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ORIGINAL ARTICLE

GnRH-agonist triggering for final oocyte maturation in GnRH-antagonist IVF cycles induces decreased LH pulse rate and amplitude in early luteal phase: a possible luteolysis mechanism

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ABSTRACT

The use of GnRH agonist to trigger final oocyte maturation in GnRH-antagonist *in vitro* fertilization (IVF) cycles has been shown to significantly reduce or even eliminate the risk of ovarian hyperstimulation syndrome (OHSS) by inducing rapid luteolysis early in the luteal phase. The exact mechanism of this early luteolysis is still widely unknown. Since luteinizing hormone (LH) has a major role in corpus luteum support, we sought to explore the pattern of LH secretion early in the luteal phase. Ten high risk patients for developing OHSS and triggered with GnRH agonist were included. Frequent blood sampling (every 20 min for 6 h) to measure LH, estradiol and progesterone was done on the day of oocyte collection ($n = 5$, Group 1) and on the day of embryo transfer, 48 h after oocyte collection ($n = 5$, Group 2). We found that the mean LH concentration and its secretion rate decreased significantly in Group 2 compared to Group 1. Both groups had similar number of LH pulses characterized by very small amplitude. In Group 2, there was a steady significant decrease in estradiol and progesterone over time. The results of this study show that LH secretion deviates significantly from normal physiologic pattern, which can explain, at least in part, the post-GnRH-agonist trigger early luteolysis mechanism.

Keywords

GnRH agonist, GnRH antagonist, luteolysis, ovarian stimulation, ovulation triggering

History

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Introduction

Controlled ovarian stimulation in *in vitro* fertilization and embryo transfer (IVF-ET) cycles results in the formation of multiple corpora lutea. Routinely, hCG is used to induce final oocyte maturation. Unlike the physiological mid-cycle surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), terminating 48 h after its onset, the hCG-mediated LH activity spans several days into the luteal phase. This supra-physiological LH activity stimulates the multiple corpora lutea, leading to high early luteal serum progesterone and estradiol concentrations, which in turn decrease the endogenous LH secretion by the pituitary gland [1]. Overstimulation of the multiple corpora lutea may cause ovarian hyperstimulation syndrome (OHSS), a prominent risk of ovarian stimulation.

GnRH agonist (GnRHa) can potentially substitute hCG for inducing final oocyte maturation. Indeed, the very first publication of such use in the context of OHSS prevention appeared in *Gynecological Endocrinology* in 1988 [2]. GnRHa generates LH and FSH surges from the pituitary gland. Although these surges are shorter than those seen in a normal cycle, they are sufficient to allow retrieval of mature oocytes [3]. From a physiologic point of view, GnRHa trigger separates the two events of oocyte maturation and maintenance of luteal function, which traditionally were undertaken together by hCG.

The development of GnRH antagonists in the late 1990s helped to disseminate the option of using GnRHa for final oocyte maturation in IVF, while controlling for premature luteinization during ovarian stimulation. IVF cycles triggered by GnRHa are associated with lower luteal phase levels of estradiol and progesterone and shorter luteal phase duration [4,5]. Moreover, Beckers et al. [6], reported extremely low implantation rate (7.5%) in non-supplemented GnRH-antagonist cycles triggered with GnRHa. However, self-limited gonadotropin release associated with the GnRHa trigger represents a major advantage—an ability to significantly reduce, if not eliminate, OHSS, as was first suggested [1] and repeatedly confirmed subsequently [7]. The mechanism underlying reduced OHSS risk with GnRHa relates to complete luteolysis [8]. Thus, GnRHa has become a viable alternative for the gold standard hCG trigger [9]. A recent survey indicated that as many as 36% of IVF GnRH-antagonist cycles in Europe are triggered with GnRHa [10], suggesting wide adoption by practitioners.

