

Individualized Treatment from Theory to Practice: The Private Case of Adding LH during GnRH Antagonist-based Stimulation Protocol

Shahar Kol

IVF Unit Rambam Health Care Campus, Maccabi Healthcare Services and the Rappaport Faculty of Medicine, Technion Israel Institute of Technology, Haifa, Israel.

ABSTRACT: The study evaluated the proportion of patients whose pituitary glands respond with a sharp decrease in luteinizing hormone (LH) levels when exposed to a conventional dose of 0.25 mg gonadotropin releasing hormone (GnRH) antagonist in a prospective, single-center, non-randomized, proof-of-concept study. Fifty women eligible for in vitro fertilization (IVF) received recFSH (Gonal-F) from day 2 or 3 of menstrual period. Basal estradiol, progesterone, and LH were measured on the same day and 4–5 days later—immediately before GnRH antagonist 0.25 mg administration, and 24 hours after its administration. Responders were defined as “normal” if 24 hours after the first GnRH antagonist injection, LH level was $\geq 50\%$ of the pre-injection level and as “over-suppressed” if it was $< 50\%$ of the pre-injection level. Twelve patients (26% of the total) were “over-suppressed” with a mean LH level of 37% of the level 24 hours earlier. These patients also demonstrated a significant decrease in estradiol rise during the first 24 hours after initial antagonist administration. This effect was reversed for the rest of the stimulation period during which recLH (Luveris, 150 IU/day) was added to the “over-suppressed.” If proven advantageous in terms of pregnancy rate, this approach to individualized treatment would be easy to implement. Trial registration: ClinicalTrials.gov Identifier: NCT01936077.

KEYWORDS: GnRH antagonist, luteinizing hormone, assisted reproduction technology, ovarian stimulation, individualized treatment

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CORRESPONDENCE: skol@rambam.health.gov.il

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Introduction

The ideal ovarian stimulation protocol is under constant debate, as we gain more pharmacological control over the subject hormonal milieu. Specifically, the debate focuses around the ideal luteinizing hormone (LH) levels; hence, the concept of an “LH window” was suggested for adequate follicular development.¹ The need for a threshold LH level is clearly demonstrated in hypogonadotropic hypogonadism subjects² as well as in cycling subjects receiving high doses of gonadotropin-releasing hormone (GnRH) antagonists. The Ganirelix dose-finding study showed very low implantation rates in the high-dose groups (1 mg, 2 mg).³ In that study, the stimulation dynamics in LH-suppressed subjects was remarkable for very low estradiol (E_2)

and LH levels during the GnRH antagonist treatment period up to and including human chorionic gonadotropin (hCG) trigger day. In fact, a functional state of hypogonadotropic hypogonadism was achieved, explaining the poor clinical results (1.5% implantation rate in subjects treated with 2 mg Ganirelix).

Huirne et al⁴ showed that if the changes in LH levels during the antagonist administration period are too large, the chance of achieving a clinical pregnancy decreases. Hence, subjects with profound suppression of LH production, irrespective of the antagonist dose, did not achieve pregnancy. The concept of change over time as a significant hormonal milieu determinant, rather than the level at a given time point, was previously reviewed.^{5,6}



The recommended daily GnRH antagonist dose is 0.25 mg, which on average provides protection from premature LH surge, with moderate suppression of LH secretion. Therefore, most subjects do not need supplemented LH after the antagonist is initiated. Indeed, previous studies performed on a general subject population did not show any benefit in terms of clinical outcome when recombinant LH was added in parallel to the GnRH antagonist.⁷⁻¹⁰ Moreover, when subjects were stratified into specific time points during ovarian stimulation, again, there was no association between endogenous LH levels and clinical outcome.^{11,12}

A more individualized approach to the question of adding LH was suggested by Marrs et al¹³ who found that adding LH to subjects >35 years of age treated with the “long GnRH agonist” protocol is beneficial. However, when this approach was implemented in the GnRH antagonist-based stimulation setting, no benefit was documented.¹⁴

Studies that described dose-dependent antagonist pharmacodynamics and pharmacokinetics indicate that the immediate response to all doses of GnRH antagonists is a drop in LH levels, which is similar in its extent among all doses. However, a large difference in LH levels is observed for the pituitary recovery 24 hours later.^{3,15} While low antagonist doses allow a quick recovery to almost pre-treatment LH levels 24 hours after injection, high doses result in incomplete recovery.

