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# Endocrine Profiles after Triggering of Final Oocyte Maturation with GnRH Agonist after Cotreatment with the GnRH Antagonist Ganirelix during Ovarian Hyperstimulation for *in Vitro* Fertilization

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**In a randomized multicenter study, the efficacies of two different GnRH agonists were compared with that of hCG for triggering final stages of oocyte maturation after ovarian hyperstimulation for *in vitro* fertilization. Ovarian stimulation was conducted by recombinant FSH (Puregon), and the GnRH antagonist ganirelix (Orgalutran) was coadministered for the prevention of a premature LH rise. Luteal support was provided by daily progestin administration. Frequent blood sampling was performed at midcycle in the first 47 eligible subjects included in the current study, who were randomized for a single dose of 0.2 mg triptorelin (n = 17), 0.5 mg leuprorelin (n = 15), or 10,000 IU hCG (n = 15). Serum concentrations of LH, FSH, E2, and progesterone (P) were assessed at variable intervals.**

**LH peaked at 4 h after both triptorelin and leuprorelin administration, with median LH levels of 130 and 107 IU/liter ($P < 0.001$), respectively. LH levels returned to baseline after 24 h. Subjects receiving hCG showed peak levels of 240 IU/liter hCG 24 h after administration. A rise in FSH to 19 IU/liter ($P < 0.001$) was noted in both GnRH agonist groups 8 h after injection. Within 24 h the areas under the curve for LH and FSH were significantly higher ($P < 0.001$) in both GnRH agonist groups compared with that for hCG. E2 and P levels were similar for all groups up to the day of oocyte retrieval. Luteal phase areas under the curve for P and E2 were significantly elevated ($P < 0.001$) in the hCG group. The mean (±SD) numbers of oocytes retrieved were 9.8 ± 5.4, 8.7 ± 4.5, and 8.3 ± 3.3; the percentages of metaphase II oocytes were 72%, 85%, and 86%; and fertilization rates were 61%, 62%, and 56% in the triptorelin, leuprorelin, and hCG group, respectively ($P$ = NS for all three comparisons). These findings support the effective induction of final oocyte maturation in both GnRH agonist groups.**

**In summary, after treatment with the GnRH antagonist ganirelix for the prevention of premature LH surges, triggering of final stages of oocyte maturation can be induced effectively by a single bolus injection of GnRH agonist, as demonstrated by the induced endogenous LH and FSH surge and the quality and fertilization rate of recovered oocytes. Moreover, corpus luteum formation is induced by GnRH agonists with luteal phase steroid levels closer to the physiological range compared with hCG. This more physiological approach for inducing oocyte maturation may represent a successful and safer alternative for *in vitro* fertilization patients undergoing ovarian hyperstimulation. (*J Clin Endocrinol Metab* 87: 709–715, 2002)**

hCG REPRESENTS the standard of care for the substitution of the endogenous LH surge to induce final stages of oocyte maturation in ovarian hyperstimulation protocols for *in vitro* fertilization (IVF). Unfortunately, hCG is also believed to contribute to the occurrence of the ovarian hyperstimulation syndrome (OHSS), a potentially life-threatening complication (1–3). Exogenous hCG is implicated in the development of multiple corpora lutea and sustained luteotropic effects due to its prolonged circulating half-life compared with native LH (4–6). Moreover, the occurrence of OHSS is associated with continued late luteal phase hCG production in the case of pregnancy. An alternative to exogenous hCG could be the administration of a GnRH agonist inducing an endogenous rise in both LH and FSH levels due to the initial flare effect (5, 7). The capacity of GnRH agonists to trigger ovulation after gonadotropin treatment of anovulatory women (8, 9) or to induce final stages of oocyte maturation after ovarian hyperstimulation for IVF (5, 7, 10–12) has been well established. The induction of an endogenous LH (and FSH) surge is more physiological compared with the administration of exogenous hCG and may reduce the risk of OHSS due to the much shorter half-life of LH (10, 13, 14). Moreover, under these conditions luteal phase steroid levels seem closer to normo-ovulatory cycles (15), which may improve endometrial receptivity (16, 17). Effects on oocyte quality and chances for subsequent fertilization remain uncertain at this stage.

