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The Importance of Mid-Follicular Phase Luteinizing Hormone Rise in GnRH Antagonist-Based Ovarian Stimulation for IVF

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Ovarian stimulation · Luteinizing hormone · IVF · Ovarian response · GnRH antagonist

Abstract
Introduction: Previous publications examined the endocrinology of follicular stimulation, focusing on luteinizing hormone (LH) levels changes. In selected, good prognosis IVF patients, a sharp drop in LH serum level was demonstrated between cycle days 2 and 6. ***Objective:*** The purpose of this study was to examine if this finding holds true for unselected patients. ***Methods:*** We retrospectively included 165 consecutive patients treated with a GnRH antagonist-based ovarian stimulation protocol during the year 2015. ***Results and Conclusions:*** In 33% of the patients an increase in LH, rather than the expected decrease, was demonstrated after 5 stimulation days. There was no difference in pregnancy outcome. Our results suggest that an increase in LH levels during ovarian stimulation occurs mainly in "high responders", or "low responders". LH rise in mid follicular phase may result in a sharp LH drop once a GnRH antagonist is given, and the possible need for LH supplementation. © 2020 S. Karger AG, Basel

Introduction

Luteinizing hormone (LH) plays an important physiologic role during the follicular phase. It assures proper estradiol (E_2) production, promotes selection of the dominant follicle, and contributes to oocyte maturation. LH levels increase progressively during the natural follicular phase [1] that ends with a sharp and rapid LH surge, the biochemical signal for final oocyte maturation and ovulation.

The follicular phase endocrine characteristics during ovarian stimulation with GnRH antagonist co-treatment were thoroughly studied. Focusing on LH levels during the first half of the follicular phase, a sharp drop in its serum level was demonstrated between cycle days 2 and 6 [2]. On cycle day 2 mean LH level was 5 IU/L, dropping to 1.7 IU/L 4 days later, before GnRH antagonist was given. This study examined selected, good prognosis patients obeying very restrictive criteria (first treatment cycle of in-vitro fertilization (IVF); age between 18 and 36 years; body mass index between 18 and 29 kg/m^2; history of regular menstrual cycle [25–35 days]; normal follicle stimulating hormone (FSH) serum levels on cycle day 2 [<12 U/L]; no major uterine or ovarian abnormalities; no en-

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docrine or metabolic abnormalities; no polycystic ovary syndrome; and no severe endometrioses). Similar results were reported in other publications, using comparable inclusion criteria [3]. In summary, the "normal" response to 5 days of gonadotropin stimulation is a drop in LH level.

While the above studies represent a sub-set of "model" patients, there is an interest to investigate if these findings hold true for "real world" patients. Based on our previous publication, only 37% of the "real world" patients meet the inclusion criteria as defined by 9 clinical trials [4]. Importantly, in the era of individualized treatment, attention must be given to those patients whose LH level increases during the first half of the ovarian stimulation follicular phase, before a GnRH antagonist is given. Based on previous publication, there is a direct correlation between the LH levels just before GnRH antagonist administration and the magnitude of drop in LH level after [5]. Therefore, an increase in LH levels (rather than the expected decease), during the first half of ovarian stimulation, could be associated with a sharp decrease in LH immediately after GnRH antagonist administration, and a need to offset this decrease with LH supplementation.

The purpose of the current study is to assess the frequency of this "abnormal" LH dynamics in un-selected IVF patients. We sought to determine the percentage of patients demonstrating an increase in LH serum level from start day of stimulation to mid-follicular phase, just before GnRH antagonist is given.

Materials and Methods

We included 165 consecutive patients treated with a GnRH antagonist-based ovarian stimulation protocol during the year 2015.

Inclusion Criteria

Known hormonal levels (E_2, Progesterone, LH) on the day gonadotropin treatment was initiated, and 5 days later, before GnRH antagonist was given.

Exclusion Criteria

Patients with missing data were excluded from the study.

