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ORIGINAL ARTICLE

Endocrine effects of hCG supplementation to recombinant FSH throughout controlled ovarian stimulation for IVF: a dose–response study

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Summary

Objective To analyse the endocrine response in relation to the Δ-4 and Δ-5 pathways of ovarian steroidogenesis after different doses of human chorionic gonadotrophin (hCG) supplementation to recombinant FSH from Day 1 of controlled ovarian stimulation for IVF.

Design A randomized dose–response pilot study.

Patients A total of 62 IVF patients aged 25–37 years with regular cycles and FSH <12 IU/l were treated with a fixed dose of rFSH 150 IU/day and randomized to four hCG dose groups: Dose 0: 0 IU/day, Dose 50: 50 IU/day, Dose 100: 100 IU/day and Dose 150: 150 IU/day.

Results A significant hCG dose-dependent incremental increase was found for progesterone (49–160%), 17-OH-progesterone (223–614%), androstenedione (91–340%) and testosterone (95–338%) from Dose 0 to Dose 150, respectively. Dehydroepiandrosterone (DHEA) showed minor changes during stimulation and no differences between the groups. The highest oestradiol concentrations were observed in Dose 100 and Dose 150. Sex hormone-binding globulin (SHBG) increased similarly in all groups at the end of stimulation. No difference was observed for anti-müllerian hormone (AMH) concentration between the groups, but a 50% decline from the start to the end of the stimulation was found.

Conclusion Supplementation with hCG resulted in a clear dose-related response for androgens, progesterone and 17-OH-progesterone. Oestradiol concentration reached maximum levels with an hCG dose of 100 IU/day, suggesting saturation of aromatase function. No difference between the groups was observed for DHEA, supporting that the stimulatory effects of hCG doses on androgens and oestrogen production were mainly induced via the Δ-5 pathway. SHBG, being a biomarker of oestrogen/androgen balance, was not changed by increasing hCG.

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Introduction

According to the two-cell, two-gonadotrophin concept of ovarian function, LH/human chorionic gonadotrophin (hCG) promotes androgen production from cholesterol and pregnenolone in theca cells, and FSH stimulates granulosa cell aromatase function of androgens to oestrogens.[1–4] In the natural cycle, the synthesis of androgens is partially provided by ovary and adrenal gland. However, each endocrine gland is under stimulation of other trophic hormones: LH or hCG for the ovary and adrenocorticotrophic hormone (ACTH) for the adrenal gland. When studying the effects by administration of increasing hCG doses, it is thus expected that mainly androgens of ovarian origin will be amplified. During controlled ovarian stimulation, it has been demonstrated that LH/hCG influences steroidogenesis in the production of androgens and oestradiol,[5,6] whereas the effect of the LH component (being either LH or hCG) on the preovulatory progesterone levels is debated. Despite uncertainty about the cause of elevated progesterone, it seems that even subtle progesterone rises result in impaired endometrial receptivity[7–9] and decreased live birth rates.[10–13]

In a large, prospective, clinical study using GnRH agonist down-regulation, LH/hCG activity–containing drugs were less often associated with higher preovulatory progesterone.[6,14] It has been suggested that hCG could promote low preovulatory progesterone levels through a more efficient progesterone conversion to androgens, by the action of 17α-hydroxylase in the theca cells, thus preferring the Δ-4 pathway.[10,15] Other groups have argued that in humans, the enzyme uses 17-OH-pregnenolone almost exclusively, as the 17,20-lyase activity is much more efficient for the conversion of 17-OH-pregnenolone into dehydroepiandrosterone (DHEA) than for the conversion of 17-OH-progesterone into androstenedione. Thus, the Δ-5 pathway seemed to be the preferred route in the production of androgens.[16–18] When Δ-5 metabolites are converted into Δ-4 metabolites by

3β-hydroxysteroid dehydrogenase (3βHSD), there is no conversion back into Δ-5 metabolites.[19] In theca cells and granulosa cells, LH bioactivity increases the expression of 3βHSD.[2,20] If the Δ-5 pathway in humans is the preferred route to androstenedione, the hCG effect would rather enhance the production of progesterone and 17-OH-progesterone as terminal products of the Δ-4 pathway.[21]

The purpose of the study was to clarify the role of hCG supplementation to rFSH during controlled ovarian stimulation on the endocrine response in IVF patients. This study is based on an earlier published prospective, randomized, dose–response study with top-quality embryos as the primary end-point.[22] In the study, supplementation with 50, 100 and 150 IU hCG increased the number of top-quality embryos per patient while the pregnancy rates in three groups were similar to the control group. However, it should be kept in mind that the study was not powered to detect differences in pregnancy rates. In addition, we found that oestradiol and androstenedione levels increased after hCG supplementation, and preovulatory progesterone increment increased with increasing doses of hCG.[22] To further scrutinize the action of hCG on the steroidogenic pathways, additional analyses were made after the initial study, and we present in this publication the results of 17-OH-progesterone, DHEA, testosterone, Sex hormone-binding globulin (SHBG) as well as anti-müllerian hormone (AMH).