These pharmacodynamics and pharmacokinetics considerations dictate that there must be a subgroup of subjects who hyper-respond to the antagonist when it is administered in the conventional 0.25 mg dose. Obeying a bell-shape curve, most women have an average response; however, “hypo-responders” might ovulate prematurely, while “over-suppressed” patients may behave as if they were exposed to a higher antagonist dose. Hence, it is reasonable to speculate that “over” response to a conventional 0.25 mg dose will lead to a slow or incomplete LH levels recovery 24 hours after the injection. LH levels in high Ganirelix doses (1 mg, 2 mg) 24 hours after the injection were <50% of the pre-injection LH levels.³ Therefore, this cut-off level was used in this study.

The basic hypothesis of this study is that there is a wide range of pituitary responses to GnRH antagonists. If indeed we wish to apply an “individualized approach” to the question of adding LH during antagonist-based stimulation, as suggested by Griesinger and Diedrich,¹⁶ we must specifically identify those subjects whose pituitary glands are “over-suppressed” when exposed to the 0.25 mg antagonist dose. Therefore, the primary objective of this study was to find the frequency of GnRH antagonist “over-suppression,” and to assess if subjects defined as antagonist “over-suppressed” may benefit from supplemental LH.

Material and Methods

Study design and setting. The study was a prospective, single center, non-randomized, proof-of-concept study.

Patient recruitment, ovarian stimulation (up to and including trigger day), and pregnancy follow-up were performed in a community fertility center (Maccabi Healthcare Services, Women Health Center, Haifa, Israel). This center performs general infertility work-up. If patients are found to need in vitro fertilization (IVF), they are referred to IVF units of their choice. Patients who chose to have their IVF procedure performed either in a private IVF clinic (Elisha Hospital, Haifa, Israel), or in a public, university-affiliated, tertiary medical center (Rambam Health Care Campus, Haifa, Israel), were asked to enter the study after a thorough explanation of its purpose and procedures.

Patient population. From July 2010 to June 2013, 50 patients eligible for IVF or intracytoplasmic sperm injection (ICSI) treatment were recruited. All patients were under 39 years of age, had spontaneous regular cycles, and had body mass index (BMI) under 32 kg/m². Patients with polycystic ovarian syndrome were excluded. Also excluded were patients with ovarian, uterine, or mammary cancer; tumors of the hypothalamus and pituitary gland; uterine myoma requiring treatment; ovarian enlargement or cyst of unknown etiology; a clinically significant systemic disease; abnormal gynecological bleeding of undetermined origin; and known allergy or hypersensitivity to human gonadotropin preparations. Patients were thoroughly informed about the study and all signed informed consent prior to recruitment.

The study was approved by the ethics committee of Maccabi Healthcare Services and was registered at ClinicalTrials.gov Identifier: NCT01936077. The research was conducted in accordance with the principles of the Declaration of Helsinki.

Ovarian stimulation protocol. Ovarian stimulation was started on day 2 or 3 of a spontaneous menstrual period with recombinant follicle-stimulating hormone (FSH) (follitropin alfa; Gonal-F®, Merck Serono, Herzliya, Israel). The starting dose was determined based on the subject's age, weight, antral follicular count (AFC), and previous exposure to gonadotropins (if available). On the same day, a blood test was performed for measuring basal E₂, progesterone, and LH. A repeated blood test was performed in the morning, 4 or 5 days later, and cetrorelix 0.25 mg (Cetrotide®, Merck Serono, Herzliya, Israel) was given subcutaneously immediately after blood was withdrawn.

Vaginal ultrasound was done to document follicular recruitment and growth. Twenty-four hours after the first cetrorelix injection, another blood test was performed for E₂, progesterone, and LH. If the LH level was less than 50% of the level measured 24 hours earlier, the subject was defined as “over-suppressed” to 0.25 mg cetrorelix, and a daily dose of 150 units of recombinant LH (lutropin alpha; Luveris®, Merck Serono, Herzliya, Israel) was added from that day (in parallel to follitropin alfa) until ovulation trigger. Final oocyte maturation was induced by choriogonadotropin alfa 250 µg (Ovitrelle®, Merck Serono, Herzliya, Israel) or by triptorelin 0.2 mg (Decapeptyl®, Ferring Pharmaceuticals Pvt. Ltd., Caesarea,



Israel) in subjects at risk for ovarian hyperstimulation syndrome (OHSS). Oocyte retrieval was performed 35 hours after the trigger dose, and the embryos were transferred 2–3 days later. Daily vaginal progesterone (Crinone® 8%, Fleet Laboratories Ltd., Herts, UK) was given as luteal support after choriogonadotropin alfa triggering. Luteal support after triptorelin trigger was a single bolus of hCG 1,500 IU on oocyte retrieval day, followed by daily Crinone® 8%.

