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Single and multi-dose pharmacology of recombinant and urinary human chorionic gonadotrophin in men

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Abstract

Objective: Human choriogonadotrophin (hCG) treatment of gonadotrophin-deficient infertile men uses hCG of urinary (uhCG) or recombinant (rhCG) origin, but these treatments have not been compared nor are there studies defining rhCG dosing in men.

Design: hCG products were studied in randomized cross-over single-dose studies of standard (Study 1, 1500 IU and 62.5 μg, respectively) or high (Study 2, 5000 IU and 250 μg) dose and a multi-dose population pharmacology study of hCG use.

Participants: Eight (Study 1) and seven (Study 2) volunteers in cross-over and 52 gonadotrophin-deficient men in the multi-dose study

Measurements: In cross-over studies, serum testosterone (T), dihydrotestosterone (DHT) and estradiol by liquid chromatography-mass spectrometry (LCMS) and serum hCG, LH, FSH, SHBG and T (observational study) by immunoassays.

Results: After standard and high-dose injection, serum hCG and testosterone responses had similar timing and peak concentrations except for a mildly lower early (<48 h) serum testosterone with uhCG. In the multi-dosing study, both hCGs had similar pharmacokinetics (pooled half-life 5.8 days, $p < .001$), while serum testosterone concentrations were stable after injection and did not differ between hCG products. Bench testing verified that 20% of pens from 4/10 individuals were used inappropriately.

Conclusions: Although hCG pharmacokinetics are not formally bioequivalent, the similar pharmacodynamic effects on serum testosterone indicate that at the doses tested both hCGs provide comparable clinical effects. The starting dose of rhCG for treating gonadotrophin-deficient men should be 62.5 μg (6 clicks) of the rhCG pen.

KEYWORDS

estradiol, DHT, liquid chromatography-mass spectrometry, recombinant choriogonadotrophin alfa, testosterone, urinary choriogonadotrophin

1 | INTRODUCTION

Gonadotrophin treatment to induce puberty, spermatogenesis and fertility in men with congenital or acquired gonadotrophin deficiency is based on treatment with human chorionic gonadotrophin (hCG), a placental heterodimeric glycoprotein hormone and natural, long-acting analogue of pituitary luteinizing hormone (LH).[1,2] hCG can conveniently be administered once to three times weekly, whereas LH would require multiple injections daily.[3] hCG fulfills an indispensable role for inducing spermatogenesis and fertility for gonadotrophin-deficient infertile men as well as triggering ovulation in infertile women.[4–6] Used clinically for over seven decades, hCG extracted from pregnancy urine (uhCG) had at least nine commercial brands in 1947,[7] but recently only a single product (Pregnyl) remained on international markets with some products still available in Europe and India. Recombinant hCG (rhCG, choriogonadotrophin alfa) was first approved by the FDA in 2000 and by the EMA in 2001 but only licensed for use in women. The patent-based marketing monopoly for rhCG, coupled with the sponsor's failure to undertake studies in men, precluded its registration for treatment of gonadotrophin-deficient males, including defining appropriate dosing. So, when Pregnyl (uhCG) was withdrawn from the market abruptly in 2021, treatment of gonadotrophin-deficient infertile men seeking fertility was severely compromised.

Single-dose clinical pharmacology studies of uhCG and rhCG in anovulatory female infertility have shown comparable efficacy and safety, but at much higher doses than used in men.[8] Only a single, non-randomized study in healthy eugonadal men has compared uhCG with rhCG[9]; however, the non-suppressed endogenous serum testosterone makes it difficult to interpret specific hCG effects in stimulating serum testosterone. The present study therefore aimed to (a) determine the time course of serum testosterone, dihydrotestosterone (DHT) and estradiol responses to a single, standard or high dose of uhCG or rhCG doses in a randomized sequence cross-over study of healthy men, (b) estimate the population pharmacokinetics (serum hCG) and pharmacodynamics (serum testosterone) of uhCG and rhCG during multi-dose ongoing treatment of gonadotrophin-deficient men and (c) adapt the single-use prefilled rhCG (Ovidrel) syringe to the more frequent lower dose uses required for treatment of gonadotrophin-deficient men.

2 | MATERIALS AND METHODS

2.1 | Single-dose, randomized cross-over studies

2.1.1 | Design

Study 1 and 2 were prospective, randomized sequence, cross-over study of uhCG and rhCG using single standard (Study 1) or high-dose (Study 2) hCG in healthy volunteers to investigate the time course of serum hCG, testosterone, DHT and estradiol responses to a single-dose hCG injection (Figure 1).

2.1.2 | Entry criteria and consent

Healthy men aged over 18 years with no history of chronic disease or requiring regular medical management, male infertility, pituitary–testicular dysfunction, androgen abuse or allergy to hCG or nandrolone injections were recruited. Both studies were approved by the Sydney Local Health District Human Ethics Committee (Concord zone) consistent with the