Surprisingly, the exact mechanism by which a single bolus of GnRHa induces quick and complete luteolysis is not known, in spite of its worldwide use for many years now. Classical experiments [11,12], conducted decades ago, showed that natural cycle luteal physiology is based on pulsatile LH secretion.

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Specifically, Filicori et al. [12], showed that mean LH pulse frequency declines from 15.2 pulses/24 h in the early to 8.4/24 h in the late luteal phase. A trend towards reduction in the amplitude of LH pulses was also observed [12]. In the context of ovarian stimulation for IVF and GnRHa trigger, corpora lutea function reflects endogenous LH secretion only, as is the case in a natural cycle. We therefore hypothesized that GnRHa-induced luteolysis may reflect altered LH secretion. Since the luteolytic process is complete during the first half of the luteal phase after GnRHa trigger, we sought to explore LH secretion pattern in two time points in early luteal phase post GnRHa trigger in IVF-ET cycles.

Subjects and methods

Patients

Ten healthy patients ''hyper-responders'' (based on previous ovarian response, or antral follicular count $\geq$15) treated in the IVF Unit, Rambam Health Care Campus, were recruited for the study. Patients with hypogonadotropic hypogonadism, known or suspected prolactin secreting tumor, or body mass index (BMI) > 35 kg/m^2 were excluded from the study. The study was approved by the Rambam Health Care Center IRB, and informed consent was obtained from each participant.

Ovarian stimulation was performed using highly purified gonadotropin (Menopur, Ferring, Switzerland) with a starting dose of 150 IU daily, followed by daily injections of a GnRH antagonist (0.25 mg Cetrotide, Merck, Darmstadt, Germany) once the leading follicle reached a size of $\geq$14 mm in diameter. Patients were considered to be at high risk for developing severe OHSS in the presence of either (i) an E_2>12 000 pmol/L or (ii) $\geq$15 follicles of >12 mm in diameter on the trigger day (Day 2) [13]. All patients were triggered with a bolus of 0.2 mg triptorelin (Decapeptyl, Ferring, Saint-Prex, Switzerland) when $\geq$3 follicles were $\geq$17 mm in diameter. Oocyte retrieval was performed 36 h later. Embryo transfer was performed 2 days after oocyte retrieval.

Hormone measurements

Repeated blood sampling was performed either (i) on day of oocyte retrieval ($n = 5$) or (ii) on the day of embryo transfer ($n = 5$). Patients did not receive any luteal hormonal support until after blood sampling was complete. Blood was withdrawn from an indwelling intravenous cannula at 20-min intervals for 6 h, starting in the morning at 9 AM and ending at 3 PM. Blood samples were analyzed immediately for LH, estradiol (E_2) and progesterone (P). All samples were analyzed in duplicate in the same assay for each hormone. Plasma LH, estradiol and progesterone were measured by chemiluminescent microparticle immunoassay, CMIA (ARCHITECT i2000, Abbott Diagnostics, Sligo, Ireland); assay sensitivities for LH, estradiol and progesterone were 0.1 IU/L, 36 pmol/L and 0.31 nmol/L, respectively. Intra-assay coefficients of variation (CV) for LH, estradiol and progesterone, 2.9, 3.2 and 4.2%, respectively, and inter-assay CVs were 3.8, 3.5 and 5.7%, respectively.

Luteal support

Luteal support with a single bolus of hCG (1500 IU) was given on the day of embryo transfer (after blood sampling in Group 2). A pregnancy test was performed 14 days after oocyte retrieval measurements; vaginal ultrasound 1 month after embryo transfer ascertained viable pregnancy.