Materials and methods

Study design

The study was designed as a prospective, randomized, controlled, dose–response pilot study conducted between February 2009 and June 2010 at the Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Denmark with approval from The Danish Medicines Agency (EudraCT number 2008-008355-42) and The Danish National Committee on Biomedical Research Ethics (HB-2008-146). The study was registered in the ClinicalTrial.gov (NCT00844311) and monitored from the unit of Good Clinical Practice, Copenhagen Region (2008-257).

Study population

A total of 62 patients were randomized, and we obtained 60 patients for the per protocol (PP) analysis. The patients were 'standard' patients scheduled for IVF such as tubal or unexplained infertility, including endometriosis Stage I/II, and mild male factor. The women were 25–37 years of age and had BMI >18 and <30 kg/m^2 and a regular menstrual cycle. Their early follicular phase serum FSH levels were 1–12 IU/l and early follicular phase total antral follicle (2–10 mm) count ≥ 6. Patients with history of PCOS, endometriosis Stage III/IV, severe male factor requiring ICSI or history of severe ovarian hyperstimulation syndrome (OHSS) were excluded from participation. A detailed description of the study population along with the clinical outcome of the study has been published previously.[22]

Treatment protocol and study end-points

Patients were assessed for eligibility on cycle Days 2–5 and had endocrine testing (s-FSH, s-LH, s-AMH). The patients underwent controlled ovarian stimulation in a GnRH agonist protocol. Once down-regulation had been confirmed, the patients were randomized to one of four treatment arms: (i) control arm (Dose 0): 150 IU/day of rFSH alone, (ii) hCG low dose (Dose 50): 150 IU/day of rFSH + 50 IU/day of hCG, (iii) hCG medium dose (Dose 100): 150 IU/day of rFSH + 100 IU/day of hCG and (iv) hCG high dose (Dose 150): 150 IU/day of rFSH + 150 IU/day of hCG. All randomized patients were treated with rFSH 150 IU/day (Puregon®, N.V. Organon, Oss, the Netherlands) in a fixed-dose regimen on Day 1 of stimulation. Supplementation with different doses of hCG (Predalon® 500 IU, Organon, Berlin, Germany) started on Day 1 of stimulation after the randomization. HCG, 10 000 IU s.c., (Pregnyl, N.V. Organon, Oss, the Netherlands) was administered to induce final follicular maturation within one day when three or more follicles of $\geq$17–18 mm diameter were observed. Oocyte retrieval took place 36 h ($\pm$2 h) after hCG administration. Subsequently, IVF was performed. Transfer of preferably one embryo was carried out on Day 3 after oocyte retrieval. The primary end-point was the total number of top-quality embryos on Day 3.[22]

Main outcome in this publication was the endocrine responses in relation to the Δ-4 and Δ-5 pathways.

Serum endocrinology

Blood samples were drawn for assays of baseline FSH, LH and AMH on cycle Days 2–5. HCG and oestradiol, FSH and LH were measured before the daily drug injection on stimulation Days 1, 3, 6 and thereafter every second day until the day of hCG administration. Progesterone was measured on Days 1, 6 and every second day until the day of hCG triggering. On Days 1, 6 and on the day of hCG triggering serum for 17-OH-progesterone, DHEA, androstenedione, testosterone, SHBG and AMH were analysed. Blood samples were collected before the daily drug injection and centrifuged for 12 min at 3 000 ***g***. Serum was stored individually at −24 °C and later analysed with all samples quantified in the same run. The analyses of hCG, androstenedione and progesterone were carried out at the Laboratorium für Klinische Forschung (LKF; Raisdorf, Germany). Oestradiol, FSH, LH and AMH were analysed at the Department of Clinical Biochemistry, Rigshospitalet, Denmark. DHEA, 17-OH-progesterone, testosterone and SHBG were analysed at the Centre for Reproductive Medicine, Vrije Universiteit Brussel UZ Brussel, Belgium.