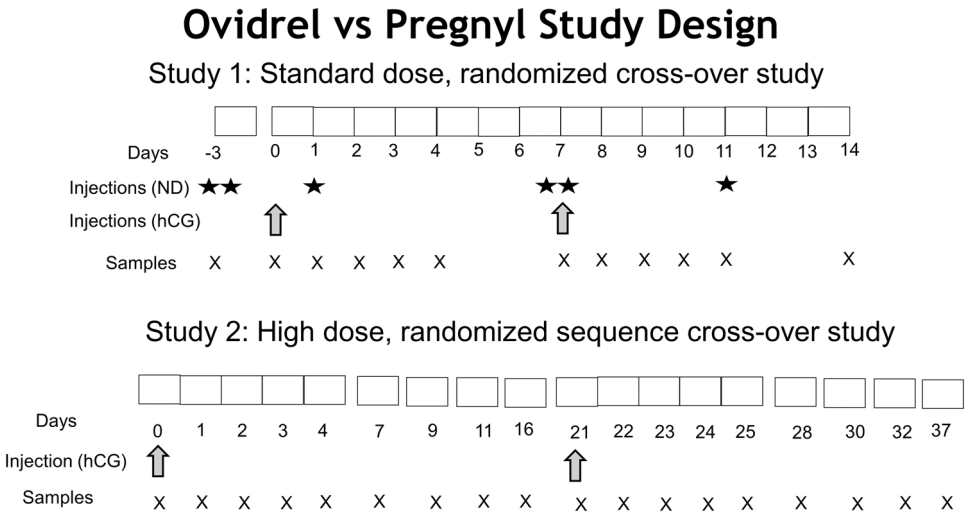

FIGURE 1 Design of Study 1 and 2. In Study 1 (upper panel), participants are randomly assigned to receive either treatment A followed by treatment B or vice versa by subcutaneous injection administered by the study nurse at 7-day intervals. Treatment A = uhCG (Pregnyl 1500 IU) and Treatment B = rhCG (Ovidrel 62.5 μg). In Study 2 (lower panel), participants are randomly assigned to receive either treatment A followed by treatment B or vice versa by subcutaneous injection administered by the study nurse at 21-day intervals. Treatment A = uhCG (Pregnyl 6000 IU) and Treatment B = rhCG (Ovidrel 250 μg). hCG, human chorionic gonadotropin; ND, nandrolone decanoate.

Declaration of Helsinki. Before entry, eligible participants were provided with verbal and written study information and signed the approved consent form. Participants were warned about possible temporary reduction in fertility and of potential adverse findings in work-related urine drug testing due to participation in this study. Both studies were registered through the Australia and New Zealand Clinical Trials Registry—Study 1 (ACTRN12609000462280) and Study 2 (ACTRN12621001053819). All treatments and testing were undertaken in the morning (usually 8 and 10 am) in the Andrology Department, CRGH.

2.1.3 | Study procedures

Participants were randomized to start with uhCG or rhCG injection before subsequent cross-over after washout. Randomization was based on a computer-generated list prepared by someone not involved with the study hormone administration or blood sampling. The sequence assignment was supplied in opaque envelopes marked with the participant number given sequentially as they were recruited. All injections were administered subcutaneously by the study nurse in the clinic. Treatment was open and unblinded as all pharmacological endpoints were based on objective serum hormone measures undertaken by laboratory scientists unaware of treatment assignment.

Study 1 was undertaken in 2009–2010 when irregular availability of uhCG required determining a reasonable alternative using rhCG when uhCG was not available. This was despite the lack of rhCG registration studies and appropriate dosing of rhCG dosage, noting the incommensurate units of gravimetric rhCG dose and bioassay-based uhCG units without known equivalence. We estimated that the 250-µg single syringe dose of rhCG was equivalent to 6000 IU uhCG making the standard uhCG dose of 1500 IU equivalent to 62.5 µg rhCG. The standard 1500 IU dose of uhCG increased serum testosterone with return to baseline by 7 days after injection.[10–13] In this study, uhCG and rhCG injections were given a week apart in random sequence with blood sampling before and Days 1, 2, 3, 4 and 7 days after hCG injection (Figure 1).

To clarify the time course of serum testosterone after hCG injection, participants had endogenous testosterone suppressed by nandrolone decanoate (ND) injections throughout the 2-week cross-over study achieved by four intramuscular ND injections comprising 200 mg on Study days −3 and 7 with 100 mg on study days 1 and 11 (Figure 2) based on prior experience.[3,15] Blood samples were taken before the first ND dose (Day −3) with hCG injected subcutaneously under the abdominal skin on Days 0 and 7 with venous blood sampled on Days 0, 1, 2, 3, 4, 7, 8, 9, 10, 11 and 14.

Study 2 was undertaken in 2021 primarily to administer a high hCG dose to obtain urine and serum samples for calibration of hCG