Protocol

Controlled ovarian stimulation was performed with a GnRH antagonist protocol. Cycles were monitored according to the policy of the clinic. Menopur (Ferring, Saint Apex, Switzerland) was used for ovarian stimulation, at a dose decided by individual patients' characteristics.

Baseline hormones (E_2, progesterone, LH) measurement was obtained in the morning of the first Menopur administration. Menopur dose was kept constant for 5 days, after which repeated hormones measurement was obtained before adding GnRH antagonist.

Table 1. Baseline characteristics in the study and control groups

	Group 1 (n = 110)	Group 2 (n = 55)	p value
Age, years, mean ± SD	33.5±6.3	32.8±7.0	0.5
BMI, mean ± SD	25.5±5.1	25.8±5.1	0.8
Etiology of infertility, %			0.3
Male factor	30.3	30.9	
Mechanical	11.0	18.2	
Unexplained	48.6	45.5	
Combined	10.1	3.6	
Primary	0	1.1	
Fertilization procedure, %			0.9
ICSI	54.1	52.7	
IVF	45.9	47.3	
Bassline FSH, IU/L, mean ± SD	7.0±3.0	7.2±3.5	0.7
AFC, mean ± SD	10.3±5.5	10.6±5.6	0.7

BMI, body mass index; AFC, antral follicular count.

Ovulation trigger (Decapeptyl 0.2 mg, Ferring, or Ovitrelle 250 μg, Merck, Darmstadt, Germany) was administered as soon as 3 leading follicles reached ≥17 mm in diameter, oocyte retrieval was performed 34–36 h later. Oocytes were fertilized with conventional IVF or intra-cytoplasmic sperm injection (ICSI), according to individual patient criteria.

Twenty-two women were excluded from the pregnancy outcome statistics (11 women from each group) because embryos transfer was not done (e.g., oocytes/embryos cryopreservation for fertility preservation, no oocytes retrieved, and failed fertilization).

Statistical Analysis

The association of the groups (groups 1, 2) and outcome (live birth, miscarriage) was examined using Pearson chi-square. The comparisons between groups and other continuous data were done using independent samples t test and the comparisons of groups and other categorical data were done using Pearson chi-square.

Significance was set at $p < 0.05$ for all tests.

Statistical analysis was done using SPSS software package (Release 24.0.0.0, SPSS Inc., Chicago, IL, USA, 2011).

The Rambam Health Care Campus IRB approved the research (0061-18).

Results

Of the 165 patients, in 110 patients (67%, group 1) an LH decrease was documented between days 1 and 5 of ovarian stimulation, as expected. In 55 patients (33%), an increase in LH was noted during the same period (group 2). Table 1 shows the basic demographic parameters of the two groups.

Two patients were not included in group 2, although a sharp increase in LH was demonstrated because the cycles were aborted due to no ovarian response.

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Table 2. Hormonal levels, stimulation parameters and cycles outcome in the study and control groups

	Group 1 (n = 110)	Group 2 (n = 55)	p value
Number of oocytes retrieved	8.4±5.7	9.42±6.6	0.3
Number of fertilizations	4.7±3.4	5.0±4.0	0.6
Number of embryos obtained	3.4±2.6	3.3±2.7	0.8
Number of embryos transferred	1.6±0.8	1.5±0.9	0.3
LH_1, IU/L	6.6±6.9	3.6±2.5	<0.001
LH_2	3.0±2.2	7.0±8.1	0.001
P_1, nmol/L	1.7±0.9	1.8±1.2	0.4
P_2	1.7±0.8	2.0±1.0	0.03
E_1, pmol/L	358.8±336.6	434.7±279.1	0.2
E_2	1,737.7±1,315.9	2,788.3±1,752.3	<0.001
Total number of stimulation days	9.2±2.6	8.8±1.8	0.3
Sum of FSH units used in stimulation	2,080.6±745.0	1,991.2±746.3	0.5
Pregnancy outcome for patients with embryo transfer, %			
Live birth	28.3	27.3	0.5
Miscarriage	4	6.8	

Values are represented by mean ± SD.
LH_1, P_1, E_1: hormonal levels on day 1 of stimulation.
LH_2, P_2, E_2: hormonal levels after 5 days of stimulation.
LH, luteinizing hormone.