The sensitivity, specificity and total imprecision for the endocrine parameters are presented in Table 1. The total imprecision refers to total variation by combining between-run and within-run variation. It denotes the total precision within the same facility using the same equipment.[23]

Statistical analysis

The sample size calculation and randomization procedure are previously described.[22]

Table 1. Analytical methods for the parameters measured in serum

Parameter	Method	Sensitivity	Specificity (%)	Total imprecision (% CV)
AMH	AMH/MIS EIA kit, Immunotech	0·7 pmol/l	Non-detectable*	14
FSH	Electrochemiluminescence immunoassay	<0·1 IU/l	<0·1	<5
LH	Electrochemiluminescence immunoassay	0·1 IU/l	<0·1	<3
hCG	Electrochemiluminescence immunoassay	<0·1 IU/l	<0·1	<8
Oestradiol	Electrochemiluminescence immunoassay	0·02 nmol/l	<0·3	5
Progesterone	Electrochemiluminescence immunoassay	0·03 nmol/l	<1·0[†]	5
Androstenedione	Radioimmunoassay	0·03 nmol/l	<1·0	10
Total testosterone	Electrochemiluminescence immunoassay	0·087 nmol/l	≤ 3·2[‡]	7
SHBG	Electrochemiluminescence immunoassay	0·35 nmol/l	Non-detectable	6
17-OH-progesterone	Competitive RIA (coated tube)	0·06 nmol/l	<1·0	<7
DHEA	Competitive RIA (coated tube)	1·0 nmol/l	<0·1[§]	<10

*Within ±10% of control when adding potential interferents.
[†]20·7% for 5β-dihydroprogesterone, 1·3% for 17-hydroxyprogesterone.
[‡] ≤ 18% for 11β-hydroxytestosterone.
[§]7·1% for androsterone, 25·3% for epiandrosterone, 1·13% for progesterone, 1·03% for 11-hydroxyprogesterone, 0·26% for oestrone.
Total imprecision is denoting the total precision within the same facility using the same equipment (Clinical and Laboratory Standards Institute (CLSI)); Specificity, cross-reactivity with other relevant hormones; CV, coefficient of variation; RIA, radioimmunoassay; EIA, enzyme-linked immunosorbent assay; SHBG, sex hormone-binding globulin; DHEA, dehydroepiandrosterone; AMH, anti-müllerian hormone.

Data are presented as mean and 95% confidence interval. For between-group analyses, comparisons of continuous variables were carried out by analysis of variance (ANOVA) or Kruskal–Wallis test where appropriate. A two-sided P-value of <0·05 was considered statistically significant. Bonferroni correction was used for multiple comparison corrections. In addition, ANOVA with repeated measures was performed to compare the multiple time-points in the hCG dose groups. If sphericity was violated because of a significant Mauchly's test, the results were corrected by the Greenhouse–Geisser correction. Serum analyses were logarithmically transformed before the between-group analyses were carried out. The logarithmic means were transformed to the original scale; thus, the presented means are the geometric means. The increments were calculated by dividing the actual hormone values on the day of hCG triggering and stimulation Day 6 with the actual hormone level on stimulation Day 1. These relations were logarithmically transformed, the between-group analyses were performed, and finally, the relations were back transformed into the original scale.

The statistical analysis of the data was performed in collaboration with statisticians affiliated to the Juliane Marie Center Rigshospitalet from the Biostatistics Department Copenhagen University using the Statistical Package for the Social Sciences 18.0 software for Windows (SPSS Inc., Chicago, IL, USA).

Results

A total of 75 patients were assessed for eligibility; 62 patients were randomized into the four hCG dose groups: Dose 0 (n = 16), Dose 50 (n = 15), Dose 100 (n = 16) and Dose 150 (n = 15). The results are based on the 60 patients who fulfilled the PP criteria. The reason for withdrawal of two initiated patient cycles from the analysis was that 10- to 30-fold dosing errors occurred with the main investigational drug (hCG). No statistical differences were observed between treatment groups of the baseline characteristics regarding demographics, clinical and sonographic characteristics and endocrine profile at screening on cycle Days 2–5 as reported previously.[22] The mean age of the patients was 32·3 years (CI: 31·6–33·0), BMI 22·1 kg/m^2 (CI: 21·5–22·8), and duration of infertility 2·4 years (CI: 2·0–2·7), and on cycle Days 2–5, the mean FSH was 6·6 IU (CI: 6·2–7·0), LH 6·1 IU (CI: 5·5–6·6) and AMH 15·7 pmol/l (CI: 12·7–19·4).

Concerning the follicular development of follicles >10 mm measured by ultrasound, no difference was found between the number of different-sized follicles in the four groups. The mean number of follicles >10 mm was 14·2 (CI: 12·4−16·1).

Serum endocrinology

Table 2 presents serum hormone levels of progesterone, 17-OH-progesterone, DHEA, androstenedione, testosterone, SHBG, free androgen index (FAI), oestradiol and AMH on Day 1, Day 6 and at the end of stimulation (day of hCG triggering), as well as the ratios of oestradiol/progesterone, oestradiol/androstenedione and oestradiol/testosterone on the day of hCG triggering. When comparison of multiple time-points in the four groups was performed, we found an overall significant effect of time for all the different hormones. Further, for progesterone, 17-OH-progesterone, androstenedione, testosterone and FAI, the hCG dose in the participant induced a significant effect on the hormone level during stimulation. Serum increments are presented in Supplementary Table S1 and graphically in Fig. 1 as mean values; for reasons of clarity, confidence intervals (CI) were not plotted. The increments were calculated to compensate for the slight differences in baseline values and were defined as the percentage difference in the hormone level on Day 1, Day 6 and the day of hCG triggering.