Definitions of responders to cetrorelix. The study focused on the degree of pituitary LH secretion suppression following exposure to cetrorelix. In that context, a “normal responder” was defined if LH level 24 hours after the first cetrorelix injection was $\geq 50\%$ of the immediate pre-injection LH level. An “over-suppressed” patient was defined if the LH level 24 hours after the first cetrorelix injection was $< 50\%$ of the immediate pre-injection LH level.

Study endpoints. The study primary endpoint was the proportion of “over-suppressed” patients as defined above. The secondary endpoints were the E_2 level increment per retrieved oocyte during 24 hours following the initial cetrorelix dose; the E_2 level increment per retrieved oocyte during the remaining ovarian stimulation days, including trigger day; and the ongoing pregnancy rate.

Data analysis. The normality of the quantitative parameters was tested by Kolmogorov–Smirnov test.

As some of the parameters were not normally distributed, Mann–Whitney U test was used; otherwise, we used t -test to analyze differences between the two groups (normal responders vs. over-suppressed patients).

Fisher’s exact test was used for differences between categorical parameters.

Repeated measure analysis was conducted to determine whether there was a statistical significant between the two groups (normal responders vs. over-suppressed) in order to understand the change in E_2 before cetrorelix (pmol/L) and 24 hours after cetrorelix (pmol/L).

The logistic regression model was used for prediction clinical pregnancy with several independent parameters.

$P < 0.05$ was considered as significant.

Statistical analysis was evaluated by SPSS software, version 21 (SPSS Inc., Chicago, IL, USA).

Results

Fifty subjects were included in the study. Of these, 1 conceived spontaneously and 3 dropped out for personal reasons; therefore, 46 subjects completed the study. The mean (\pm SD) age was 30.8 ± 3.7 years, and the mean BMI was 21.5 ± 2.9 kg/m². The mean duration of infertility was 33 months (Table 1).

Response to ovarian stimulation. The ovarian stimulation characteristics are described in Table 2. Twelve subjects (26.1%) were defined as “over-suppressed.” In this group, the mean LH level after the first cetrorelix injection was 37% of the LH level 24 hours earlier. Thirty-four subjects (73.9%) were defined as “normal responders.” In this group, the mean LH level after the first cetrorelix injection was 70% of the LH level 24 hours earlier. Prior to cetrorelix administration, E_2 and progesterone levels were similar in both groups (Table 2). Mean immediate pre-antagonist LH levels were 3.05 and 2.08 in the “over-suppressed” and “normal responders” groups, respectively ($P = 0.051$). As expected, LH levels dropped sharply in the “over-suppressed” group 24 hours after the first cetrorelix injection by a mean of 63% compared to a mean decrease of 29.5% in the “normal responders” group ($P < 0.001$).

Estradiol-level increment per retrieved oocyte during 24 hours after administration of the initial cetrorelix dose.

In order to assess the effect of the decrease in LH levels on E_2 biosynthesis, its increment during the first 24 hours after exposure to cetrorelix was normalized to the number of oocytes retrieved. The results show that in the “normal responders” group, mean per-oocyte E_2 increased by 57.7 pmol/L, while the corresponding value in the “over-suppressed” group was only 25.4 pmol/L ($P = 0.009$; Table 2).

Estradiol-level increment per retrieved oocyte during the remaining ovarian stimulation days. The “over-suppressed” group was supplemented with exogenous LH (lutropin alpha) as described in the Material and methods section. The E_2 increment from that time point (24 hours after the first cetrorelix dose) to the triggering day was measured,

Table 1. Subjects baseline characteristics.