For obvious reasons, a GnRH agonist to induce oocyte maturation can only be used when a GnRH agonist has not been applied for pituitary down-regulation to prevent a premature LH rise during ovarian stimulation. As the GnRH agonist long protocol has been the standard of care for over

Abbreviations: ANCOVA, Analysis of covariance; AUC, area under the curve; ET, embryo transfer; ICSI, intracytoplasmatic sperm injection; IVF, *in vitro* fertilization; OHSS, ovarian hyperstimulation syndrome; P, progesterone.

a decade, alternative approaches to induce oocyte maturation has received little attention in recent years. The mid to late follicular phase administration of a GnRH antagonist may also be used to prevent a premature LH rise during ovarian hyperstimulation. The recently introduced GnRH antagonist ganirelix (Orgalutran, Antagon) competes with native GnRH in binding to its receptor, resulting in the immediate and dose-dependent suppression of endogenous gonadotropin release (18, 19). In previous trials the daily administration of 0.25 mg ganirelix has been shown to be safe and effective (20), requiring, on the average, 5 d of treatment and a reduced total dose of recombinant FSH compared with a long GnRH agonist protocol (21–24). hCG has been used to trigger final oocyte maturation in these first studies applying GnRH antagonist. However, in contrast to GnRH agonist, the suppressive effect of the GnRH antagonist can be reversed immediately by administering native GnRH or GnRH agonist, resulting in a surge of endogenous LH and FSH. This new concept of triggering final oocyte maturation after GnRH antagonist treatment was successfully tested in five patients undergoing ovarian hyperstimulation for intrauterine insemination using a single dose of 0.1 mg triptorelin (25). No information on the maturity of oocytes could be obtained in this first pilot study. In addition, a single dose of 0.2 mg triptorelin was effective in triggering an endogenous LH surge and final oocyte maturation in eight high responder IVF patients cotreated with ganirelix (26). None of these patients developed OHSS.

The current randomized study was designed to examine whether, after daily late follicular phase treatment with 0.25 mg ganirelix, administration of a single dose of GnRH agonist is at least as effective as hCG in inducing final oocyte maturation in patients undergoing ovarian hyperstimulation for IVF. Here we report on the endocrine profile and clinical outcome of the first subset of patients in whom blood sampling was performed at regular intervals after the administration of triptorelin, leuprolide, or hCG.

## Subjects and Methods

### *Subjects*

The first 57 of 200 subjects participating in this comparative randomized trial signed informed consent, which included hospitalization to monitor hormonal changes after GnRH agonist was given to trigger final oocyte maturation. All subjects underwent ovarian hyperstimulation with recombinant FSH for IVF plus intracytoplasmatic sperm injection (ICSI) allowing for the assessment of oocyte maturation (metaphase II stage). Subjects had a regular indication for ICSI, were between 18 and 39 yr of age, had a regular menstrual cycle (24–35 d) and body weight was normal (body mass index, 18–29 kg/m$^2$).

### *Study design*

Six international centers participated in this study, and the protocol was approved by the local ethics review committees. This study was an open label, randomized, three-arm trial in subjects who started ovarian hyperstimulation with recombinant FSH (Puregon, NV Organon, Oss, The Netherlands) starting at cycle d 2 or 3 and continued treatment until the day of inducing final oocyte maturation (Fig. 1). The FSH dose was 150 or 225 IU/d, sc, for the first 5 d of treatment. Thereafter, the daily dose could be adjusted based on the follicular growth as observed by ultrasound. To prevent premature LH surges, ganirelix (Orgalutran/Antagon, NV Organon; 0.25 mg in 0.5 ml daily) was administered sc starting on d 6 of FSH stimulation until and including the day of triggering final oocyte maturation. On that day, randomization was performed by means of an interactive telephone randomization system that stratified for age, primary or secondary infertility, and number of follicles at least 11 mm in diameter. Subjects were randomized in a ratio of 1:1:1 to treatment with 0.2 mg triptorelin (Decapeptyl, Ferring Pharmaceuticals Ltd., Copenhagen, Denmark), 0.5 mg leuprorelin (Lupron, TAP Pharmaceuticals, Inc., Lake Forest, IL), or 10,000 IU hCG (Pregnyl, NV Organon). Subjects were only randomized if at least three follicles 17 mm or larger were observed. High responders (defined as having >25 follicles beyond 11 mm) were considered drop-outs from the study. Approximately 30–36 h after triggering of final oocyte maturation, oocyte retrieval was performed, followed by ICSI. No more than three embryos were transferred at 2–5 d after oocyte retrieval. Exogenous progesterone (P) was administered im for luteal support (Progestine, NV Organon; 50 mg in 2 ml daily) from the day of embryo transfer (ET) for at least 2 wk or until menses.