Table 2. Serum levels and ratios of progesterone, the androgens, oestradiol, SHBG and AMH during the stimulation

	Dose 0 (n = 16)	Dose 50 (n = 15)	Dose 100 (n = 16)	Dose 150 (n = 13)	P–value
Progesterone* (nmol/l)					
Stimulation Day 1	1·5 (1·2–1·9)	1·9 (1·4–2·4)	1·4 (1·1–1·7)	1·4 (1·1–1·9)	0·24
Stimulation Day 6	1·5 (1·2–1·8)[a]	2·3 (1·8–2·9)[a,b]	1·5 (1·2–1·9)[b]	1·6 (1·2–2·1)	0·02
Day of hCG	2·3 (1·8–2·8)[c]	3·3 (2·5–4·3)	2·9 (2·5–3·5)	3·7 (2·6–5·3)[c]	0·03
17-OH-progesterone (nmol/l)					
Stimulation Day 1	1·9 (1·5–2·4)	2·8 (2·2–3·4)	2·1 (1·7–2·6)	2·2 (1·7–2·7)	0·10
Stimulation Day 6	2·5 (2·1–3·1)[d,e,f]	5·3 (4·4–6·4)[d]	3·8 (3·3–4·5)[e]	4·1 (3·1–5·4)[f]	<0·001
Day of hCG	6·2 (4·7–8·1)[g]	11·0 (7·9–15·5)	11·0 (8·2–14·7)	15·4 (9·3–25·7)[g]	<0·01
DHEA (nmol/l)					
Stimulation Day 1	22·5 (18·9–26·7)	27·5 (20·5–36·8)	22·1 (16·4–29·8)	24·0 (17·7–32·4)	0·59
Stimulation Day 6	21·8 (17·5–27·1)	29·8 (22·7–39·0)	21·7 (16·8–28·0)	21·5 (17·1–27·0)	0·14
Day of hCG	25·8 (22·8–29·1)	30·4 (24·0–38·6)	23·4 (18·3–29·9)	28·6 (23·5–35·0)	0·27
Androstenedione* (nmol/l)					
Stimulation Day 1	3·8 (3·2–4·4)	4·6 (3·8–5·5)	4·0 (3·3–4·7)	3·7 (3·1–4·3)	0·27
Stimulation Day 6	4·2 (3·7–4·9)[h]	6·1 (5·1–7·4)[h]	5·4 (4·4–6·6)	5·1 (4·1–6·4)	0·03
Day of hCG	7·2 (5·6–9·2)[i,j]	11·2 (8·1–15·6)	14·1 (11·3–17·5)[i]	16·1 (12·3–21·2)[j]	<0·001
Testosterone (nmol/l)					
Stimulation Day 1	0·68 (0·56–0·84)	0·83 (0·63–1·10)	0·62 (0·47–0·81)	0·53 (0·31–0·92)	0·26
Stimulation Day 6	0·78 (0·66–0·91)	1·08 (0·87–1·34)	0·84 (0·61–1·14)	0·85 (0·59–1·21)	0·27
Day of hCG	1·33 (1·01–1·75)	1·98 (1·45–2·69)	1·95 (1·42–2·68)	2·34 (1·60–3·40)	0·06
SHBG (nmol/l)					
Stimulation Day 1	85 (72–100)	70 (59–83)	78 (62–98)	64 (51–80)	0·16
Stimulation Day 6	82 (73–94)	66 (54–81)	71 (57–88)	59 (47–74)	0·08
Day of hCG	112 (94–132)	108 (86–136)	114 (90–144)	97 (72–120)	0·78
FAI					
Stimulation Day 1	0·81 (0·62–1·05)	1·19 (0·81–1·73)	0·79 (0·56–1·14)	0·84 (0·45–1·57)	0·39
Stimulation Day 6	0·94 (0·77–1·14)	1·63 (1·12–2·37)	1·18 (0·83–1·68)	1·43 (0·89–2·30)	0·10
Day of hCG	1·19 (0·97–1·46)[k]	1·83 (1·44–2·34)	1·71 (1·27–2·31)	2·39 (1·62–3·53)[k]	0·01
Oestradiol* (nmol/l)					
Stimulation Day 1	0·05 (0·05–0·07)	0·05 (0·03–0·08)	0·06 (0·04–0·08)	0·05 (0·04–0·08)	0·31[†]
Stimulation Day 6	0·80 (0·55–1·16)	1·87 (1·18–2·95)	1·28 (0·71–2·28)	1·15 (0·67–1·99)	0·19
Day of hCG	6·32 (3·69–10·80)	10·01 (6·28–15·96)	12·77 (9·49–17·18)	12·00 (7·19–20·02)	0·09
Ratios on the Day of hCG					
E2//Progesterone	2·77 (1·83–4·19)	2·97 (2·02–4·37)	4·35 (3·26–5·81)	3·23 (2·09–4·98)	0·28
E2/Androstenedione	0·88 (0·61–1·26)	0·87 (0·68–1·11)	0·91 (0·79–1·04)	0·74 (0·54–1·03)	0·73
E2/Total testosterone	4·75 (3·35–6·73)	5·07 (4·13–6·21)	5·84 (4·82–7·07)	5·14 (3·89–6·81)	0·68
AMH (pmol/l)					
Stimulation Day 1	17·7 (11·3–27·8)	16·3 (11·0–24·2)	19·0 (11·8–30·4)	17·0 (9·5–30·6)	0·97
Stimulation Day 6	15·7 (11·0–22·5)	11·2 (6·8–18·4)	13·8 (8·5–22·1)	12·7 (7·3–22·1)	0·73
Day of hCG	8·2 (5·2–12·8)	6·1 (3·8–9·8)	8·1 (4·9–13·3)	7·0 (3·7–13·2)	0·79