Assessment of pulsatile LH secretion

LH pulse analysis was performed by a single investigator (C.R.M.) who was blinded to the timing of assessment (i.e. subject group). For each LH concentration time series, estimates of LH measurement uncertainty (i.e. experimental measurement error) were assigned. Assuming a minimal detectable concentration (MDC) of 0.1 mIU/mL and 3% CV, the following variance model was employed: $(MDC/2)^2 + [CV \times Y(i)/100]^2$. LH secretion characteristics were then characterized using AutoDecon [14]. This statistically based and fully automated (i.e. non-subjective) multi-parameter deconvolution algorithm identifies initial parameter estimates and then iteratively inserts and tests the significance of presumed secretion events, with removal of presumptive secretory events that fail to achieve statistical significance. This iterative process is terminated when no additional statistically significant secretion events can be identified.

Statistical analyses

Mean values and standard error of mean (SEM) were calculated for LH, estradiol and progesterone. Pearson correlation was used to assess change over time for estradiol and progesterone levels. Paired *t*-test was used to compare LH secretion parameters between Groups 1 and 2. A two-sided $p \leq 0.05$ was used as the null hypothesis rejection rule. All statistical calculations were carried out in SAS version 8.0.

Results

Patient's characteristics: mean age= 29.1 ± 5.0, BMI = 26.3 ± 5.1 antral follicle count (AFC) = 19.5 ± 13.9.

Cycle stimulation and outcome: stimulation days = 9.3 ± 0.8, total gonadotropin dose = 1575 ± 450, oocytes retrieved = 15.8 ± 5.3, normal fertilizations = 10.0 ± 4.2, embryos transferred = 1.8 ± 0.4, embryos frozen = 4.8 ± 3.8, positive pregnancy test = 5 (50%) and ongoing pregnancies = 4 (40%).

LH secretion parameters are shown in Table 1. Six-hour mean LH concentration and basal LH secretory rate were significantly lower in Group 2, but the number of LH pulses over 6 h and LH amplitude was not significantly different between groups. The number of LH pulses ranged from 0–2 pulses/6 h. No pulses were detected in one patient in each group, while all other subjects had 1 or 2 pulses in 6 h. Although mean LH pulse amplitude appeared to be lower in Group 2 compared to Group 1, this apparent difference was not statistically significant.

Mean LH levels in both groups (5.17 and 0.6 in Groups 1 and 2, respectively) were lower than those described for a natural cycle (21.5 ± 5.2) [10]. Similarly, LH pulse amplitudes in both groups (1.13 and 0.44 in Groups 1 and 2, respectively) were significantly lower than those described for a natural cycle (12.3 ± 2.2) [10].

E_2-levels in Group 1 is shown in Figure 1(A). The mean E_2-levels in Group 1 decreased significantly over 6 h from 5442 ± 907 to 3376 ± 612 pmol/L (38% decrease, $r = -0.95$, $p < 0.0001$). Decreasing E_2-levels over 6 h were also observed in Group 2; 3036 ± 481 pmol/L to 2642 ± 580 pmol/L (13% decrease, $r = -0.78$, $p < 0.01$), although the decrease was more moderate (Figure 1(B)).

Table 1. LH secretion parameters.

Parameter	Group 1	Group 2	*p*
Mean LH (IU/L)	5.2 ± 2.0	0.6 ± 0.1	0.049
Basal LH secretory rate (IU/L/min)	0.39 ± 0.036	0.0042 ± 0.0027	0.0001
No. of LH pulses	1.0 ± 0.7	1.2 ± 0.8	NS
Amplitude	1.13 ± 0.79	0.44 ± 0.44	NS

Data presented as mean ± standard deviation.

There was a modest apparent increase in mean P-levels over time in Group 1, from 17.14 ± 3.73 nmol/L to 18.80 ± 5.23 nmol/L (8% increase, $r = 0.53$, $p = 0.023$) (Figure 2(A)). Group 2 exhibited a significant decrease of P from 50.52 ± 8.97 nmol/L to 35.90 ± 6.25 nmol/L over 6 h (27% decrease, $r = -0.94$, $p < 0.00001$) (Figure 2(B)).