### *Assessments*

Before the start of ovarian stimulation, pregnancy was excluded by means of a hCG test, a blood sample was taken for hormone assessments, and ultrasound was performed. The subject returned to the clinic at least once every 2 d for ultrasound investigation and blood sampling from d 6 of stimulation until the day of triggering of final oocyte maturation. Blood sampling was performed before ganirelix and FSH administration. On the day of triggering final oocyte maturation, blood samples were taken just before GnRH agonist or hCG administration; at 2, 4, 8, 12, and 24 h after GnRH agonist injection; and at 12 and 24 h after hCG injection. Additional blood samples of all subjects were taken on the day of oocyte pick-up, on the day of ET, and 1 and 2 wk after ET. hCG concentrations were not assessed at 4 and 8 h because previous pharmacokinetic studies (27) demonstrated a $c_{max}$ at 20 h.

Serum FSH, LH, E2, hCG, and P levels were assessed by a central laboratory using fluoroimmunoassays (Delfia, Wallac, Inc. OY, Turku, Finland). Detection limits of these assays were less than 1 IU/liter for FSH, less than 0.6 IU/liter for LH, less than 14 pg/ml for E2, less than 2.0 IU/liter for hCG, and less than 0.3 ng/ml for P. Intra- and interassay coefficients of variation were 1.6% and 4.1% for FSH, 11.0% and 13.0% for LH, 7.8% and 10.3% for E2, 6.9% and 9.3% for hCG, and 5.0% and 6.9% for P, respectively.

Embryo quality was assessed as grade 1 (excellent embryos with absent fragmentation), grade 2 (1–20% fragmentation), grade 3 (21–50% fragmentation), or grade 4.

### *Statistical methods*

The primary objective of this study was to compare endocrine characteristics of both GnRH agonist groups *vs.* hCG to induce final oocyte

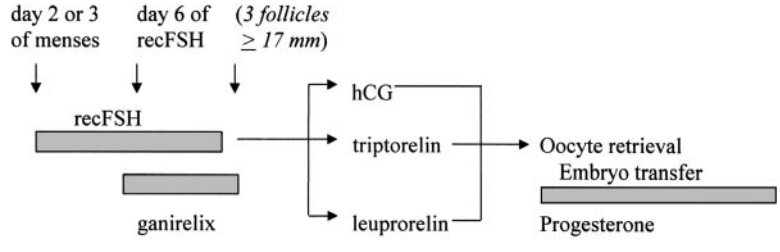


FIG. 1. Schematic representation of different treatment regimens for ovarian hyperstimulation for IVF using recombinant FSH and ganirelix to prevent a premature rise in LH, followed by the triggering of final oocyte maturation by a single dose of two GnRH agonists (triptorelin or leuprorelin) or hCG.

maturation. Relevant data of leuprorelin, triptorelin, and hCG have been statistically compared using analysis of covariance (ANCOVA) with center and treatment as factors and endocrine or ultrasound features as covariate. In case the overall treatment factor was statistically significant (*i.e.* the three treatment groups differ statistically significant), pairwise comparisons were made between hCG and leuprorelin or triptorelin, respectively. These pairwise comparisons were performed using the same analysis of covariance.

Individual areas under the curve (AUC) were calculated using the linear trapezoidal rule. For LH and FSH the concentrations obtained on the day of triggering final oocyte maturation up to 24 h after GnRH agonist or hCG injection were included in the AUC. For E2 and P the applied timeframe was from the day of triggering of final oocyte maturation until 2 wk after ET. To correct for possible differences in the timeframes, each AUC value was divided by the actual timeframe, resulting in individual estimates of the mean hormone concentration over time. Descriptive statistics were calculated for these derived concentrations per treatment (Table 1), and a one-way ANCOVA was performed to compare the treatment effects of both leuprorelin and triptorelin *vs.* hCG. In case the ANCOVA showed statistically significant differences among the three treatment groups, two-sample *t* tests were performed to compare both leuprorelin and triptorelin *vs.* hCG, respectively.