*Presented in the Supplementary Table in Thuesen *et al.* 2012.
Values are mean and 95% CI. ANOVA or [†]nonparametric test.
[a–k]Significant differences (P < 0·05) in Bonferroni-corrected *post hoc* tests.
DHEA, dehydroepiandrosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; AMH, anti-müllerian hormone; E2, oestradiol; hCG, human chorionic gonadotrophin.

Progesterone. Serum progesterone levels on the day of hCG triggering were significantly different between the treatment arms. The lowest serum progesterone was found in Dose 0 group and the highest in the Dose 150 group. The progesterone increments increased significantly with increasing doses of hCG from 49% (CI: 18−90%) in Dose 0–160% (CI: 95−247%) in Dose 150 (P = 0·02).

17-OH-progesterone. The level of 17-OH-progesterone increased significantly with increasing doses of hCG when comparing the control group with the Dose 150 group. A significant dose-related increase in the increments was found from 223% (CI: 132−350%) in Dose 0 to 614% (CI: 320−1113%) in Dose 150 (P = 0·02).

Dehydroepiandrosterone (DHEA). No differences were observed for DHEA between the groups. From Day 1 to Day 6, no consistent changes were seen, but on the day of hCG triggering, DHEA increased 6–20% compared with the start of stimulation in all dose groups (P = 0·75).

