recombinant
573 human follicle-stimulating hormone in men with isolated hypogonadotropic
574 hypogonadism. *Fertility and Sterility*, **77**, 270-273.
575
576 6. Liu L, Banks SM, Barnes KM & Sherins RJ. (1988) Two-year comparison of
577 testicular responses to pulsatile gonadotropin-releasing hormone and exogenous
578 gonadotropins from the inception of therapy in men with isolated hypogonadotropic
579 hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **67**, 1140-1145.
580
581 7. Schopohl J. (1993) Pulsatile gonadotrophin releasing hormone versus gonadotrophin
582 treatment of hypothalamic hypogonadism in males. *Hum Reprod*, **8**, 175-179.
583
584 8. Barrio R, De Luis D, Alonso M, Lamas A & Moreno JC. (1999) Induction of puberty
585 with human chorionic gonadotropin and follicle- stimulating hormone in adolescent
586 males with hypogonadotropic hypogonadism. *Fertility and Sterility*, **71**, 244-248.
587
588 9. Raivio T, Wikström AM, Dunkel L. (2007) Treatment of gonadotropin-deficient boys
589 with recombinant human FSH: long-term observation and outcome. *Eur J Endocrinol.*,
590 **156**, 105-111.
591

10. Sinisi AA, Esposito D, Maione L, Quinto MC, Visconti D, De Bellis A, Bellastella A, Conzo G & Bellastella G. (2008) Seminal anti-Mullerian hormone level is a marker of spermatogenic response during long-term gonadotropin therapy in male hypogonadotropic hypogonadism. *Hum Reprod, **23**, 1029-1034*

11. Zacharin M, Sabin MA, Nair VV & Dagabdhao P. (2012) Addition of recombinant follicle-stimulating hormone to human chorionic gonadotropin treatment in adolescents and young adults with hypogonadotropic hypogonadism promotes normal testicular growth and may promote early spermatogenesis. *Fertility and Sterility*, **98**, 836-842.

12. Shiraishi K, Oka S, Matsuyama H. (2014) Assessment of quality of life during gonadotrophin treatment for male hypogonadotrophic hypogonadism. Clin Endocrinol (Oxf). **81**, 259-265.

13. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT & Vogelsong KM. (2010) World Health Organization reference values for human semen characteristics. *Hum Reprod Update*, **16**, 231-245.

14. Wurst E, Herle M, Fuiko R, Hajszan M, Katkhouda C, Kieboom A, Schubert MT. (2002) The quality of life of chronically ill and psychiatrically disturbed children. Initial experiences with an inventory for assessing quality of life in children and adolescents.*Z Kinder Jugendpsychiatr Psychother*., **30**, 21-28.

15. Steck B, Grether A, Amsler F, Dillier AS, Romer G, Kappos L, Bürgin D. (2007) Disease variables and depression affecting the process of coping in families with a somatically ill parent. *Psychopathology*., **40**, 394-404.

16. Cracco E, Van Durme K, Braet C. (2015) Validation of the FEEL-KJ: An Instrument to Measure Emotion Regulation Strategies in Children and Adolescents. *PLoS One*, **2**, 10(9):e0137080. doi: 10.1371/journal.pone.0137080.

17. Behre HM, Nashan D & Nieschlag E. (1989) Objective measurement of testicular volume by ultrasonography: evaluation of the technique and comparison with orchidometer estimates. *Int J Androl*, **12**, 395-403.

18. Siris ES, Nisula BC, Catt KJ, Horner K, Birken S, Canfield RE & Ross GT. (1978) New evidence for intrinsic follicle-stimulating hormone-like activity in human chorionic gonadotropin and luteinizing hormone. *Endocrinology*, **102**, 1356-1361.

19. Matsumoto AM, Karpas AE & Bremner WJ. (1986) Chronic human chorionic gonadotropin administration in normal men: Evidence that follicle-stimulating hormone is necessary for the maintenance of quantitatively normal spermatogenesis in man. *Journal of Clinical Endocrinology and Metabolism*, **62**, 1184-1192.