Preliminary clinical outcome parameters are described for all randomized patients (using descriptive statistics unadjusted for center) showing no major differences (Table 2). Definitive statistical evaluation will be performed separately on the extended series of women (as described above under *Subjects*).

$P < 0.05$ was considered to represent a statistically significant difference.

## Results

### *Disposition and cancellations*

A total of 57 subjects started treatment with FSH, of whom 47 were randomized. Eight subjects (14%) were not randomized due to insufficient ovarian response to stimulation. Two subjects were not randomized due to high response. One subject in the hCG group did not undergo ET due to a fertilization failure.

### *Characteristics of randomized subjects*

The overall mean age was 30.4 ± 4.2 yr, height was 1.67 ± 0.07 m, weight was 64.6 ± 7.7 kg, and body mass index was 23.3 ± 2.5 kg/m$^2$. The vast majority of subjects (98%) participating in this study were Caucasian. The three treatment groups were similar with respect to age, height, weight, and body mass index (data not shown).

The total amount of exogenous FSH required (mean ± SD, 1579 ± 395, 1665 ± 455, and 1605 ± 544 IU for triptorelin, leuprorelin, and hCG, respectively), the duration of FSH stimulation (8.6 ± 1.1, 9.2 ± 1.6, 9.3 ± 2.0 d), and the duration of ganirelix treatment (4.6 ± 1.1, 5.1 ± 1.7, and 5.3 ± 2.0 d) were similar ($P$ = NS for all these prerandomization parameters) in the three treatment groups. The overall median duration of stimulation was 9 d (range, 6–14 d), and the median total amount of exogenous FSH administered was 1500 IU (range, 900-2625 IU). The median duration of ganirelix treatment was 5 d (range, 2–10 d). The mean numbers of follicles 11 mm or larger per treatment group assessed on d 1, 6, and 8 of stimulation and on the day of randomization for induction of final oocyte maturation are presented in Fig. 2. On the day of the last ultrasound investigation before triggering of final oocyte maturation the mean numbers of follicles 17 mm or larger were 4.8 ± 2.2, 4.6 ± 1.8, and 4.3 ± 1.4 ($P$ = NS) for the triptorelin, leuprolide, and hCG groups, respectively.

**TABLE 1.** Area under the curve divided by the actual time period for serum hormone concentrations (mean + SD) during triggering of final oocyte maturation with the GnRH agonists (triptorelin and leuprorelin) or hCG following ovarian hyperstimulation for IVF (0–24h for LH and FSH) and during the subsequent luteal phase (0–2 wk for E2 and P)

| | Triptorelin (n = 15) | Leuprorelin (n = 15) | hCG (n = 15) |
|---|---|---|---|
| $LH_{(0-24h)}$ (IU/L)[a] | 59 ± 14[b] | 53 ± 14[b] | 2.7 ± 2.2 |
| $FSH_{(0-24h)}$ (IU/L)[a] | 14.5 ± 3.8[b] | 14.3 ± 3.7[b] | 5.0 ± 1.2 |
| $E2_{(0-2\ wk)}$ (pg/ml)[a] | 252 ± 154[b] | 196 ± 111[c] | 515 ± 286 |
| $P_{(0-2\ wk)}$ (ng/ml)[a] | 12.5 ± 7.7[b] | 15.2 ± 7.1[b] | 37.8 ± 17.1 |

[a] $P_{ANCOVA} < 0.001$ comparing all three groups.
[b] $P = 0.0001$ *vs.* hCG group (paired *t* test).
[c] $P = 0.0006$ *vs.* hCG group (paired *t* test).

**TABLE 2.** Clinical outcome (mean ± SD) of a randomized comparison between two GnRH agonists (triptorelin and leuprorelin) *vs.* hCG in inducing final oocyte maturation after ovarian hyperstimulation with FSH and cotreatment with ganirelix in 47 IVF patients

| | Triptorelin (n = 17) | Leuprorelin (n = 15) | hCG[a] (n = 15) |
|---|---|---|---|
| Number of oocytes/subject | 9.8 ± 5.4 | 8.7 ± 4.5 | 8.3 ± 3.3 |
| Proportion of metaphase II oocytes | 72 ± 18% | 85 ± 17% | 86 ± 17% |
| Fertilization rate | 61 ± 30% | 62 ± 23% | 56 ± 18% |
| Number of embryos obtained/subject | | | |
| Good quality (grade 1) | 0.5 ± 0.9 | 1.6 ± 1.8 | 0.8 ± 0.9 |
| Good quality (grade 2) | 1.9 ± 1.8 | 2.7 ± 2.5 | 1.7 ± 1.9 |
| Grades 1 and 2 pooled | 2.7 ± 1.9 | 3.2 ± 2.6 | 3.3 ± 2.0 |
| Implantation rate | 15 ± 34% | 18 ± 37% | 7 ± 14% |
| Ongoing pregnancy rate[b] | 18% | 20% | 13% |