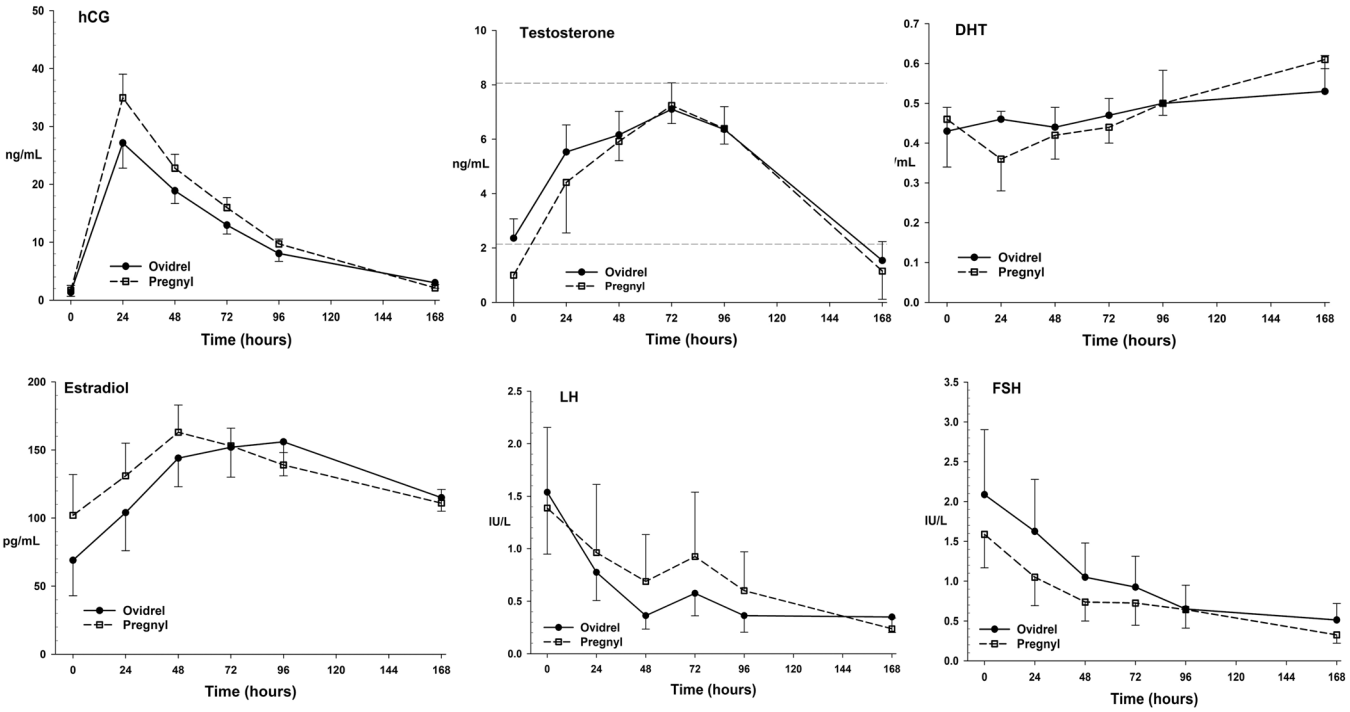

FIGURE 2 Plots of serum human choriogonadotrophin (hCG) (upper left panel), testosterone (middle upper panel), dihydrotestosterone (DHT) (right upper panel), estradiol (left lower panel), luteinizing hormone (LH) (middle lower panel), follicle-stimulating hormone (FSH) (right lower panel) against time since standard dose of hCG injection (62.5 µg rhCG or 1500 IU uhCG). Data are plotted as mean and standard error of the mean for rhCG (Ovidrel, filled symbol, solid line) and uhCG (Pregnyl, open symbol, dashed line) of seven men with endogenous testosterone suppressed by nandrolone decanoate injections in a randomized sequence cross-over study. The horizontal dashed lines indicate the 95% confidence limits for serum testosterone using this liquid chromatography-mass spectrometry assay in 423 young men from the Raine birth cohort.[14]

assays used in anti-doping testing. Secondarily, as this is equivalent to a single weekly therapeutic hCG dose, this study also provided blood samples to investigate the time -course of serum hCG, testosterone, DHT and estradiol after a single high dose of hCG (uhCG 5000 IU or rhCG 250 µg). Subcutaneous hCG injections were administered on Days 0 and 21 without suppression of endogenous testosterone and blood sampling before and at 1, 2, 3, 4, 7, 9, 11 and 16 days after each injection.

2.2 | Multidose population pharmacology study

Study 3 was an ongoing population pharmacokinetic and pharmacodynamic study conducted from 2010 onwards as an observational study of routine hCG treatment used to stimulate spermatogenesis and fertility of gonadotrophin-deficient infertile men.[16–19] The participants had gonadotrophin deficiency due to either congenital hypogonadotrophic hypogonadism,[6] usually presenting with failed puberty, or acquired gonadotrophin deficiency due to pituitary tumours and their surgical and/or radiotherapy treatment, all diagnosed by standard clinical criteria.[20] hCG treatment was based on subcutaneous injections of 1500 IU uhCG or 62.5 µg (6 clicks) rhCG to stimulate spermatogenesis and induce fertility.[16] In some men, if serum testosterone responses were suboptimal, rhCG doses were up titrated with increased to 83.3 (8 clicks) or 125 µg (12 clicks) or to higher uhCG doses (3000 or 5000 IU). Men were usually treated with uhCG unless it was unavailable due to intermittent supply shortages when rhCG was used instead. All blood sampling was conducted in the Andrology Department, CRGH with recording the time since last hCG injection (in hours) together with anthropometric variables.

2.3 | In vitro study of fluid delivery from Ovidrel pens

Study 4 was a bench experiment evaluating delivery of solution from the prefilled rhCG (Ovidrel) pen injector prompted by reports of some men claiming to have run out of injectable fluid prematurely. Although rhCG is marketed as a single-use injection of 250 µg as an ovulation trigger, the prefilled pen injector has a click-based dosage delivery system which can be adapted for the lower doses per injection required for treating gonadotrophin-deficient men, with or without changing the needle between doses. Thus 25 clicks were reported in product literature to be available from each Ovidrel pen injector; however, some patients returned used Ovidrel pens notionally after dispensing 24 clicks (three doses of eight clicks or four doses of six clicks) with some or no residual fluid content with no fluid remaining. To evaluate pen fluid delivery volumes, unused as well as used rhCG (Ovidrel) pens were studied by repeatedly collecting the fluid dispensed into clean, pre-weighed tubes till fluid delivery ceased. Each fluid-containing collecting tube was weighed on a regularly calibrated laboratory balance (EJ-410, sensitivity 10 mg) to calculate the fluid volume dispensed by subtracting the known empty tube weight. The Ovidrel pens were also weighed before and after the experiment.

2.4 | Study drugs

Lyophilized uhCG (Pregnyl, MSD) purified from urine of pregnant women was supplied in vials containing 1500 or 5000 IU with a 1 mL diluent. Recombinant hCG (choriogonadotrophin alfa, Ovidrel, Merck-Serono) is supplied in a prefilled syringe containing 250 µg in 0.5 mL solution for subcutaneous injection. Nandrolone decanoate (ND; Deca-Durabolin, MSD) is an injectable ester of 19 nortestosterone provided in an arachis oil vehicle at a concentration of 50 mg/mL for deep intramuscular injection.

2.5 | Hormone measurements

For Study 1 and 2, serum testosterone, DHT and estradiol were measured by liquid chromatography-mass spectrometry (LCMS) in a single batch at the end of each study using a method free from cross-reactivity of nandrolone.[21,22] These steroid assays have well-established reproducibility (all CVs <10% for at least three quality control samples per analyte spanning the working range) and lower limits of quantifiability of 25 pg/mL (testosterone), 100 pg/mL (DHT) and 2.5 pg/mL (estradiol).[23] The reference range for testosterone in this LCMS assay was derived from the Raine birth cohort study of 423 young men.[14] In Study 1 and 2, serum hCG, LH, FSH and SHBG using Roche reagents by established commercial immunoassays subject to routine external and internal quality control. Additionally for Study 3, testosterone (Roche) was measured by routine immunoassays because over the 12 years of that study, steroid LCMS (including testosterone) was not available for routine clinical use.