	ALL SUBJECTS	OVER SUPPRESSED n = 12 MEAN \pm SD	NORMAL RESPONDERS n = 34 MEAN \pm SD	P^a
Age (years)	30.8 \pm 3.7	32.0 \pm 2.5	30.2 \pm 4.1	0.17
BMI (kg/m ²)	21.5 \pm 2.9	20.6 \pm 1.9	21.9 \pm 3.2	0.23
Infertility duration (months)*	33 (19.8–42)	39 (24.3–60)	30 (18.8–36.8)	0.16
Basal E_2 (pmol/L)	179 \pm 48	169 \pm 46	180 \pm 49	0.51
Basal P (nmol/l)	3.0 \pm 1.5	2.7 \pm 0.9	2.8 \pm 0.9	0.61
Basal LH (IU/l)	5.9 \pm 2.7	7.0 \pm 2.6	5.5 \pm 2.7	0.09

Notes: ^a P value by Student’s t -test. *Statistical significant by Mann–Whitney U test (median, 25%–75% Interquartile).

Abbreviations: SD, standard deviation; BMI, body mass index; E_2 , estradiol; P, progesterone; LH, luteinizing hormone.

Table 2. Comparison of ovarian stimulation parameters between the responder groups.

	OVER SUPPRESSED n = 12 MEAN ± SD	NORMAL RESPONDERS n = 34 MEAN ± SD	P ^c
E ₂ before cetorelix (pmol/L)	2138 ± 1500	1749 ± 736	0.24
*P before cetorelix (nmol/l)	1.57 (1.19–2.4)	2.36 (1.9–3.0)	0.053
*LH before cetorelix (IU/l)	3.05 (1.5–5.8)	2.08 (1.40–2.80)	0.051
E ₂ 24 hours after cetorelix (pmol/L)	2452 ± 1755	2384 ± 1021	0.88
*P 24 hours after cetorelix (nmol/l)	1.60 (1.19–2.09)	2.40 (1.81–3.48)	0.006
*LH 24 hours after cetorelix (IU/l)	1.1 (0.8–1.55)	1.5 (1.07–2.01)	0.11
*Decrease in LH levels 24 hours after cetorelix (%)	63 (55.3–79.0)	29.5 (14.5–33.0)	<0.0001
*E ₂ increment per oocyte first 24 h ^a (pmol/L)	25.4 (4.04–51.05)	57.7 (27.8–102.1)	0.009
E ₂ trigger day (pmol/L)	6571 ± 3678	5454 ± 2436	0.25
E ₂ increment per oocyte total ^b (pmol/L)	428.4 (155.5–704.5)	276.5 (185.4–394.2)	0.29
Total FSH dose (units)	1703 ± 452	1597 ± 467	0.5
Endometrial width on triggering day (mm)	9.7 ± 1.7	9.4 ± 2.1	0.66

Notes: ^aE₂ level 24 hours after the first cetorelix dose, minus E₂ before the first cetorelix dose divided by oocytes retrieved. ^bE₂ level on trigger day minus E₂ level 24 hours after the first cetorelix dose divided by oocytes retrieved. ^cP value by Student's *t*-test. *Statistical significant by Mann–Whitney *U* test (median, 25%–75% Interquartile).

Abbreviations: SD, standard deviation; E₂, estradiol; P, progesterone; LH, luteinizing hormone; FSH, follicle stimulating hormone.

and the result was normalized per retrieved oocyte. Mean E₂ increment per-oocyte for this time interval was 428.4 pmol/L in the “over-suppressed” group and 276.5 pmol/L in the “normal responders” group (*P* = 0.29; Table 2).

The total FSH unit consumption in the two groups was comparable, as was endometrial width on the day of triggering (Table 2).

Ongoing pregnancy rate. The IVF treatment outcome is described in Table 3. Oocyte number, fertilization rate, embryos transferred, embryos frozen, and clinical pregnancy rates were all comparable between the two groups.

Discussion

It is widely accepted that to secure the best clinical results of assisted reproductive technology, an individualized approach is required.^{17–19} It makes sense, therefore, to determine the need for exogenous LH during GnRH

antagonist-based ovarian stimulation, based on specific patient characteristics. The results of the current study show that 26% of the patients hyper-responded to 0.25 mg of the GnRH antagonist, cetorelix. Administration of exogenous LH to these patients led to an increase in E₂ increment per-oocyte retrieved.