[a] $P$ = NS for all features comparing the triptorelin or the leuprorelin group *vs.* women receiving hCG.
[b] Defined as a pregnancy per IVF cycle, confirmed by detection of positive heart beat on ultrasound between 12 and 16 wk of gestation.

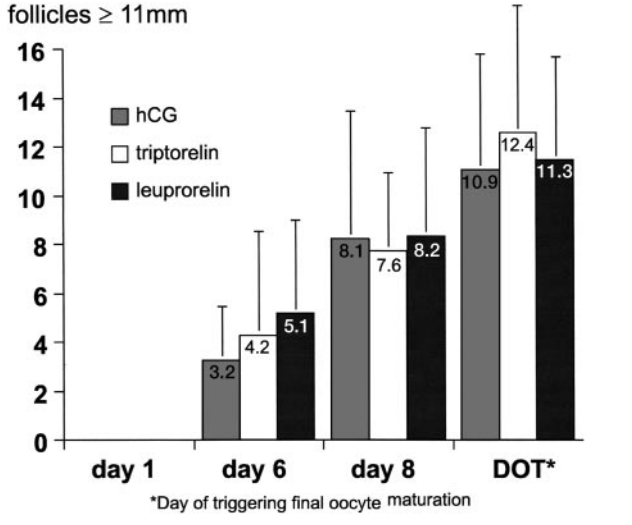


FIG. 2. Number of follicles 11 mm or larger (mean ± SD) assessed on stimulation d 1, 6, and 8 and on the day of triggering of final oocyte maturation (by triptorelin, leuprorelin, or hCG) during ovarian hyperstimulation for IVF with exogenous FSH.

*Serum hormone levels*

Serum concentrations of LH, FSH, E2, and P measured at regular intervals after triggering final oocyte maturation up to 2 wk after ET are presented in Fig. 3. An endogenous LH rise was observed in all subjects who received either triptorelin or leuprorelin. In both treatment groups serum LH levels rapidly increased and reached peak levels 4 h after GnRH agonist administration. Mean (±SD) LH levels increased from 0.9 ± 0.4 IU/liter before triggering oocyte maturation to peak levels of 130 ± 60 IU/liter after triptorelin administration ($P < 0.001$) and from 0.9 ± 0.8 to 107 ± 55 IU/liter after leuprorelin administration ($P < 0.001$). On the day of oocyte pick-up LH levels returned to baseline (4.8 ± 2.5 in the triptorelin group and 2.6 ± 0.4 IU/liter in the leuprorelin group). In subjects randomized to hCG treatment, serum hCG levels increased gradually and reached a peak of 240 ± 101 IU/liter at 24 h after administration. Thereafter, serum hCG levels declined to 5.0 ± 1.6 IU/liter 1 wk after ET. After hCG administration, serum LH remained low or undetectable during the entire luteal phase. In contrast to hCG-treated subjects, FSH levels increased after GnRH agonist administration. FSH levels increased from 5.8 ± 1.6 IU/liter before induction of final oocyte maturation to 19.2 ± 5.2 IU/liter at 8 h after GnRH agonist administration in the triptorelin group. In the leuprorelin group these levels increased from 5.2 ± 1.6 to 19.7 ± 5.1 IU/liter ($P <$ 0.001 for both groups), whereas in the hCG group serum FSH levels declined after hCG administration from 5.8 ± 1.6 to 3.4 ± 0.8 IU/liter ($P < 0.001$) on the day of oocyte pick-up, reflecting the clearance of exogenous FSH. The individual AUCs for LH and FSH within the first 24 h are depicted in Table 1, confirming the major difference comparing both GnRH agonist groups *vs.* hCG.