2.6 | Data analysis

Study 1 and 2 were analysed by standard cross-over study methods, accounting for carry-over or sequence effects, and estimating standard pharmacokinetic (serum hCG) and pharmacodynamic (serum steroids) endpoints parameters.[15,24] The pharmacological endpoints were derived from serum concentrations of hCG and testosterone to calculate the maximal concentrations (C_{max}), the time of maximum concentrations (T_{max}), effective terminal half-life and the area under the concentration-time curve (AUC). Secondary endpoints were analyses of serum DHT and estradiol. Additionally, linear mixed model analysis for repeated measures was used to evaluate differences between hCG type and the influence on those regressions of covariables comprising age, weight, and height in derived variables body mass index (BMI, kg/m^2) or body surface area (BSA, m^2) using the Gehan–George formula.[25]

Study 3 was analysed by standard population pharmacology methods,[26,27] which are valuable for analysis of observational studies of

real-world uses of drugs where prospective studies are not feasible or realistic. In this study, serum hCG (on log scale) and testosterone concentrations were regressed on the hours since last hCG injection in a multiple linear regression considering the main effects (hCG type), time since last hCG injection (log scale) and their interactions as well as anthropometric variables as covariates. Multiple regression findings were interpreted as standardized (beta) regression coefficient to compare the influence of analytes with different scales. Due to collinearity, models including height and weight, BSA or BMI were run separately. For evaluating dose responses, doses of rhCG (Ovidrel) and uhCG (Pregnyl) were divided into standard (6 clicks [62.5 μg], 1500 IU), increased (8 clicks [83.3 μg], 3000 IU) and high dose (12 clicks [125 μg], 4500 or 5000 IU), respectively.

Study 4 was analysed by descriptive statistics and one-way analysis of variance.

All data analysis was undertaken using NCSS 2023 software (NCSS). Data are presented as mean and standard error of the mean unless non-normally distributed when it was expressed as median and quartiles. Pharmacological bioequivalence calculations of AUC used the conventional 90% confidence limits (80%–125%) of log transformed AUC.[28] Statistical significance was considered present for findings with a $p < .05$ with adjustment for multiplicity testing, if applicable.

3 | RESULTS

3.1 | Single dose cross-over pharmacokinetics and pharmacodynamics

In Study 1 the eight male participants were aged 34 ± 11 years with height 176 ± 6 cm, weight 80.5 ± 7.4 kg, BMI 25.9 ± 2.6 m^2 and BSA 1.98 ± 0.10 m^2. One individual was excluded from the analysis because he was accidentally administered one injection of testosterone instead of nandrolone. There were no differences in the pharmacokinetic variables for testosterone and hCG (Table 1 and Figure 2) and no significant sequence or cross-over effects. The ratio (rhCG/uhCG) of AUC for testosterone was 1.27 ± 0.20 (median 1.21, interquartile range [IQR]: 0.94, 1.33; $p = .23$) and for hCG was 0.81 ± 0.05 (0.89, IQR: 0.77, 0.90; $p = .007$).

Linear mixed model regression analysis indicated that the time course of serum testosterone ($p = .69$), DHT ($p = .42$), estradiol (0.38), LH (0.87) and FSH (0.85) were not significantly different for uhCG or rhCG treatment. Similarly, for each analyte, the study sequence or the interaction of hCG type and study sequence were not significant as were all covariate effects of age, BSA or BMI (all $p > .44$) nor for hCG pharmacokinetics (C_{max}, T_{max}).

In study 2 the seven male participants were aged 37 ± 3 years with height 178 ± 2 cm, weight 84.9 ± 5.5 kg, BMI 26.7 ± 1.8 m^2 and BSA 2.06 ± 0.07 m^2. One individual developed COVID after one hCG injection and his samples from that phase of the study were not analysed. There were no differences in the pharmacokinetic variables for testosterone and hCG (Table 2 and Figure 3). The time course of serum DHT ($p = .81$) and estradiol ($p = .60$) did not differ according to hCG type, nor did they differ according to study sequence or their interaction (data not shown). There were no significant effects of age, BSA, or BMI on hCG pharmacokinetics (C_{max}, T_{max}).

Other than the incidental diagnosis of COVID19 in one Study 2 participant, there were no emergent clinical or biochemical adverse effects reported by, or detected on routine laboratory safety testing, in either Study 1 or 2.

TABLE 1 Pharmacokinetic (serum hCG) and pharmacodynamic (serum testosterone) responses to a single dose hCG injection using either recombinant or urinary hCG with nandrolone-induced suppression of endogenous testosterone (study 1).

Hormone	rhCG (Ovidrel)	uhCG (Pregnyl)	p	Correlation[a]
Testosterone (nmol/L)				
Time of peak (T_{max}), mean ± SD, hours (median)	69 ± 8 [72]	69 ± 3 [72]	1.00	−0.07
Peak concentration (C_{max}), mean ± SD, ng/mL (median)	7.4 ± 0.9 [6.7]	7.3 ± 1.1 [6.6]	0.90	0.74
Area under curve (AUC), mean ± SD, hours, ng/mL (median)	840 ± 129 [778]	750 ± 136 [643]	0.38	0.74
Ratio of AUCs[a]	1.27 [0.65–2.38]			
hCG (ng/mL)				
Time of peak (T_{max}), mean ± SD, hours (median)	31 ± 4 [24]	24 ± 0 [24]	0.17	0
Peak concentration (C_{max}), mean ± SD, ng/mL (median)	26.2 ± 4.9 [24.3]	35.2 ± 4.7 [36.6]	0.001	0.95
Half-time, days, mean (95% confidence limits)	3.5 (2.3, 5.8)	3.4 (2.4, 4.9)	0.06	--
Area under curve (AUC), mean ± SD, hours, ng/mL (median)	1928 ± 249 [2244]	2337 ± 244 [2451]	0.003	0.94
Ratio of AUCs[b]	0.81 [0.57–0.92]			

[a]For each pharmacokinetic measure (Tpeak, Cmax, AUC), this variable represents the correlation of individual values for Ovidrel and for Pregnyl for that variable.

[b]AUC ratio rhCG/uhCG [10th–90th centile].

TABLE 2 Serum testosterone response to a single high-dose recombinant and urinary hCG administration in healthy eugonadal men without suppression of endogenous testosterone (Study 2).