Discussion

The present study, in which a single dose of GnRHa was used to trigger ovulation, disclosed two primary findings of interest:

(1) Although pulsatile LH secretion continues, mean LH concentrations and LH pulse amplitude are lower than those described for a natural cycle.
(2) The process of luteolysis starts very early in the luteal phase as indicated by falling progesterone and estradiol levels 2 days after ovulation.

Maintenance of adequate corpus luteum function is dependent on pulsatile LH secretion. Filicori et al. [11], studied gonadotropin secretion in 51 normal menstrual cycles in 48 women and found that, in early luteal phase, average inter-pulse interval was 103 ± 8 min, average LH pulse amplitude was 12.3 ± 2.2 IU/L and mean LH concentration was 21.5 ± 5.2 IU/L [11]. In a similar study, LH pulses were detected every 90 min in the early luteal phase, and average LH pulse amplitude was 10.3 IU/L [15]. These published findings differ significantly from our results; in our study, the frequency of LH pulses ranged from 0–2 cycle in 6 h in the whole study group, and the pulse amplitude was lower. Moreover, mean LH levels were markedly lower, especially in Group 2. Importantly, since no luteal support was given to our patients until blood sampling was completed, corpora lutea function was completely dependent on endogenous LH secretion, which was skewed significantly from physiology

Patterns of LH secretion change throughout the normal luteal phase. In the early luteal phase, LH secretion is characterized by a relatively high frequency and high amplitude, but LH pulse frequency decreases to one pulse every 216 min in the mid-luteal phase. This presumably reflects the negative feedback actions of progesterone and estradiol, which peak in the mid-luteal phase [16]. In our study, the LH pulse frequency in the early luteal phase was similar to pulse rates previously reported in the mid-luteal phase of natural cycles, but mean LH and LH pulse amplitude were significantly lower. These differences in our study may reflect the depressive effect of early luteal supra-physiologic levels of progesterone and estradiol on LH secretion, and/or changes in endogenous GnRH secretion induced by the single bolus of GnRGa given to trigger final oocyte maturation.

The mechanism by which a single dose of GnRHa causes luteal phase deficiency has been debated for more than three decades. GnRHa induces an endogenous pituitary gonadotropin surge of LH and FSH, which is considered more physiologic compared to exogenous hCG trigger [5]. However, unlike the