Serum E2 and P levels were comparable from the time of triggering ovulation until oocyte pick-up (see Fig. 3). Thereafter, serum E2 and P levels remained higher in hCG-treated subjects compared with both GnRH agonist groups. Respective mean (±SEM) E2 levels were 279 ± 48, 204 ± 30, and 609 ± 115 pg/ml on the day of ET ($P_{ANCOVA} = 0.0002$; $P_{triptorelin}$ *vs.* hCG = 0.0006; $P_{leuprorelin}$ *vs.* hCG = 0.0001), and 46 ± 4, 45 ± 9, and 490 ± 145 pg/ml 1 wk thereafter ($P_{ANCOVA} = 0.0001$; $P_{triptorelin}$ *vs.* hCG = 0.0001; $P_{leuprorelin}$ *vs.* hCG = 0.0001) in triptorelin-, leuprorelin-, and hCG-treated subjects, respectively. Mean P levels were 7.2 ± 1.7, 8.0 ± 1.5, and 58.6 ± 9.6 ng/ml on the day of transfer ($P_{ANCOVA} = 0.0001$; $P_{triptorelin}$ *vs.* hCG = 0.0001; $P_{leuprorelin}$ *vs.* hCG = 0.0001) and 18.0 ± 3.6, 23.2 ± 3.7, and 45.9 ± 11.2 ng/ml 1 wk later ($P_{ANCOVA} = 0.0006$; $P_{triptorelin}$ *vs.* hCG = 0.0002; $P_{leuprorelin}$ *vs.* hCG = 0.0054). At the end of the luteal phase, levels of both hormones were comparable in the three treatment groups. The luteal phase AUCs (Table 1) confirmed elevated E2 and P levels in the hCG group.

*Clinical outcome*

The clinical outcome (number of oocytes retrieved, percentage of metaphase II oocytes, fertilization rates, embryo quality, and implantation rates) is presented in Table 2. The numbers of miscarriages within the first 12 wk after transfer were 3, 2, and 3 in the triptorelin-, leuprorelin-, and hCG-treated subjects, respectively.