20. Tapanainen JS, Aittomaki K, Min J, Vaskivuo T & Huhtaniemi IT. (1997) Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nat Genet,* **15**, 205-206.

21. Maddock WO & Nelson WO. (1952) The effects of chorionic gonadotropin in adult men: increased estrogen and 17-ketosteroid excretion, gynecomastia, leydig cell stimulation and semi-niferous tubule damage. *Journal of Clinical Endocrinology and Metabolism*, **12**, 985-1014.

22. Lytton B & Kase N. (1966) Effects of human menopausal gonadotrophin on a eunuchoidal male. *N Engl J Med*, **274**, 1061-1064.

23. Kliesch S, Behre HM, Nieschlag E. (1995) Recombinant human follicle-stimulating hormone and human chorionic gonadotropin for induction of spermatogenesis in a hypogonadotropic male. *Fertil Steril.;* **63**, 1326-1328.

24. Warne DW, Decosterd G, Okada H, Yano Y, Koide N & Howles CM. (2009) A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle-

stimulating hormone and human chorionic gonadotropin. *Fertility and Sterility*, **92**, 594-604.

25. Matsumoto AM, Snyder PJ, Bhasin S, Martin K, Weber T, Winters S, Spratt D, Brentzel J & O'Dea L. (2009) Stimulation of spermatogenesis with recombinant human follicle-stimulating hormone (follitropin alfa; GONAL-f®): long-term treatment in azoospermic men with hypogonadotropic hypogonadism. *Fertility and Sterility*, **92**, 979-990.

26. Bistritzer T, Lunenfeld B, Passwell JH & Theodor R. (1989) Hormonal therapy and pubertal development in boys with selective hypogonadotropic hypogonadism. *Fertil Steril*, **52**, 302-306.

27. Gong C, Liu Y, Qin M, Wu D & Wang X. (2015) Pulsatile GnRH is superior to hCG in therapeutic efficacy in adolescent boys with hypogonadotropic hypogonadodism. *J Clin Endocrinol Metab*, **100**, 2793-2799. doi: 10.1210/jc.2015-1343

28. Dwyer AA, Quinton R, Morin D & Pitteloud N. (2014) Identifying the unmet health needs of patients with congenital hypogonadotropic hypogonadism using a web-based needs assessment: implications for online interventions and peer-to-peer support. *Orphanet J Rare Dis*, **9**, 83. doi: 10.1186/1750-1172-9-83.

29. Miyagawa Y, Tsujimura A, Matsumiya K, Takao T, Tohda A, Koga M, Takeyama M, Fujioka H, Takada S, Koide T & Okuyama A. (2005) Outcome of gonadotropin therapy for male hypogonadotropic hypogonadism at university affiliated male infertility centers: A 30-year retrospective study. *Journal of Urology*, **173**, 2072-2075.

30. Liu PY, Gebski VJ, Turner L, Conway AJ, Wishart SM & Handelsman DJ. (2002) Predicting pregnancy and spermatogenesis by survival analysis during gonadotrophin treatment of gonadotrophin-deficient infertile men. *Human Reproduction*, **17**, 625-633.

31. Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT & Crowley WF, Jr. (2002) The role of prior pubertal development, biochemical markers

of testicula maturation, and genetics in elucidationg the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **87**, 152-160.

32. Rohayem J, Sinthofen N, Nieschlag E, Kliesch S, Zitzmann M. (2016) Causes of hypogonadotropic hypogonadism predict response to gonadotropin substitution in adults *Andrology* in press

33. Rastrelli G, Corona G, Mannucci E, Maggi M. (2014) Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study. Andrology, **2**, 794-808.

34. Liu PY, Baker HWG, Jayadev V, Zacharin M, Conway AJ & Handelsman DJ. (2009) Induction of spermatogenesis and fertility during gonadotropin treatment of Gonadotropin-Deficient infertile men: Predictors of fertility outcome. *Journal of Clinical Endocrinology and Metabolism*, **94**, 801-808.

35. Kirk JMW, Savage MO, Grant DB, Bouloux PMG & Besser GM. (1994) Gonadal function and response to human chorionic and menopausal gonadotrophin therapy in male patients with idiopathic hypogonadotrophic hypogonadism. *Clinical Endocrinology*, **41**, 57-63.