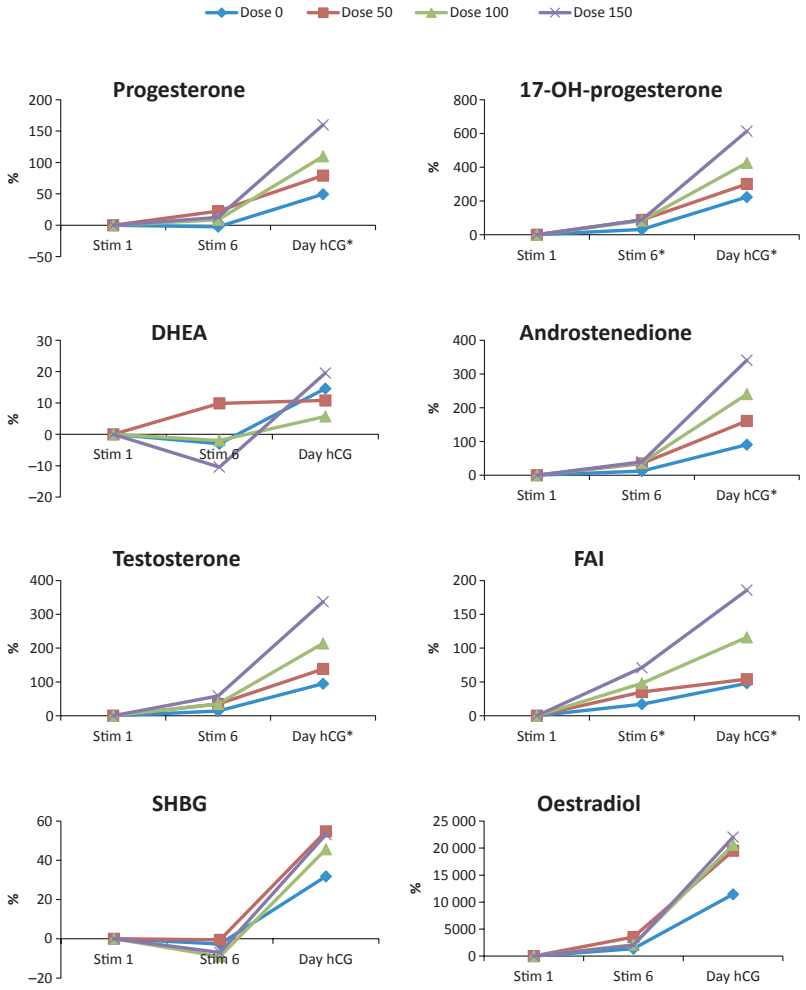

Fig. 1 Increments of progesterone, the androgens, oestradiol and SHBG. The increments are defined as the relation between the hormone level on the Day 6 of stimulation and the Day 1 of stimulation, and the relation between the hormone level on the day of human chorionic gonadotrophin (hCG) triggering in relation to stimulation Day 1. Serum increments were calculated for progesterone, 17-OH-progesterone, dehydroepiandrosterone (DHEA), androstenedione, testosterone, free androgen index (FAI), sex horomone-binding globulin (SHBG), and oestradiol. *Statistical significance ($P < 0{\cdot}05$) between the groups using analysis of variance (ANOVA). Stim, stimulation day; Day hCG, the day of hCG triggering.

Androstenedione. The levels of androstenedione increased significantly with higher doses of hCG. The preovulatory level was more than twofold higher after 150 IU/day of hCG compared with no hCG supplementation. The incremental increase was 91% (CI: 48−145%) to 340% (CI: 242−468%) from Dose 0 to Dose 150 ($P < 0{\cdot}001$).

Testosterone and FAI. The level of testosterone followed the doses of hCG with the highest value for the Dose 150; however, the differences were not statistically significant ($P = 0{\cdot}06$). The testosterone increments increased significantly when the hCG dose was increased. The increments ranged from 95% (CI: 50−153%) in Dose 0–338% (CI: 188−567%) in Dose 150 ($P < 0{\cdot}01$). The FAI levels followed the same pattern as testosterone, and an incremental increase from 48% (CI: 20−81%) (Dose 0) to 186% (CI: 86−318%) (Dose 150) was observed ($P < 0{\cdot}01$).

Sex hormone-binding globulin (SHBG). In all groups, SHBG increased towards the end of stimulation, but no differences were found in either the levels of SHBG or in the increments between the groups. Still, the control group had the lowest increment compared with the hCG dose groups ($P = 0{\cdot}52$).

Oestradiol. On the day of hCG triggering, the two groups given the highest hCG doses also had the highest oestradiol levels; however, the differences were not statistically significant. The peak preovulatory levels were twice as high in Dose 100 and Dose 150 compared with Dose 0 ($P = 0{\cdot}09$). No difference in increment was observed for oestradiol, and from Day 1 of stimulation to the day of hCG triggering oestradiol increased 11 450% in Dose 0 to 21 833% in Dose 150 ($P = 0{\cdot}21$).

Anti-Müllerian hormone (AMH). Levels of AMH did not differ between the groups before or during the stimulation. A decline of around 50% was observed for all the groups from the beginning to the end of stimulation.

Human chorionic gonadotrophin (hCG). As reported earlier, steady state level of s-hCG was reached on Day 6 of stimulation. On the day of hCG triggering, serum hCG levels (IU/l) were Dose 0: <0·1, Dose 50: 3·1 (CI: 2·6–3·6), Dose 100: 5·5 (CI: 4·1–7·4) and Dose 150: 11·0 (CI: 8·9–13·6) ($P < 0{\cdot}001$).[22]

Gonadotrophins. The levels of FSH and LH did not vary between the groups. On the day of hCG triggering, the LH levels (IU/l) were Dose 0: 2·1 (CI: 1·5–2·9), Dose 50: 1·9 (CI: 1·3–2·6),

Dose 100: 2·1 (CI: 1·6–2·8) and Dose 150: 1·5 (CI: 1·1–2·1) (P = 0·41). The FSH (IU/l) levels on the day of hCG were Dose 0: 11·2 (CI: 10·2–12·2), Dose 50: 11·7 (CI: 10·4–13·2), Dose 100: 10·9 (CI: 9·6–12·4) and Dose 150: 11·7 (CI: 10·4–13·1) (P = 0·73).

Discussion

To our knowledge, this is the first dose–response study analysing in detail the endocrine effects of hCG supplementation to rFSH throughout controlled ovarian stimulation for IVF. The clinical data published earlier from this study showed that there was an hCG dose-related increase in both oestradiol and androstenedione.[22] In a previous study of patients treated with the long GnRH agonist protocol, the hCG containing gonadotrophin highly purified menotrophin (HP-hMG) resulted in a lower frequency of preovulatory progesterone levels than the non-hCG containing recombinant FSH.[6] Based on our earlier observation that daily administration of hCG in the dose range of 50–150 IU had a stimulatory effect on progesterone,[22] we have made supplementary analysis of ovarian steroids as well as SHBG to further analyse the effects of adding hCG to FSH.