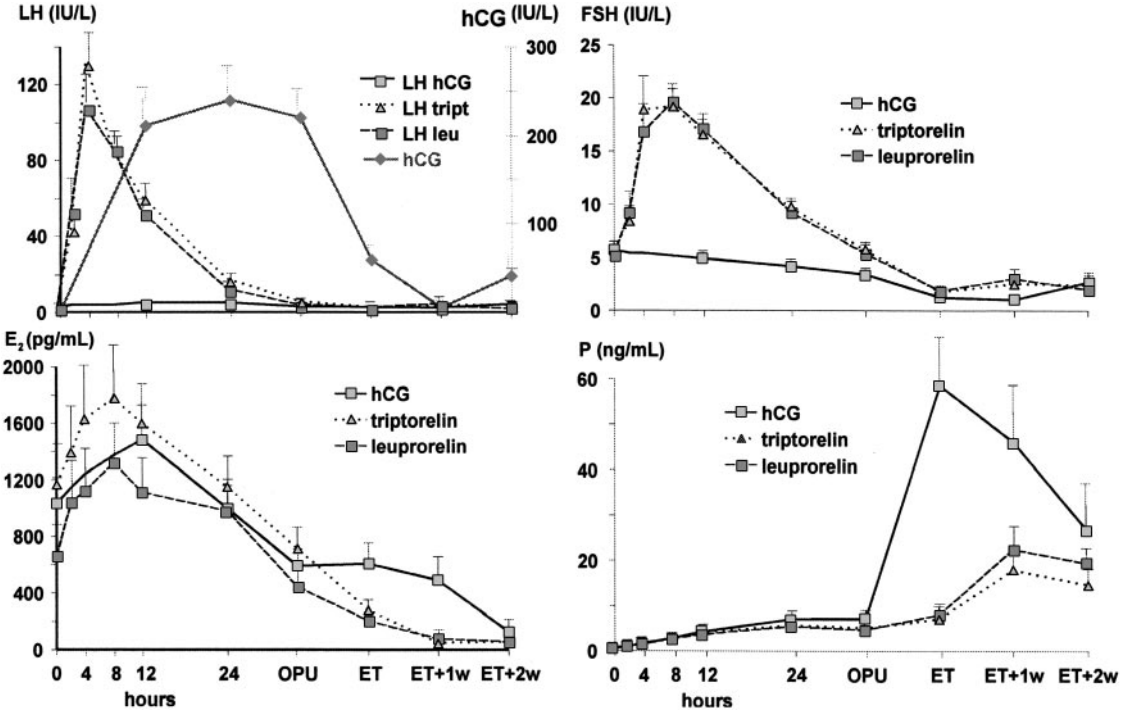


FIG. 3. Serum concentrations of LH (hCG), FSH, E2, and P (mean ± SEM) during triggering of final stages of oocyte maturation (0, 4, 8, 12, and 24 h) with two GnRH agonist (triptorelin and leuprorelin) or hCG after ovarian hyperstimulation for IVF and during the subsequent luteal phase [day of oocyte pick-up (OPU), embryo transfer (ET), and 1 and 2 wk after ET].

## Discussion

In the normal menstrual cycle the midcycle LH and FSH surge (lasting for ~48 h) is a complex and carefully orchestrated event elicited in the late follicular phase by persistently elevated estrogen concentrations in combination with a small, but distinct, rise in P (28). Exposure to a transient massive stimulation by LH elicits the resumption of meiotic maturation of the oocyte, rupture of the dominant follicle resulting in the release of the oocyte, and luteinization of thecal and granulosa cells resulting in the formation of the corpus luteum. The timing of the endogenous LH surge is frequently disrupted if exogenous FSH is administered either for ovarian hyperstimulation for IVF or for the treatment of anovulation. Therefore, exogenous hCG (which activates the LH receptor due to structural and biological similarities to LH and which is easy to extract from urine of pregnant women) has been used for decades to replace endogenous LH during FSH stimulation protocols. hCG has been shown to effectively induce ovulation, final oocyte maturation, and corpus luteum formation. However, hCG has an extended MCR (4, 27), and therefore hCG can still be detected in the serum 10 d after the preovulatory bolus injection. Consequently, continued support of the corpus luteum by hCG elicits supraphysiological luteal phase steroid concentrations. Moreover, hCG in the late follicular phase may stimulate the growth of medium-sized follicles (3, 29, 30), which may subsequently ovulate. A midcycle bolus dose of hCG induces elevated follicular fluid P levels, suggesting changes in the microenvironment of the oocyte just before ovulation compared with the endogenous LH surge (31).

Next to a hyperresponse of the ovary to stimulation (*i.e.* large late follicular phase number of follicles and very high E2 levels), both exogenous and endogenous hCG have clearly been associated with OHSS (32–34). The induction and stimulation of multiple corpora lutea may represent a key phenomenon in this regard. Although luteal phase support by repeated hCG injections has been clearly shown to be beneficial in IVF (35), randomized comparative trials have also convincingly demonstrated a higher incidence of OHSS in these patients (36, 37). Therefore, luteal function in IVF cycles is currently supplemented by exogenous P. Moreover, it has been shown that the late and most severe form of OHSS occurs especially in multiple pregnancies (coinciding with elevated hCG production) (32, 34). Indeed, withholding hCG along with discontinuation of ovarian stimulation effectively prevents ovulation, pregnancy, and the development of OHSS in women presenting with an excessive ovarian response. Other approaches for the prevention of OHSS include withholding gonadotropins for some days during the late follicular phase (also referred to as coasting), reducing the hCG bolus dose, replacing hCG with GnRH agonist as addressed in the current study, the administration of glucocorticoids or albumen, the avoidance of luteal support by hCG, and cryopreservation of embryos and transfer in subsequent cycles.

It has been clearly shown in several animal models that the midcycle gonadotropin surge is accompanied by a major rise in endogenous GnRH in the portal circulation (38). Indeed, several initial studies in IVF showed that a midcycle endogenous LH rise could be induced by the late follicular phase administration of exogenous GnRH or GnRH agonist. Induction of final oocyte maturation with GnRH agonists in patients undergoing ovarian hyperstimulation is believed to be more physiological and of special benefit to high responders with an increased risk of developing OHSS. In the current study very high hCG levels (median, >200 IU/liter) lasted for 24 h (from 12–36 h after triggering of oocyte maturation), and clearance of the 10,000 IU hCG required around 10 d.