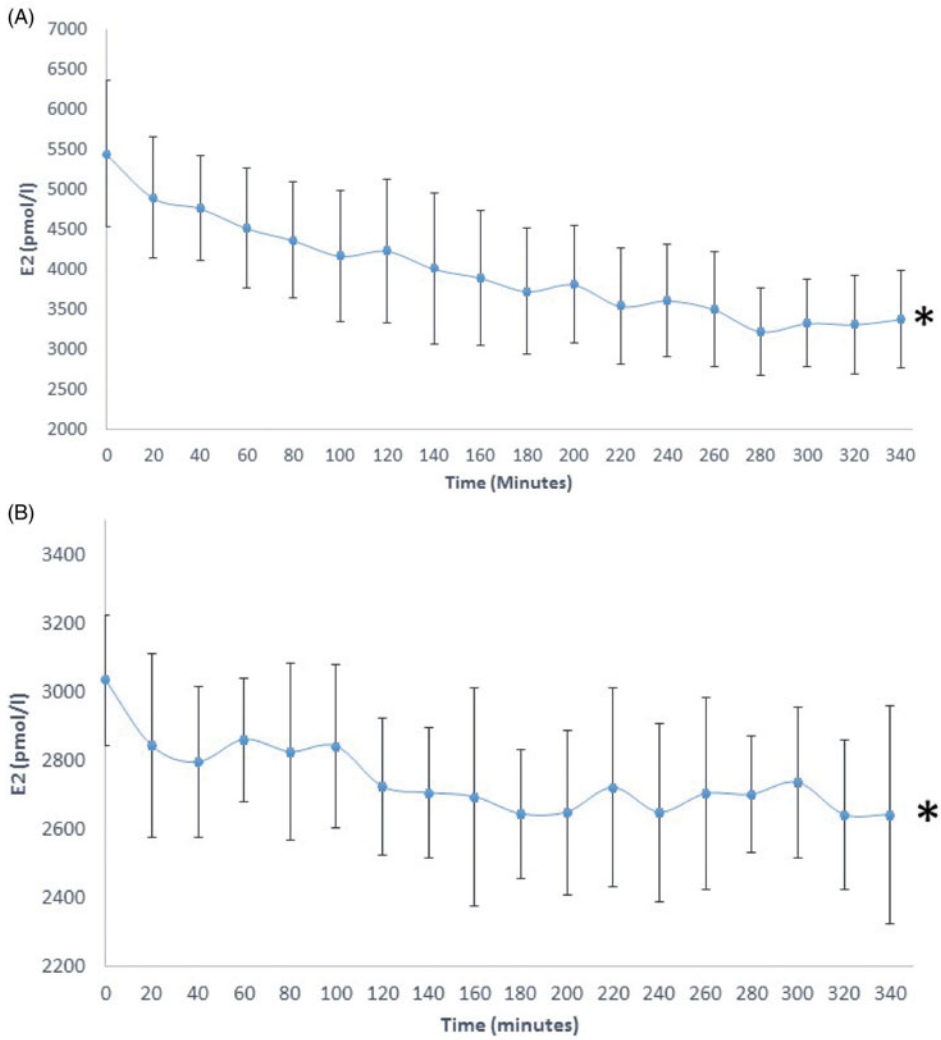

Figure 1. Plasma E_2-level change over time in Group 1 (A) and Group 2 (B). Data are expressed as mean ± SEM. *, Pearson correlation $p < 0.05$ for change of P-levels over time.