This study showed that supplementation with hCG resulted in a dose-dependent increase in the levels of both progesterone and 17-OH-progesterone. The augmentation of both hormones in relation to increasing hCG doses supports the concept that addition of hCG does not lower, but rather increases, preovulatory progesterone levels. In contrast to the dose-related increase in androstenedione and testosterone, no significant rise was seen for DHEA, which may suggest that DHEA, being an intermediate product in the preferred Δ-5 pathway, is rapidly converted into androstenedione. HCG increased both oestrogens and androgens, but the oestrogen/androgen balance remained unaltered, and the SHBG concentration unchanged by the different hCG doses.

Studies on ovarian steroid-converting enzyme reactions on the production cascade of androgens have shown that there are marked species-dependent differences in the utilization of either 17-OH-pregnenolone or 17-OH-progesterone. The major species-dependent differences have been observed in 17, 20-lyase activity. The enzymes from rodent and guinea pig utilize both 17-OH-pregnenolone and 17-OH-progesterone,[24] and bovine enzyme utlizes mainly 17-OH-progesterone. Human enzyme converts almost exclusively 17-OH-pregnenolone.[16,17,25] Figure 2 provides a schematic overview of the two main steroidogenic pathways in humans and supports the concept that the vast majority of androgens synthesized in the human ovary goes through the Δ-5 pathway[18,21,25]; however, this is not in line with other authors suggesting that progesterone can be converted into androgens.[10,15]

Human chorionic gonadotrophin binds to the LH/hCG receptor located on the theca cells throughout the follicular phase resulting in the production of androgens. The binding of FSH to the FSH receptor on the granulosa cells promotes oestrogen secretion from androgens through stimulation of aromatase. In

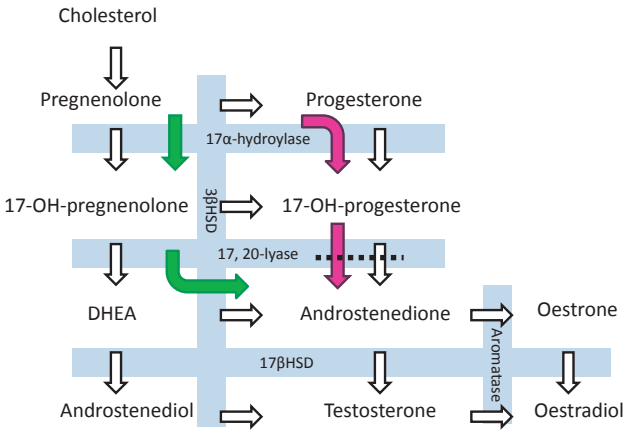

Fig. 2 The steroid biosynthetic pathway. Δ-4 pathway represented with purple and Δ-5 pathway with green arrows. Dotted line represents limited enzymatic activity in humans. HSD, hydroxysteroid dehydrogenase; OH, hydroxy; DHEA, dehydroepiandrosterone.

the presence of oestradiol, FSH stimulates the formation of LH/hCG receptors on granulosa cells in follicles larger than 10 mm in diameter.[26] Both theca cells and granulosa cells are able to produce progesterone[2] through activation of the 3βHSD activity mediated by FSH and LH/hCG.[2,20] Furthermore, LH/hCG enhances the expression of CYP17 and hence the activity of 17-hydroxylase and 17,20-lyase. In the ovary, CYP17 expression is restricted to theca cells, which are the site of androgen production.[27]Supplementation of hCG in the dose range from 50 to 150 IU/day augmented the production of both androgens and progesterone. A dose-dependent increase in the levels of progesterone and 17-OH-progesterone was observed consistent with an accumulation of these as end-products in the Δ-4 pathway. In contrast, no significant differences were seen between the four hCG dose groups for DHEA. This could support that DHEA is an intermediate product in the Δ-5 pathway and that immediate conversion into androstenedione takes place. For androstenedione and testosterone, the marked dose-dependent increase may present an accumulation in theca cells before diffusion over the basal membrane into the granulosa cells for conversion into oestradiol. *In vitro*, it has been shown that granulosa cell aromatase induction/activation by FSH is an androgen receptor-regulated process.[28] However, no further increase in the oestradiol level was observed after supplementation of 100 IU/day and above, suggesting that despite an increasing pool of androgen precursors, the aromatase system was saturated at around 100 IU of hCG per day. By not including the analyses of androstenediol, pregnenolone and 17-OH-pregnenolone, we are aware that we do not present a complete analysis of the two pathways.