36. Pitteloud N, Hayes FJ, Dwyer A, Boepple PA, Lee H & Crowley Jr WF. (2002) Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **87**, 4128-4136.

37. Mitchell AL, Dwyer A, Pitteloud N & Quinton R. (2011) Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. *Trends Endocrinol Metab*, **22**, 249-258

38. Waldstreicher J, Seminara SB, Jameson JL, Geyer A, Nachtigall LB, Boepple PA, Holmes LB & Crowley WF, Jr. (1996) The genetic and clinical heterogeneity of

gonadotropin-releasing hormone deficiency in the human. *J Clin Endocrinol Metab*, **81**, 4388-4395.

39. Bougneres P, Francois M, Pantalone L, Rodrigue D, Bouvattier C, Demesteere E, Roger D & Lahlou N. (2008) Effects of an early postnatal treatment of hypogonadotropic hypogonadism with a continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone. *J Clin Endocrinol Metab*, **93**, 2202-2205.

40. Dwyer AA, Sykitios GP, Hayes FJ, Boepple PA, Lee H, Loughlin KRDM, Sluss PM, Crowley WF, Jr. & Pitteloud N. (2013) Trial of recombinant follicle-stimulationg hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **98**, E1790-E1795.

Table 1
Baseline and outcome measurements on hCG/rFSH therapy in previously pre-pubertal (A) and T-virilised (B) adolescents with HH

Cause of HH n:A/B	baseline inhibinB (pg/ml)		baseline AMH (ng/ml)		baseline BTV (ml) Prader orchiometer/ ultrasound		cryptorchidism (% of cohort)		InhibinB max. (pg/ml)		AMH min. (ng/ml)		final BTV Prader orchio. / ultrasound (ml)		final sperm conc. (mill/ml)		final total sperm count (mill)	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Kallmann syndrome n:11/9	20±8	18±18	21±13	18±12	3.5±1.4/ 1.5±0.6	4.2±3.3/ 2.1±0.6	82	56	112±66	87±52	3.7±2.7	2.8±3.2	30±8/ 16±8	31±13/ 16±5	37±36	21±52	19±22	26±43
CHH absent puberty n:10/8	31±19	28±22	21±14	26±17	3.5±1.7/ 1.9±1.0	4.6±2.4/ 2.2±1.5	33	29	198±145	266±128	6.0±5.7	5.1±2.5	39±18/ 26±15	36±16/ 24±8	37±48	24±28	39±57	68±70
CHH pubertal arrest n:2/0	120±13	-	10±2	-	20±6/ 14.0±2.8	-	0	-	219±52	-	5.0±0	-	42±12/ 31±6	-	8.8±9	-	19±27	-
MPHD congenital n:5/3	14±1	31±33	11±5	13±6	2.5±2.0/ 0.9±0.5	6.0±5.0/ 2.6±1.1	25	33	126	133±173	3.7±2.2	2.2±2.9	23±7/ 11±6	26±23/ 24±16	19.3±20	4.2	51±16	59
MPHD after tumour n:4/6	94±102	33±29	32±14	22±11	5.5±4.4/ 4.9±6.2	6.3±4.0/ 3.4±2.8	0	0	270±156	177±35	8.7±10.9	3.9±1.5	60±28/ 36±11	24±21/ 19±9	180±222	12±12	261±303	18±12
CHARGE syndrome n:2/0	48±31	-	42±47	-	4,0±2.8/ 1.4±0,9	-	100	-	49.7	-	1.85	-	36±19 11	-	8.9±11	-	2.8±4	-

Table 1
Baseline and outcome measurements on hCG/rFSH therapy in previously pre-pubertal (A) and T-virilised (B) adolescents with HH

all patients n:34/26*	**39**±45	**27**±23	**31**±32	**20**±13	**4.6**±4.7/ **2.7**±3.8	**5.0**±3.4/ **2.5**±1.6	**45**	**32**	**177**±118	**122**±73	**5.8**±4.3	**3.7**±2.7	**35**±15 **21**±12	**32**±16/ **19**±8	**40**±73	**19**±38	**60**±160	**42**±55
p-value **(A/B)**	**p=0.02**		p=0.24, n.s		p=0.89; n.s p=0.48; n.s		_		p=0.14, n.s.		p=0.80, n.s.		p=0.95, n.s.		p=0.07, n.s.		p=0.43, n.s.	