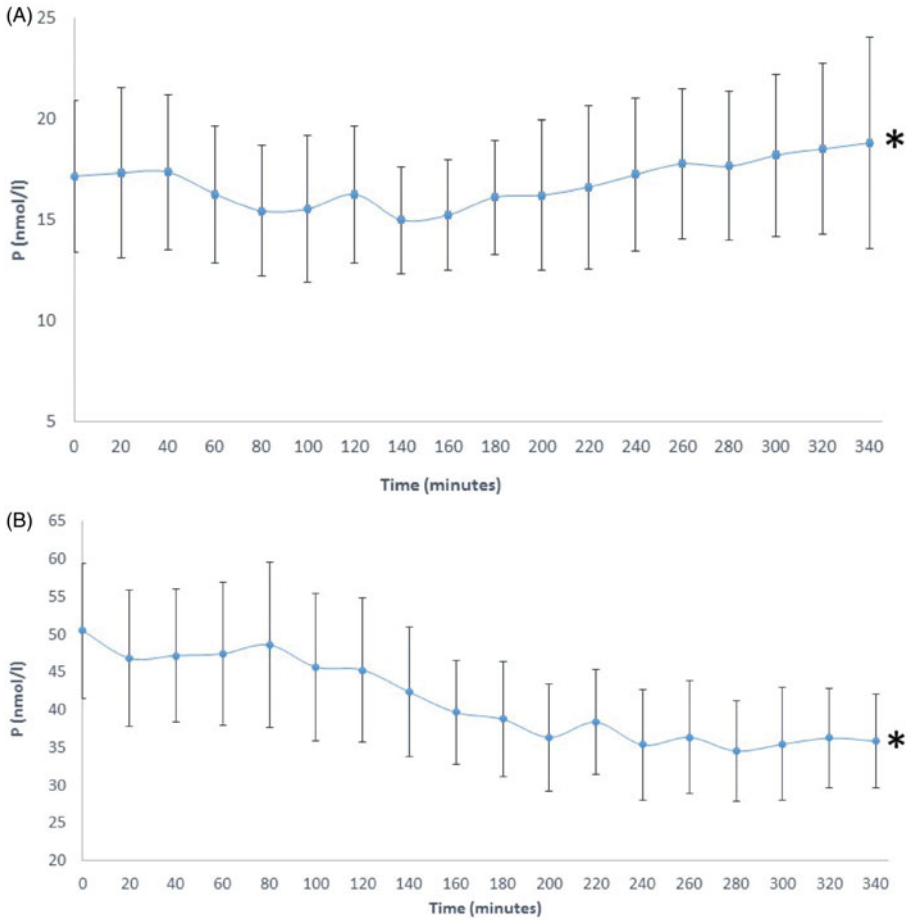

Figure 2. Plasma P-level change over time in Group 1 (A) and Group 2 (B). Data are expressed as mean ± SEM. *, Pearson correlation $p < 0.05$ for change of E_2-levels over time.

natural cycle LH surge that consists of three phases with a total duration of 48 h [17], GnRHa induced LH surge consist of two phases with a shorter duration of 24 h [3]. This notwithstanding, it is well-established that the GnRHa trigger is effective in producing mature oocytes, as well as early luteinization reflected by early increases in P-secretion (Figure 2(A)). Because of its short half-life, GnRHa is unlikely to have a long-term suppressive effect on the pituitary, or to have any stimulatory effect on the early luteal phase. Although, subtle changes in GnRH secretion pattern cannot be ruled out. This is in contrast to hCG trigger, which can stimulate the corpus luteum for several days.

Early clinical experience with GnRHa trigger in GnRH-antagonist cycles resulted in lower clinical pregnancy rate (6 versus 36%) and higher rate for early pregnancy loss (79 versus 4%) compared to hCG [18]. This and other similar studies highlighted the fact that good clinical outcomes following GnRHa trigger require high mid-luteal (implantation period) progesterone concentrations. Given complete (in most cases) luteolysis, high mid-luteal progesterone levels can be achieved by intensive E_2 and P supplementation [19], or a bolus of low dose hCG [20].

So far, different hCG-based luteal rescue strategies were reported: 1500 IU on the day of oocyte retrieval [20], three days later [21], or individually based ''luteal coasting'' [22]. Our study delineates a common change in LH secretion pattern as a possible luteolysis mechanism; thought luteolysis kinetics may vary between patients.

Interestingly, LH concentrations in the luteal phase after a GnRHa trigger are higher than in hCG triggered cycles; Humaidan et al. [18], found that the mid-luteal LH concentration was 1.5 IU/L compared to 6 IU/L in natural cycles [18]. Fatemi et al. [23], compared LH levels 5 days after triggering with GnRHa or hCG in GnRH-antagonist cycles and found that LH levels are higher (1.9 versus 0.3 IU/L) in the GnRHa group, which can be explained by higher gonadal steroid levels in the hCG group. These data suggest that resulting LH secretion is not sufficient to support the luteal phase. To our knowledge, no study so far addressed the minimal LH secretion required to support the corpus luteum.

The limitation of our study is the small sample size, 10 patients studied in two time points in the early luteal phase, and the lack of a control group. We also did not prove complete luteolysis in our patients, since all were treated with hCG to rescue the luteal phase in order to establish pregnancy. However, based on the available literature GnRH-agonist trigger leads to luteolysis in nearly 100% of cases. Indeed, when analyzing the change in hormone levels individually per patient, the decrease in P-level over time was significant in all five patients in Group 2, which implies that corpus luteum insufficiency started early in the whole group.

Our study is the first to explore the pattern of LH and gonadal steroid secretion following GnRHa trigger in the early luteal phase. We show that LH maintains its pulsatile secretion with reduced pulse frequency and markedly diminished pulse amplitude compared to normal physiology. We suggest that this can explain the mechanism by which early luteolysis ensues.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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