Hormone	rhCG (Ovidrel)	uhCG (Pregnyl)	*p*	Correlation[a]
Testosterone (nmol/L)				
Time of peak (T_{max}), mean ± SD, hours (median)	64 ± 10 [72]	60 ± 10 [60]	0.61	0.84
Peak concentration (C_{max}), mean ± SD, ng/mL (median)	9.3 ± 1.4 [7.7]	10.8 ± 0.8 [10.5]	0.16	0.78
hCG (ng/mL)				
Time of peak (T_{max}), mean ± SD, hours (median)	31 ± 5 [24]	24 ± 0 [24]	0.17	0
Peak concentration (C_{max}), mean ± SD, ng/mL (median)	72 ± 10 [76]	82 ± 12 [82]	0.35	0.95
Half-time, mean (95% confidence limits)	6.8 (3.8, 14.1)	6.6 (3.4, 14.7)	0.88	--

Note: AUC calculations for testosterone were not performed due to the residual endogenous testosterone which would have become suppressed during the study

[a]For each pharmacokinetic measure (T_{peak}, C_{max}, AUC), this variable represents the correlation of individual values for Ovidrel and for Pregnyl for that variable.

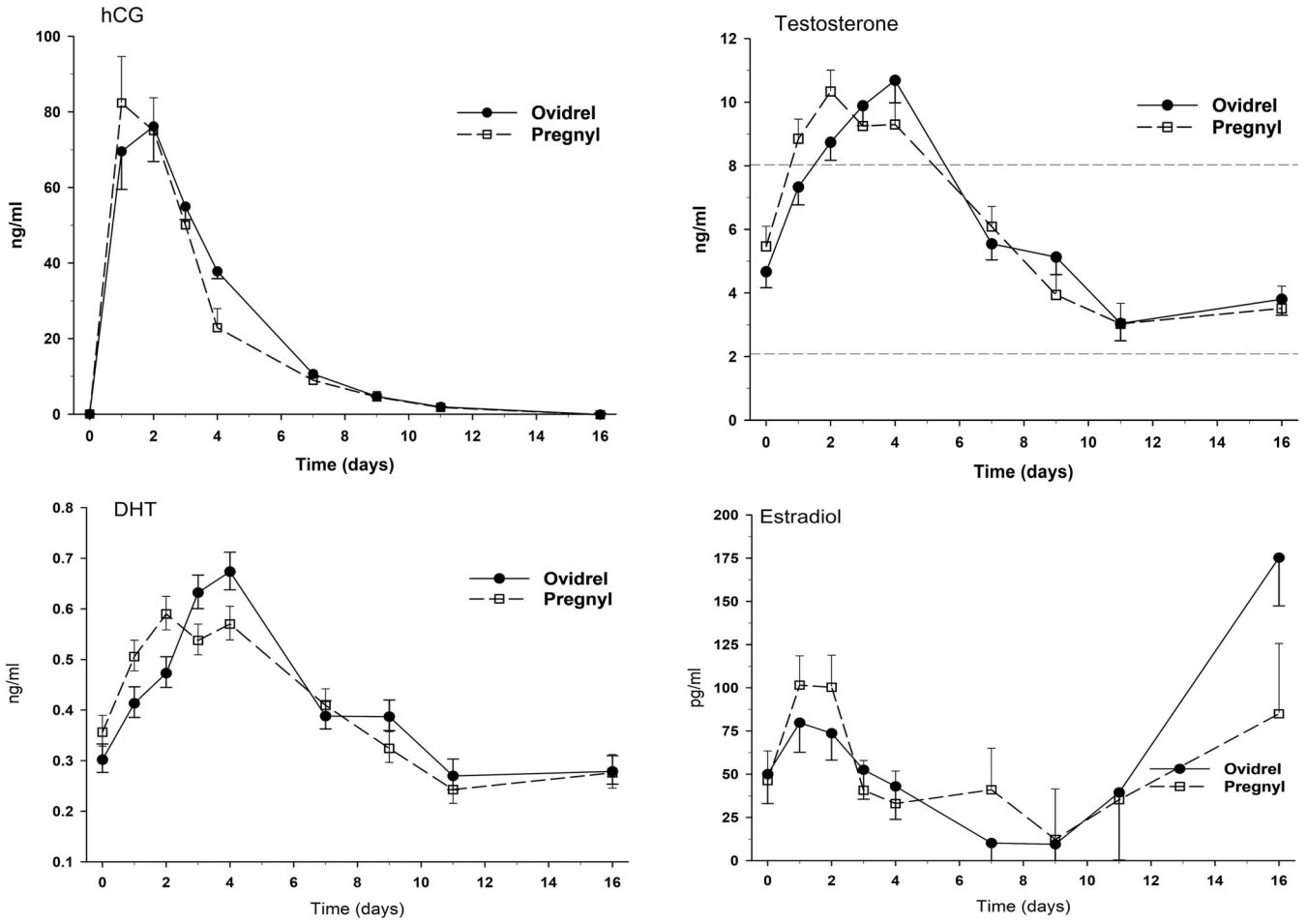

FIGURE 3 Plots of serum human choriogonadotrophin (hCG) (upper left panel), testosterone (upper right panel), dihydrotestosterone (DHT) (lower left panel), estradiol (lower right panel) against time since high dose hCG injection (250 μg rhCG or 5000 IU uhCG). Data are plotted as mean and standard error of the mean for rhCG (Ovidrel, filled symbol, solid line) and uhCG (Pregnyl, open symbol, dashed line) of seven healthy men in a randomized sequence cross-over study. The horizontal dashed lines indicate the 95% confidence limits for serum testosterone using this liquid chromatography-mass spectrometry assay in 423 young men from the Raine birth cohort.[14]

3.2 | Study 3 multidose pharmacokinetics and pharmacodynamics

Serum samples ($n = 502$) were obtained from 52 gonadotrophin-deficient men undergoing long-term therapeutic hCG treatment with a median of 6 (IQR 3, 13) samples per person who received urinary ($n = 295$) or recombinant ($n = 178$) hCG. Linear regression of log serum hCG on time since last hCG injection indicated a highly significant effect of time on serum hCG (pooled slope = −0.25 log(ng/mL)/day, $p < 0001$) creating an effective half-life of 5.8 days (Figure 4) but the slope did not differ between types of hCG (main effect hCG type $p = .15$, interaction of hCG type × time $p = .94$). The regression of log serum hCG on time was significantly influenced, according to standardized beta coefficients, by BSA (7.3), weight (7.3), BMI (6.4) and height (3.8) but not age.