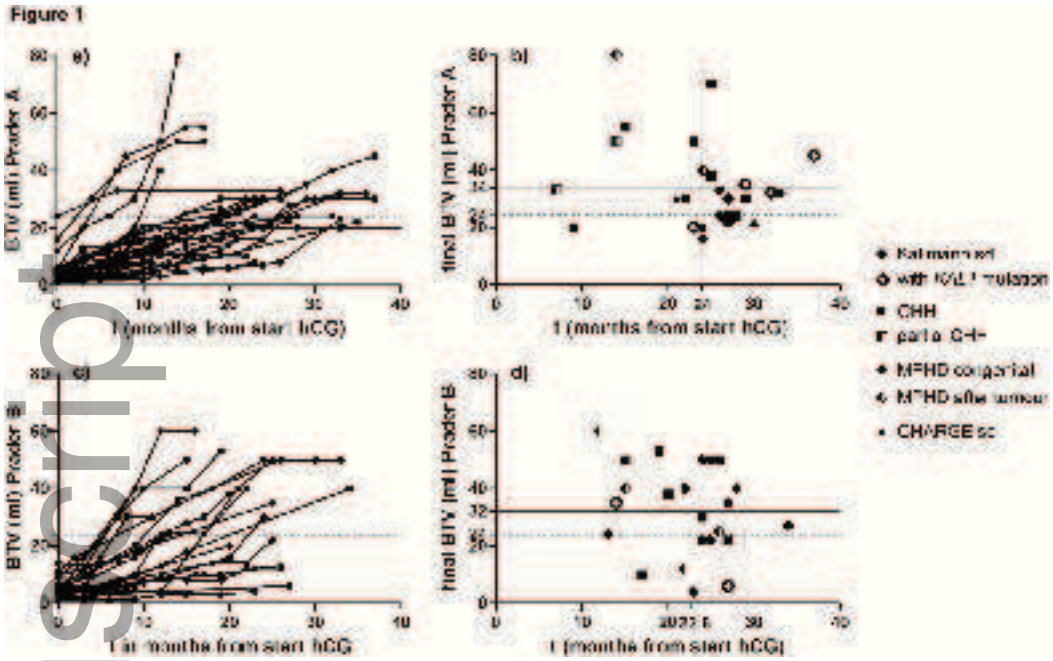
*Semen was available for analysis in 23 group A and 19 group B patients

Table 2
Outcomes of previous studies on gonadotrophin replacement in adolescents with hypogonadotrophic hypogonadism and outcomes of this study

Studies	Number of **adolescent** HH patients	Age (years)	Gonadotrophin preparations and sequence of applications	Duration of replacement (months)	Adult T levels reached (%)	Mean±SD median(range) final single TV (ml) reached	Spermato-genesis achieved	Sperm concentration achieved (mill/ml) median (range) mean±SD	Time to sperm plateau (months)
Liu L et al. (1988)[6]	3 (subset of cohort)	16-17	hCG/HMG	n.a.	100	9 ± 1	n.a. total cohort: 80%	<5	n.a.
Schopohl et al. (1993)[7]	9 (subset of cohort)	18-24	hCG/MHM	20 ± 2	100	n.a. (8-30)	n.a. total cohort: 47%	n.a. (2-26)	20±2
Barrio et al. (1999)[8]	14 IHH: 7 panhypopit: 7	13-21	hCG+rFSH	31	100	IHH: 10 ± 4 panhypopit: 15 ± 5	7/8 (87%) IHH: 4/5 panhypo: 3/3	n.a. (1.5-80)	n.a.
Raivio et al. (2007)[9]	14	10-18	rFSH→ rFSH+hCG	rFSH: 2-34 hCG+FSH: n.a	100	6 (2-37)	6/7 (86%)	8.5 (2.9-92)	n.a.
Sinsi et al. (2008)[10]	10 (subset of cohort)	11-25	hCG→ hCG+rFSH	hCG/rFSH: 12 (-24)	100	10 (7-15)	n.a. total cohort: 81%	29 (2.6-96)	n.a.