Although effective, the approach to induce oocyte maturation by midcycle GnRH agonist administration lost interest during the nineties, as ovarian hyperstimulation protocols also included GnRH agonist coadministration to suppress a premature rise in LH during the follicular phase. This renders the pituitary in a state of desensitization, precluding stimulation of the endogenous LH surge. Several recent studies have shown that short-term GnRH antagonist administration during the late follicular phase is also effective in preventing a premature LH rise. This blockage of GnRH receptors on the gonadotropic cells by the antagonist can be reversed by GnRH stimulation, allowing reevaluation of stimulating a midcycle rise in endogenous LH. The primary purpose of the current study was to investigate the dynamics of the midcycle gonadotropin surge under these circumstances. The current study also demonstrates for the first time that final maturation of oocytes can be triggered with GnRH agonists rather than hCG after ovarian hyperstimulation using ganirelix to prevent a premature LH rise. An adequate pituitary response in terms of a rise in endogenous LH and FSH was observed after the administration of either 0.2 mg triptorelin or 0.5 mg leuprorelin, and luteal phase steroid levels were closer to the physiological range.

Endogenous LH and FSH surges in the current study were comparable to those described after triggering of oocyte maturation using GnRH agonists in nonsuppressed subjects undergoing ovarian stimulation for IVF (5, 7). Thus, in the current study the doses of GnRH agonist administered 12 h after the last antagonist injection were sufficient to displace ganirelix from the GnRH receptors. The maximum LH and FSH levels were comparable to circulating midcycle LH and FSH levels in natural cycles (28). In a recent preliminary study (also applying 0.2 mg triptorelin for triggering of oocyte maturation in eight high responder patients treated with exogenous FSH and 0.25 mg ganirelix) higher maximum LH concentrations compared with the current study were reported (26). This may indicate that the magnitude of the LH surge is in part determined by the endocrine status of the patient, as high response patients were excluded from the current study. In the current study the duration of the LH surge appeared to be shorter compared with that in the natural cycle (24 *vs.* 36–48 h, respectively). This phenomenon might be explained by the immediate pituitary desensitization after the initial flare effect, as two doses of buserelin 12 h apart resulted in similar LH profiles (7). Despite the shorter duration compared with physiological conditions, the induced LH surge effectively stimulated final oocyte maturation, as reflected by the high percentage of metaphase II oocytes (72–85%) as well as good fertilization and embryo implantation rates.

An alternative means of mimicking the midcycle gonad-

otropin surge is the administration of a high dose of recombinant LH (39). However, the relatively short half-life of this compound (40) suggests that very high or multiple doses should be given. A recently published large randomized comparative trial showed the complete absence of OHSS in women receiving a single dose of recombinant LH in doses up to 30,000 IU (41). Extensive studies in the monkey using different doses of LH or hCG suggest that the duration of the midcycle LH surge is critical for inducing a normally functioning corpus luteum (42, 43). A relatively short LH surge resulted in normal oocyte maturation and ovulation, whereas luteal phase length was reduced, implying that luteal support is required under these conditions. In the current study serum E2 and P levels were comparable for all treatment groups up to the day of oocyte retrieval. However, thereafter both E2 and P levels were higher in hCG-treated subjects. This difference can be explained by the prolonged half-life of hCG compared with LH, which is responsible for continued support of the corpus luteum. These luteal phase steroid levels far above the physiological range may suppress the release of endogenous gonadotropins required for corpus luteum support (44) and may exhibit a negative impact on endometrial receptivity (16, 17). In the current study luteal phase supplementation was applied by daily progestin administration. Therefore, the dynamics of midcycle LH requirements for subsequent normal luteal function in the human require further investigation.

The physiological role of the midcycle FSH surge that occurs in the natural cycle has not been elucidated to date, but recent studies suggest that FSH may play a role in the process of nuclear maturation by actively promoting resumption of meiosis (45, 46). Moreover, studies in the rat have demonstrated that a high dose of FSH alone is capable of inducing ovulation (47). On the other hand, a midcycle FSH surge is not mandatory, as normal oocyte maturation and ovulation occur after the administration of hCG. The potential favorable impact of a GnRH agonist-induced FSH surge is unknown at present.

In summary, the application of a single dose of GnRH agonist was shown to be effective in inducing a gonadotropin surge and triggering final oocyte maturation in normal responder patients after ovarian hyperstimulation for IVF cotreated with GnRH antagonist. Effects on the dynamics of the pituitary-ovarian axis during the luteal phase along with its capacity to prevent OHSS require further evaluation. This more physiological approach to inducing oocyte maturation may provide a successful and safer alternative for patients undergoing IVF.

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