Linear regression of serum testosterone on time since last hCG injection showed a mean serum testosterone of 20.6 ± 1.0 nmol/L with no significant slope (−0.08, $p = .17$) or difference between hCG types in regression on time since injection (main effect hCG type $p = .56$, interaction of hCG type × time $p = .68$). Covariates of height (0.17, $p = .004$), weight (−0.25, $p < .0001$), BSA (−0.20, $p < .0001$) or BMI (−0.22, $p = .0001$), but not age (−0.11, $p = .07$) had small but significant influence on the regression of serum testosterone on time.

For serum hCG and testosterone concentrations, the effects of standard doses, increased or high doses of rhCG and uhCG, show reduced serum testosterone on higher hCG doses (Figure S1).

Serum SHBG concentrations were stable overall with a mean of 32.4 ± 1.3 nmol/L but significantly higher on uhCG ($n = 296$, 31.5 ± 1.0 nmol/L vs. $n = 180$, 22.3 ± 0.7 nmol/L, $p < .001$) without significant change over time since last injection ($p = .58$) or to hCG type ($p = .17$). Covariates displayed negative effects on serum SHBG by BSA (−14.2, $p < .001$) and BMI (−0.55, $p < .0001$) with no effect of age ($p = .49$).

Consistent with the diagnosis of hypogonadotrophic hypogonadism, serum LH remained very low ($n = 487$, 0.76 ± 0.01 IU/L) throughout the study. Similarly, at entry into the study (when participants may have already been on gonadotrophin treatment), serum FSH was low (2.2 ± 0.6 IU/L) and higher overall (as more started FSH treatment) with overall serum FSH ($n = 476$, 4.8 ± 0.3 IU/L) was higher and marginally correlated with serum testosterone ($n = 487$, $p = .06$).

3.3 | Study 4

Ovidrel pens ($n = 110$) were studied either as unused pens (n = 12) or pens returned by patients after completing their use ($n = 98$). Fluid volume delivery was estimated by delivering five clicks into clean, pre-weighed tubes until no further fluid was dispensed. Unused pens (batches AU031060, AU031337, BA079077) had an average weight of 31.3 ± 0.03 g (before) and 30.7 ± 0.03 g (after) delivering fluid, indicating the average total weight of fluid dispensed per pen was 0.56 ± 0.01 gm. Each of the 12 unused pens delivered six sets of five clicks with negligible delivery after the sixth delivery (Table S1). Analysis of serial volume delivery by repeated measured analysis of variance showed no significant difference between pens ($p = .97$) or batches ($p = .13$) but volume delivered was slightly but significantly different over repeated deliveries with the second set of five clicks being highest ($p = .02$) but with only 6% difference in volume from the lowest to largest volume delivered.

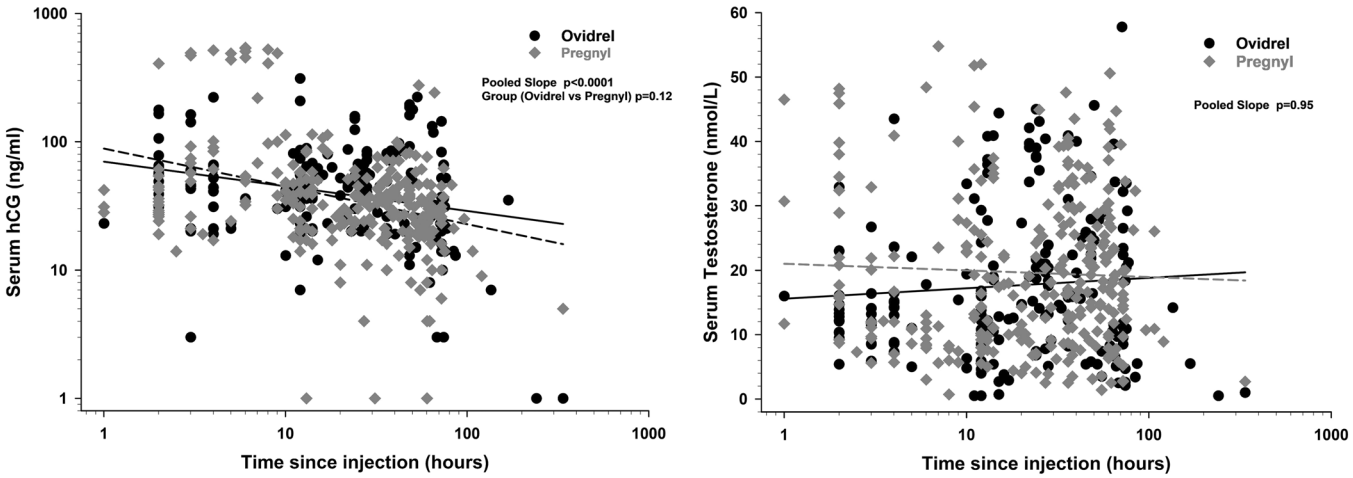

FIGURE 4 Plots of serum human choriogonadotrophin (hCG) (left panel, log scale) and serum testosterone (right panel) measured at times after the last hCG injection (in hours) with the *x* axis on a log scale for clarity. Serum samples ($n = 502$) were obtained from 52 men with a median of 6 (interquartile range: 3, 13) samples per person receiving urinary ($n = 290$) or recombinant ($n = 178$) hCG. Recombinant hCG is shown in solid symbols with regression line and urinary hCG is shown in grey circles and regression line. Serum hCG displayed an overall significant negative slope of the regression on time (pooled half-life 5.8 days) since last injection with no significant difference between hCG products for serum hCG measurement ($p = .61$) or regression slope ($p = .34$). Serum testosterone showed no significant change over time since injection nor between hCG products ($p = .21$).