De Placido et al²⁰ examined the GnRH agonist long protocol and found that in 12–14% of down-regulated subjects, the initial response to FSH is suboptimal in terms of follicular growth and E₂ rise. They suggested that these subjects should be candidates for LH supplementation. Data showed that mean LH concentrations of the “normal responders” increased from 1.5 IU to 4.3 IU after 8 days of stimulation, while the mean LH concentration in the suboptimal responders decreased from 1.2 IU to 0.7 IU during the same time period. Although the study did not focus on these changes, the data suggested that the follicular unit is not necessarily

Table 3. Comparison of IVF treatment outcome between the responder groups.

	OVER SUPPRESSED n = 12	NORMAL RESPONDERS n = 34	P
*No. of oocytes	8 (6.5–12.5)	10.5 (6.8–15)	0.48
*Fertilization rate	81.5 (67–87)	66 (40.8–83.8)	0.27
*No. of embryos obtained	6.5 (4.0–9.8)	4 (3.0–7.3)	0.18
*No. of embryos transferred	2 (1.3–2.0)	2 (2–2.25)	0.054
*No. of embryos frozen	4.5 (2–8)	3 (0–5.5)	0.15
Clinical pregnancy rate, n (%)	6 (50)	10 (29)	0.29 ^b

Notes: ^bFisher's exact test. *Statistical significant by Mann–Whitney *U* test (median, 25%–75% Interquartile).

sensitive to the current concentration of LH, but rather to the dynamic changes in these concentrations. The current study offers a similar approach to this question for the antagonist-based protocol.

In a natural cycle, E_2 biosynthesis obeys a pre-set tide to coincide with follicular growth and ovulation.²¹ Theca cell-derived, LH-dependent, aromatizable androgens (mainly androstenedione) are used to produce E_2 by FSH-induced granulosa cell aromatase activity. The extent of aromatase activity is limited by the amount of precursor available, which in turn depends on LH levels. In a natural cycle, LH levels are more or less constant during the follicular phase,²² allowing for a sufficient supply of androgens; for a continuous rise in E_2 levels, determined by the growing number of granulosa cells in the dominant follicle; and a parallel increase in aromatase activity. We hypothesized that a sharp drop in LH causes a sudden decrease in precursor availability, whereas the complex system that holds a delicate balance cannot adjust to abrupt changes. The result is insufficient E_2 production by the growing follicles, manifested in a drop or plateauing in circulating E_2 levels.

In “long” agonist-based, pituitary down-regulation ovarian stimulation, LH levels are low, but with minimal fluctuations. Since it typically takes about 2 weeks from the start of agonist treatment to ovarian stimulation, E_2 production mechanism has enough time to adjust to down-regulated LH levels. In these subjects, unless LH is completely eliminated, a steady rise in E_2 levels during stimulation is observed that depends on exogenous FSH supplied to the system. Theoretically, LH levels themselves are of less importance, as long as fluctuations are minimal. In contrast, in antagonist-based cycles, following a mild decrease in LH level during the first 5 days of stimulation, a sudden antagonist-mediated LH drop leads to depleted E_2 biosynthesis.³ We therefore suggest that the sharp drop in LH level is clinically significant, rather than the absolute level itself.

Based on the lesson learnt from the Ganirelix dose-finding study,³ it is suggested that about one-quarter of subjects treated with the antagonist-based protocol behave as if they are exposed to a higher antagonist dose. These are the subjects that should be identified, since they may benefit from additional LH.

High LH levels just before the first antagonist dose administration may predispose a subject to a sharp decrease in LH 24 hours later (over-suppressed). This, in turn, leads to suboptimal E_2 production during these 24 hours compared to “normal responders” (Table 2). However, the LH-starved system quickly recovers with exogenous LH, resulting in accelerated E_2 production that even surpasses that of the “normal responders” E_2 rise dynamics. Further research is needed to examine if this individualized approach can improve the pregnancy rate. Our study was not powered to assess this outcome, although our preliminary findings are positive in that regard.

In summary, we suggest a simple, objective test to assess individual response to a GnRH antagonist. The results show that 26% of our subjects were over-suppressed, and therefore, may be candidates for LH supplementation.

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Author Contributions

Conceived and designed the experiments: SK. Analyzed the data: SK. Wrote the first draft of the manuscript: SK. Contributed to the writing of the manuscript: SK. Agree with manuscript results and conclusions: SK. Jointly developed the structure and arguments for the paper: SK. Made critical revisions and approved final version: SK. All authors reviewed and approved of the final manuscript.

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