Table 2
Outcomes of previous studies on gonadotrophin replacement in adolescents with hypogonadotrophic hypogonadism and outcomes of this study

Zacharin et al. (2012) [11]	7 (subset of the cohort)	16-22	hCG→ hCG+rFSH	hCG/rFSH: 9	100	12 ± 7 10 (5-27)	7/7 (100%)	1.2 (0.2-15) 4.6 ± 6	n.a.
This study:	60 A:34 B:26	14-22	A: hCG→ hCG+rFSH B: Testo→ hCG→ hCG+rFSH	A: hCG: 31 ± 6 hCG/FSH: 25 ± 9 B: hCG: 30 ± 7 hCG/FSH: 25 ± 9	A: 100 B: 100	A: 17 ± 3; 15 (8-40) B: 16 ± 3 17.5 (2-30)	A: 21/23 (91%) B: 18/19 (95%)	A: 17 (0.2-337) 40 ± 73 B: 3.5 (0.1-158) 19 ± 38	A: 31 ± 6 B: 30 ± 7



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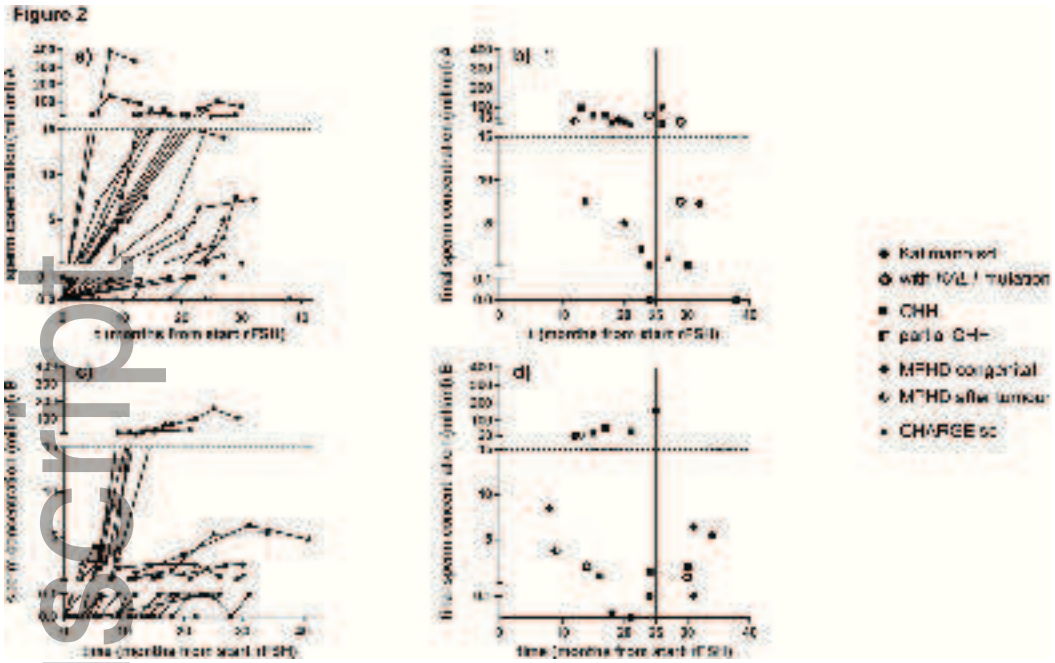