Used Ovidrel pens that had nominally completed dispensing 24 clicks either as six clicks twice weekly (i.e., 2 weeks treatment per pen) or eight clicks three times weekly (i.e., 1 week treatment per pen) were collected from patients (Batches BA 075593, 077852, 079077, 081204, 082761, 084205). Used pens were provided by 10 patients with a median of 12 (4, 23) pens per person. Further testing of residual fluid delivery in sets of five clicks revealed that, among 98 used pens, 20 pen from 4 patients had no fluid left. The remaining 78 pens delivered a further mean 21 ± 0.42 (median 22 (20, 22)) μL per click on the first test of five clicks. On a second test of five clicks, 48 pens delivered a further 6.0 ± 0.62 (median 6 (4, 8)) μL per click. There was subsequently negligible further delivery of fluid.

4 | DISCUSSION

The present study is the most comprehensive study of hCG pharmacology in men, including the first controlled studies of single and multiple dosing of rhCG administration. It demonstrates that, at the doses tested, uhCG and rhCG are not formally bioequivalent by regulatory pharmacokinetics standards[28]; however, they demonstrate such similar clinical pharmacodynamic effects on serum testosterone, their primary therapeutic objective, that the rhCG dose of 62.5 mg (6 clicks) can be considered for the first time as suitable starting dose for hCG treatment using rhCG comparable with the standard uhCG dose of 1500 IU.

The single standard dose study in healthy men with suppression of endogenous testosterone allowed a clear depiction of the time course of hCG concentrations as well as the hCG-stimulated testicular testosterone free from being obscured by ongoing LH-driven endogenous testosterone. The pharmacodynamic serum testosterone responses, based on T_{max}, C_{max} and AUC, were statistically indistinguishable between the two hCG products. Nevertheless, at the doses used, the ratio of AUCs was significantly lower for rhCG than for uhCG and did not meet conventional pharmacokinetic bioequivalence criteria, which required tighter confidence limits than were obtained.

While the serum testosterone profiles indicate that rhCG appears slightly more effective than uhCG at the doses used, these observational data cannot exclude differences between equal doses due to the lack of direct dose comparability of hCG doses because uhCG dosing is based on bioassay standardization whereas rhCG has a gravimetric dose and there is no reported equivalence between these metrics. The high single dose study indicates that both hCGs have similar effects on circulating hCG as well as testicular steroid secretion at those higher doses as well noting the inability to estimate separately the unsuppressed residual endogenous testosterone in that study.

The multi-dosing study analysis was undertaken using an observational population pharmacology approach in the therapeutic target group of gonadotrophin-deficient men undergoing gonadotrophin (including hCG) stimulation of spermatogenesis. In this real-world therapeutic analysis, the time course of serum hCG and testosterone did not differ significantly between the hCG products, although an expected log-linear decline of serum hCG over time was observed. The lower serum testosterone shown by both products at high hCG doses most likely reflects the resistance of testicular testosterone production to hCG rather than desensitization to hCG. This is because these higher hCG doses were only arrived at after individual upward dose-titration of hCG in treated men whose serum testosterone was not normalized on standard hCG doses.[16] As a result, these two hCG products at these standard doses tested can be considered pharmacologically interchangeable for treatment of gonadotrophin-deficient men. This finding overcomes the difficulty that, although uhCG was registered (grandfathered) based on bioassay units for use in stimulating spermatogenesis for gonadotrophin-deficient men, rhCG was never registered for use in men nor was any bioassay equivalence ever reported. These data also establish for the first time an evidence-based starting dose for rhCG treatment.

Our analysis of Ovidrel pen fluid delivery demonstrates that the prefilled Ovidrel pens have an overfill loading, supplying 30 rather than 25 clicks, but with highly consistent delivery of at least five, and possibly six, sets of 5 clicks, each click being equivalent to 20 μL or 10 μg of choriogonadotrophin alfa. Thus, either six clicks delivered four times or eight clicks delivered three times, both amounting to 24 clicks, are reliable and within the capacity of the Ovidrel pens. Analysis of the used pens suggests that instruction in pen usage outside the approved single-use mechanism is mostly effective with a minority using the pens incorrectly.

Limitations of this study include relatively small numbers in the two randomized controlled studies; however, the cross-over design is 4–10 times more powerful/efficient than a parallel group design.[29] Additionally, a cross-over study is preferable for testing new products against older ones in humans wherein each participant acts their own control. This reduces otherwise unexplained between-person variance as well as accommodating testing of period (sequence) and carry-over effects. The numbers we employed were adequate for findings of statistically significant effects. The multi-dose study used established population pharmacology methods using real-world data. Although a randomized controlled study design might have provided more conclusive short-term findings, that would be at the expense of not reflecting real-world usage which the population pharmacology methodology achieves. The study design limitations of the single and multiple dose studies are all traceable to the abuse of patent rights in that the monopoly patent holder for rhCG failed to conduct any clinical studies or register rhCG for use in men despite its extensive, lucrative use in IVF. Other limitations of this study are that structural differences or microheterogeneity and immunoassay cross-reactivity of the two hCG products could not be verified and required cautious interpretations of hCG immunoassay findings. Similarly, it was a limitation that a testosterone immunoassay rather than LCMS was used in the long-term multi-dosing study because steroid LCMS was not available for routine clinical practice during that 12-year period.

We conclude that the urinary and recombinant forms of hCG at the standard and high doses studied produce comparable effects on serum testosterone, DHT and estradiol although uhCG is associated with modestly higher serum hCG concentrations in regular clinical use. Despite the lack of registration of Ovidrel for men with gonadotrophin deficiency, these studies suggest it can be used with

a low-dose adaptation (62.5 μg, 6 clicks) of the click-based delivery system of Ovidrel pens as a standard starting dose of rhCG.

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CONFLICT OF INTEREST STATEMENT

Manufacturers of hCG products provided no support for this work or had any input into study design, analysis, or reporting.