Table 2 shows the hormonal variables and FSH consumption during ovarian stimulation. Of note, baseline LH in group 1 (6.6 IU/L, comparable to 5 IU/L in Blockeel et al. [2]), was higher than that in group 2 (3.6 IU/L). E_2 levels after 5 stimulation days were higher in group 2.

Figures 1 and 2 depict the distribution of oocytes retrieved in groups 1 and 2 respectively. While the distribution in group 1 obeys the normal distribution we usually see with IVF patients [6], Group B shows a very wide scatter of oocytes number. It seems that an increase in LH levels during ovarian stimulation occurs mainly in "high responders", or "low responders".

Discussion

In most patients, ovarian stimulation with gonadotropin is associated with a decrease in LH secretion during the first 5 days of stimulation. However, according to our study, in 33% of patients LH level on the fifth stimulation day is higher than the LH level just before stimulation begins (day 2 of cycle). Our study suggests that this group of patients is actually comprised of 2 distinctly different sub-populations: hyper-responders and hypo responders to ovarian stimulation.

Of note, Menopur was used for ovarian stimulation. Its LH activity is derived mainly from its hCG content, yet it may cause a bias in term of affecting the hypothalamus – pituitary-ovarian axis. A more appropriate agent would be recombinant FSH.

Hosts of players of which two play a more significant role govern pituitary LH secretion during ovarian stimulation: E_2 and ovarian gonadotrophin surge-attenuating factor (GnSAF) [7, 8]. In the natural ovulatory cycle, a rapid rising E_2 level (about 1,000 pmol/L) secreted by the dominant follicle is the cue for pituitary LH surge and ovulation. In most patients, comparable E_2 levels are reached after 5 stimulation days [2]; however, LH secretion is decreased given the dominant influence of GnSAF secreted by the small and developing follicles. This hormonal balance if offset in two situations: excessive E_2, or diminished GnSAF secretion.

In "high responders", the sharp E_2 rise probably overrides the inhibitory effect induced by GnSAF, resulting in increased LH secretion.

In the hypo-responders group, a rather modest rise in E_2 is enough to cause LH rise probably because of diminished GnSAF secretion from few small and developing follicles. In addition, this group tends to be older, with diminished oocyte and follicular quality leading to diminished GnSAF secretion. This may also explain the fact that in elderly women ovulation typically occurs at shorter dominant follicle diameter, and lower E_2 level.

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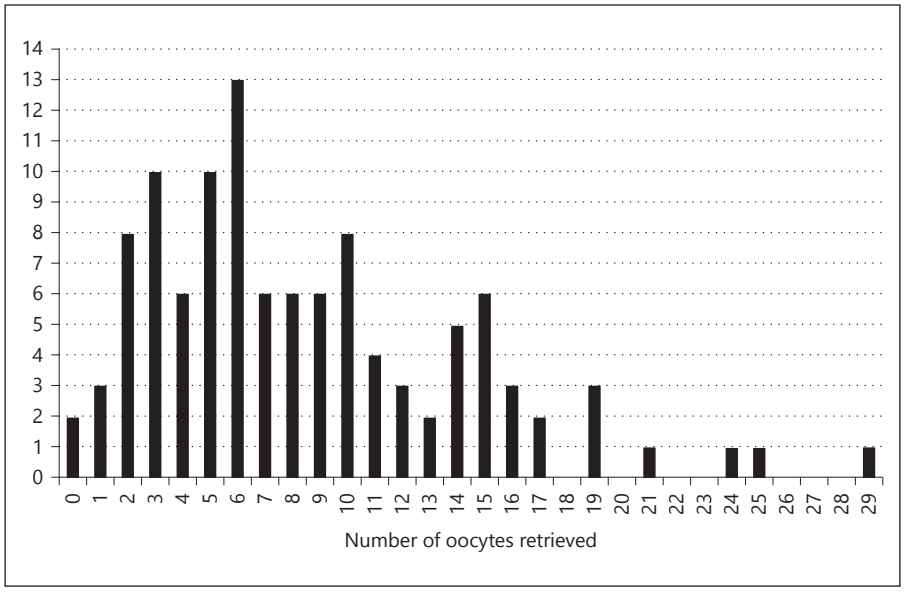