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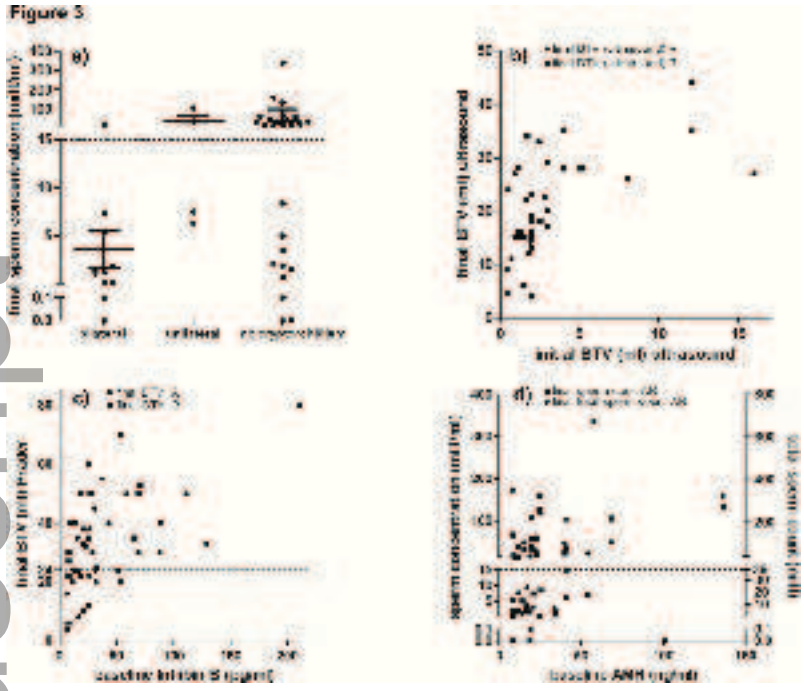

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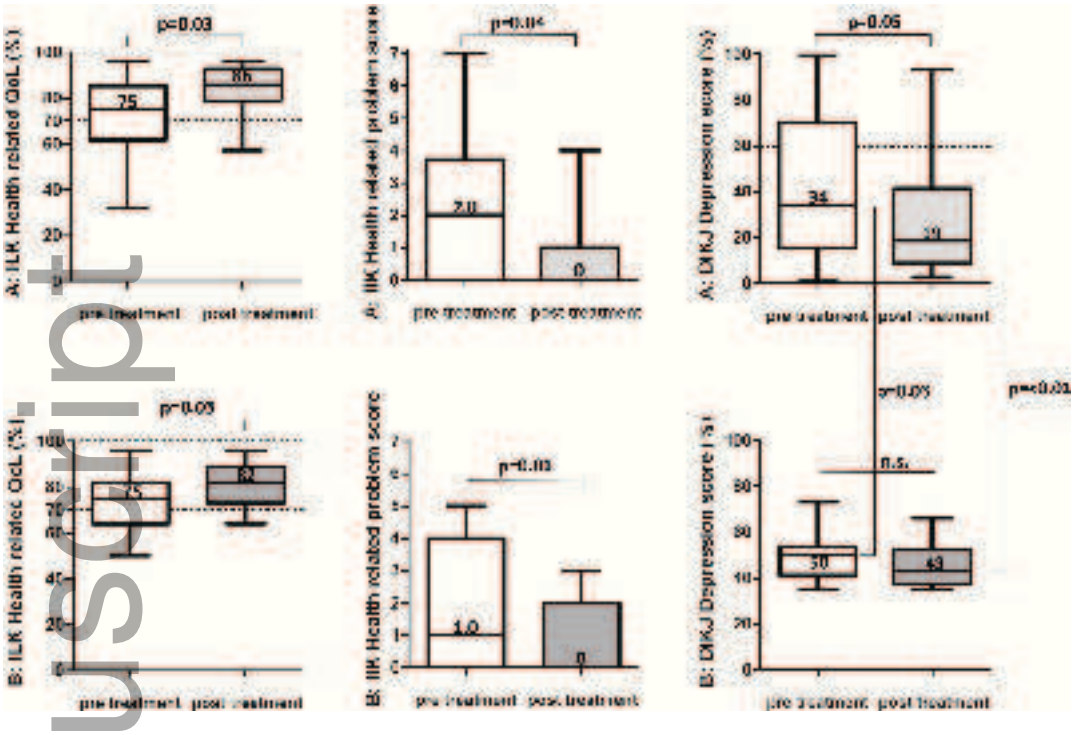

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