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REFERENCES

1. Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. *Annu Rev Biochem*. 1981;50:465-495.
2. Boime I, Ben-Menahem D. Glycoprotein hormone structure-function and analog design. *Recent Prog Horm Res*. 1999;54:271-288.
3. discussion 288-279.
4. Handelsman DJ, Goebel C, Idan A, Jimenez M, Trout G, Kazlauskas R. Effects of recombinant human LH and hCG on serum and urine LH and androgens in men. *Clin Endocrinol*. 2009;71:417-428.
5. Hayes F, Dwyer A, Pitteloud N. Hypogonadotropic hypogonadism (HH) and gonadotropin therapy. In: Feingold KR, Anawalt, B, Blackman MR, eds. *Endotext*. MDText.com, Inc.
6. Boehm U, Bouloux PM, Dattani MT, et al. European Consensus Statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol*. 2015;11:547-564.
7. Young J, Xu C, Papadakis GE, et al. Clinical management of congenital hypogonadotropic hypogonadism. *Endocr Rev*. 2019;40:669-710.
8. Heller CG, Nelson WO. Classification of male hypogonadism and a discussion of the pathologic physiology, diagnosis and treatment. *J Clin Endocrinol Metab*. 1948;8:345-366.
9. Al-Inany H. Recombinant versus urinary gonadotrophins for triggering ovulation in assisted conception. *Hum Reprod*. 2005;20:2061-2073.
10. Cailleux-Bounacer A, Reznik Y, Cauliez B, Menard JF, Duparc C, Kuhn JM. Evaluation of endocrine testing of Leydig cell function using extractive and recombinant human chorionic gonadotropin and different doses of recombinant human LH in normal men. *Eur J Endocrinol*. 2008;159:171-178.
11. Saal W, Glowania HJ, Hengst W, Happ J. Pharmacodynamics and pharmacokinetics after subcutaneous and intramuscular injection of human chorionic gonadotropin. *Fertil Steril*. 1991;56:225-229.
12. PADRÓN RS, Wischusen J, Hudson B, Burger HG, de Kretser DM. Prolonged biphasic response of plasma testosterone to single intramuscular injections of human chorionic gonadotropin. *J Clin Endocrinol Metab*. 1980;50:1100-1104.
13. Trinchard-Lugan I, Khan A, Porchet H, Munafo A. Pharmacokinetics and pharmacodynamics of recombinant human chorionic gonadotrophin in healthy male and female volunteers. *Reprod Biomed Online*. 2002;4:106-115.
14. Jones TH, Darne JF, McGarrigle HH. Diurnal rhythm of testosterone induced by human chorionic gonadotrophin (hCG) therapy in isolated hypogonadotrophic hypogonadism: a comparison between subcutaneous and intramuscular hCG administration. *Eur J Endocrinol*. 1994;131:173-178.
15. Hart RJ, Doherty DA, McLachlan RI, et al. Testicular function in a birth cohort of young men. *Hum Reprod*. 2015;30:dev244.
16. Minto CF, Howe C, Wishart S, Conway AJ, Handelsman DJ. Pharmacokinetics and pharmacodynamics of nandrolone esters in oil vehicle: effects of ester, injection site and injection volume. *J Pharmacol Exp Ther*. 1997;281:93-102.
17. Liu PY, Baker HWG, Jayadev V, Zacharin M, Conway AJ, Handelsman DJ. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. *J Clin Endocrinol Metab*. 2009;94:801-808.
18. Liu PY, Gebski VJ, Turner L, Conway AJ, Wishart SM, Handelsman DJ. Predicting pregnancy and spermatogenesis by survival analysis during gonadotrophin treatment of gonadotrophin-deficient infertile men. *Hum Reprod*. 2002;17:625-633.
19. Liu PY, Turner L, Rushford D, et al. Efficacy and safety of recombinant human follicle stimulating hormone (Gonal-F) with urinary human chorionic gonadotrophin for induction of spermatogenesis and fertility in gonadotrophin-deficient men. *Hum Reprod*. 1999;14:1540-1545.
20. Bouloux PMG, Nieschlag E, Burger HG, et al. Induction of spermatogenesis by recombinant follicle-stimulating hormone (puregon) in hypogonadotropic azoospermic men who failed to respond to human chorionic gonadotropin alone. *J Androl*. 2003;24:604-611.
21. Handelsman DJ. Androgen physiology, pharmacology, use and misuse. In: Feingold KR, Anawalt B, Boyce A, eds. *Endotext*. MDText.com Inc.; 2020.
22. Keski-Rahkonen P, Desai R, Jimenez M, Harwood DT, Handelsman DJ. Measurement of estradiol in human serum by LC-MS/MS using a novel estrogen-specific derivatization reagent. *Anal Chem*. 2015;87:7180-7186.
23. Harwood DT, Handelsman DJ. Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously measure androgens and estrogens in serum without derivatization. *Clin Chim Acta*. 2009;409:78-84.
24. Handelsman DJ, Desai R, Seibel MJ, Le Couteur DG, Cumming RG. Circulating sex steroid measurements of men by mass spectrometry are highly reproducible after prolonged frozen storage. *J Steroid Biochem Mol Biol*. 2020;197:105528.
25. Gibaldi M, Perrier D. *Pharmacokinetics*. Marcel Dekker; 1982.
26. Gehan EA, George SL. Estimation of human body surface area from height and weight. *Cancer Chemother Rep*. 1970;54:225-235.
27. Duffull SB, Wright DFB, Winter HR. Interpreting population pharmacokinetic-pharmacodynamic analyses—a clinical viewpoint. *Br J Clin Pharmacol*. 2011;71:807-814.
28. Minto C, Schnider T. Expanding clinical applications of population pharmacodynamic modelling. *Br J Clin Pharmacol*. 1998;46:321-333.
29. Morais JAG, Lobato MR. The new European Medicines Agency guideline on the investigation of bioequivalence. *Basic Clin Pharmacol Toxicol*. 2010;106:221-225.
30. Garcia R, Benet M, Arnau C, Cobo E. Efficiency of the cross-over design: an empirical estimation. *Stat Med*. 2004;23:3773-3780.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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