Fig. 1. Group 1: number of patients per oocytes retrieved.

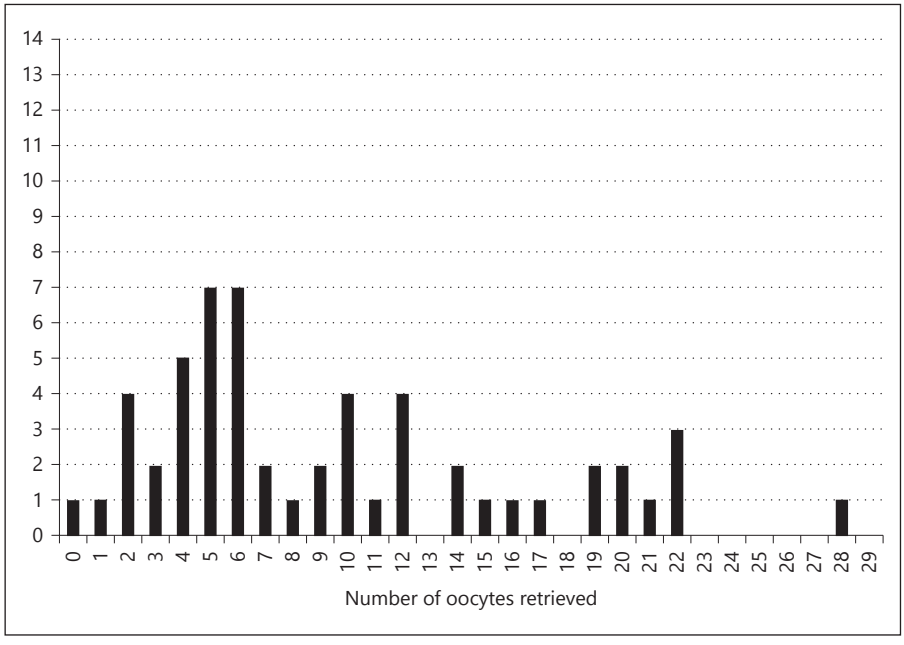

Fig. 2. Group 2: number of patients per oocytes retrieved.

Based on previous publication [5], it is important to emphasize that there is a direct correlation between LH level just before antagonist administration and the degree of LH drop 24 h later. In other words, those patients who demonstrate an increase in LH levels 5 days into stimulation (33% of our patients), are prone to experience a sharp drop in LH when the GnRH antagonist is given. We speculate that the use of LH activity bearing ovarian stimulant (Menopur) shielded the system from potential LH drop. Such a shield may not be available if recombinant FHS only is used.

Routine LH measurement does not necessarily reflect residual LH bioactivity in various clinical conditions with increased and decreased gonadotropin secretion [9]; however, from the clinical point of view, ART cycles are conducted by the available measurements.

In summary, to the best of our knowledge, this study describes, for the first time, the "irregular" behavior of LH

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secretion mode during the first 5 days of ovarian stimulation. LH rising levels may reflect either over- or diminished -ovarian response. LH rise in mid-follicular phase may result in a sharp LH drop once GnRH antagonist is given, and the possible need for LH supplementation.

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Author Contributions

S.V.: collected and recoded patients data, took part in manuscript writing. R.B.-F.: took part in writing the manuscript. L.S.: analyzed data and performed statistics. S.K.: conceived the manuscript objectives, and took part in manuscript writing.

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