Human chorionic gonadotrophin has been suggested to cause reduction in the level of progesterone by promoting the conversion of progesterone into androgens and oestradiol.[10,15] Nonetheless, in the dose range tested, our results do not support a diminishing effect of hCG, as a dose-dependent increase in the increment for progesterone as well as for 17-OH-progesterone was seen. The highest level of progesterone (3·7 nmol\l)

observed after 150 IU/day was, nevertheless, below the level of 4·8 nmol\l (1·5 ng/ml) previously reported to be related to negative pregnancy outcome.[10,13]

A recent large, prospective, multicenter study demonstrated that premature progesterone rise during stimulation with the GnRH antagonist protocol was associated with increased follicular responsiveness upon FSH administration.[11] Furthermore, progesterone increase was equally prevalent (1 in 5 patients) but more harmful (pregnancy rate reduced), when the stimulation regimen did not contain LH bioactivity. The adverse effects of progesterone were suggested to be predominantly exerted at the endometrial level.

Concerns have been published regarding the capacity of commercially available assays to measure progesterone in the follicular phase. The minor changes during the follicular phase are detectable only with assays validated in the lower measurement range.[10,15,21] Methods for progesterone on automated systems are not uniformly well calibrated to the gold standard (Isotope Dilution Gas Chromatography Mass Spectrometry, ID-GCMS); thus, some assays lack the sensitivity and precision for the low preovulatory ranges.[29] The assay used in our study had a sensitivity of 0·1 nmol\l, a precision (coefficient of variation) of 5% and was calibrated against the gold standard (ID-GCMS). Further, all samples were measured batchwise on the same analyser. This assay was found suitable to measure low progesterone concentrations in the follicular phase.

A majority of the principal gonadal steroids, including oestradiol and testosterone, are bound to the protein carrier SHBG produced in the liver. The androgens have stronger affinity than oestradiol for SHBG, but androstenedione has a much lower binding potential compared with the other androgens.[30] Oestradiol increases SHBG levels, whereas androgens decrease SHBG. In our results, no significant differences in either increment or the actual values of SHBG were observed between the different hCG dose groups. The level of SHBG decreased from stimulation Day 1 to stimulation Day 6 for all four groups with the two highest hCG dose groups having the most notable decrease, plausibly by higher androgen production mediated by hCG in the smaller follicles. The decrease in SHBG was reversed from Day 6 until the final day of hCG triggering (the period that demonstrated the highest oestradiol production). Although not statistically significant, the control group had the lowest increment in SHBG compared with the groups supplemented with hCG, possibly reflecting the lowest oestradiol level observed in that group. As hCG induced a marked increase in oestradiol, androstenedione and testosterone, it was of interest that SHBG was not influenced by hCG. It has previously been shown that an oestrogen-dominated intrafollicular milieu may be related to better embryo quality, as assessed by ability to implant.[31] In this study, no differences were seen between the groups for the serum E2/androstenedione ratios or serum E2/testosterone, so considering SHBG as a biomarker of the oestrogen/androgen balance, we conclude that this balance was not changed remarkably by hCG.

The levels of AMH did not differ between the groups, but a decline was observed for all four groups during the stimulation. The AMH level was almost halved from stimulation Day 1 to the day of hCG triggering. Fancin *et al.*[32] found a similar pattern during stimulation with recombinant FSH in a GnRH agonist protocol, reflecting the reduction in AMH-producing smaller follicles on the day of hCG triggering. This supports our clinical data where we found no influence of hCG on the number of recruited follicles.[22]

With the present knowledge, whether it is reasonable to give recommendations on the dose of hCG supplemented to rFSH in COS could be challenged. Presently, in the clinical practice, hCG is used in doses around 20–30 IU per day when HP-hMG is administered in recommended doses of 150–225 IU/day. Based on the biochemical evidence of a minor preovulatory elevation of progesterone and 17-OH-progesterone as well as a possible saturated aromatase system, supplementation of hCG in a range up to 100 IU/day could be used. This conclusion is supported by the finding of an increase in the number of top-quality embryos per patient with increasing doses of hCG.[22] Both the endocrinology and the embryological data are of course surrogate end-points compared with live birth rates. We observed no decline in the live birth rate in the clinical study,[22] but this should be interpreted cautiously as the study was not powered to detect differences in live birth rates. Hence, a trial with a larger sample size is evidently needed to be able to give firm recommendations on the addition of hCG.

In conclusion, supplementation with doses of hCG up to 150 IU/day from the first day of stimulation resulted in a dose-dependent response for androstenedione, testosterone, progesterone and 17-OH-progesterone, whereas the aromatase system producing oestrogens seemed saturated with androgen precursors using hCG doses above 100 IU/day. Considering sex hormone-binding globulin as a biomarker of the oestrogen/androgen balance, this balance was not changed by hCG. hCG induced a dose-related increase in both 17-OH-progesterone and progesterone, but no increase in the Δ-5 pathway intermediate product dehydroepiandrosterone, suggesting that hCG induces an efficient flow in the Δ-5 pathway.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Endocrine increments from stimulation Day 1 to the day of hCG.