

PROGESTERONE/ESTRADIOL RATIO IN THE LATE FOLLICULAR PHASE OF LONG GONADOTROPIN-RELEASING HORMONE AGONIST CYCLES DID NOT DIFFER BETWEEN CONCEIVED AND NOT-CONCEIVED WOMEN

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Abstract- There is a challenging debate on the effect of premature luteinization on the clinical outcome of 'controlled ovarian hyperstimulation' (COH) using long 'gonadotropin-releasing hormone agonist' (GnRHa) cycles. Premature luteinization is defined as late follicular progesterone/estradiol ratio more than 1 on the day of human chorionic gonadotropin (HCG) administration. We carried out a retrospective case-control study on 75 conceived cases versus 75 not-conceived control women, receiving long GnRHa cycles in their first cycle of treatment. Premature luteinization developed in 15% of the case group vs. 22% of the control group. Neither the late follicular progesterone/estradiol (P/E₂) ratio was significantly different between the two groups, nor the day 3 follicle stimulating hormone (FSH), serum estradiol level on the HCG day, total amount of human menopausal gonadotropins ampoules, number of follicles, retrieved oocytes and transferred embryos. Endometrial thickness was significantly more in the pregnant women than in the non-pregnant group. Premature luteinization seems not to adversely affect the clinical outcome of COH.

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INTRODUCTION

In in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles, controlled ovarian hyperstimulation (COH) using clomiphene citrate (CC) and/or gonadotropins has been replaced by gonadotropins plus gonadotropin-releasing hormone agonist (GnRHa) because the former protocol is

associated with a 20%–25% chance of premature luteinizing hormone (LH) surge (1).

Controversies on the complicated pathogenesis of premature luteinization in long GnRHa cycles has induced a challenging debate among several investigators in the past decade (2-14). Because the long GnRHa protocol ensures prevention of premature LH elevation in 95%–98% of patients (15,16), it seems unsatisfactory to invariably blame the increased preovulatory LH levels as the sole pathogenic factor of premature luteinization. Investigators have linked the appearance of premature luteinization in long GnRHa cycles with lower (3,4,8,10,12,13), higher (6,14), or similar

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(2,5,7,9,11) quality of oocyte and embryo or pregnancy rates when compared to cycles without premature luteinization (17).

Copperman and colleagues (18) comparing women with and without premature luteinization during GnRHa plus human menopausal gonadotropins (HMG) in IVF cycles, have reported higher serum human chorionic gonadotropin (HCG) levels in women who experienced subtle rise of serum progesterone (P), suggesting that premature luteinization, despite pituitary suppression with GnRHa may be due to the HCG content of HMG.

Ubaldi and colleagues (11) analyzed the clinical outcome of ones who underwent COH using GnRHa plus recombinant follicle stimulating hormone (rFSH), or urinary FSH (uFSH) for IVF–Embryo Transfer (ET) to assess whether the HCG content of HMG could be one possible cause of subtle rise of progesterone.

Based on the higher exposure of FSH and its correlation with higher levels of progesterone, they concluded that one of the possible factors inducing premature luteinization is the increased FSH–induced LH receptivity in granulosa cells and no adverse effects of premature luteinization on the clinical outcome were observed.

Premature luteinization was defined as late follicular progesterone/estradiol (P/E_2) ratio more than 1 on the day of HCG administration in long GnRHa cycles (19).

Johnny S. Younis and colleagues reported premature luteinization could be related to low ovarian reserve and this manifestation is not necessarily an LH–dependent event and seems to adversely affect clinical outcome (17).

Hofmann and colleagues failed to show such an association with the serum P/E_2 ratio on the day of HCG administration and diminished ovarian reserve in women stimulated with HMG for intrauterine insemination (IUI) or IVF as demonstrated by the clomiphene citrate challenge test, or pregnancy outcome (20).

However, in a recent study, Hasan Tayfun Ozcakir and colleagues mentioned that in their study, premature luteinization defined as P/E_2 more than 1 on HCG administration day in long GnRHa cycles, seems to adversely affect clinical outcome (21). This

study is conducted to assess the advantages of administration of a GnRHa during COH.

MATERIALS AND METHODS

This was a retrospective case–control study. Based on previously published studies (17), assuming a 15% frequency rate of premature luteinization ($P/E_2 > 1$) among women receiving COH, the odds ratio of 3, $\alpha = 0.05$, and $\beta = 0.2$, there were 75 women required in each of the cases and controls groups to detect an absolute 20% difference in the premature luteinization rate.

The inclusion criteria included serum FSH < 15 mIU/mL and serum estradiol < 50 pg/mL. The 75 cases who met these criteria were retrospectively selected among all the eligible infertile women who were conceived via their first cycle of assisted reproductive techniques (ART) referred to the IVF Unit of the Obstetrics and Gynecology Department, Dr. Shariati Hospital, Tehran University of Medical Sciences, between 13 April 2002 and 19 March 2003.

For each case, we randomly selected one eligible control from the failed ART women around the same date.

Prior to the intervention, the cases had undergone hormonal assays and screening for HBs–Ag, HIV–Ab, and HCV–Ab. Superovulation was achieved by the following regimen: prior to the induction of COH, on the 21st day of menstrual cycle, 500 μ g/day of buserelin (Suprefact, Hoechst Inc, Germany) was given subcutaneously as a GnRHa, to inhibit endogenous gonadotropins production, hence maximizing control of the cycle. Oral contraceptives in the form of HD that was started since the follicular phase of the previous cycle for GnRH down–regulation, was discontinued on the same day of GnRHa; and menstruation was expected then after 2–3 days.

To prevent a premature surge in LH secretion, HMG was begun on the day 3 of menstruation in a dose of 300–600 IU/day (2–4 vials, 75 IU FSH – 75 IU LH per vial, Menogon, Ferring) intramuscularly, to stimulate follicular growth, with a concurrent reduction in the dosage of GnRHa to 250 μ g/d.

RESULTS

After four days of stimulation, transvaginal ultrasonography was performed and subsequently the HMG dose was adjusted according to follicular growth and serum estradiol concentrations (an indicator of granulosa cell proliferation).

The criteria for follicle maturity included a follicle diameter of 18 mm and a serum estradiol concentration of 200 pg/mL (734 pmol/L) per dominant follicle. When there were at least two mature ovarian follicles detected, urinary HCG at a dose of 10,000 units was administered to stimulate ovulation. In the presence of 10 or more mature follicles, the HCG dose was reduced to 5,000 units due to subsequent risk of the ovarian hyperstimulation syndrome. On the same day of HCG injection, the administration of buserelin was discontinued.

Oocytes were retrieved by transvaginal ultrasound-guided follicle aspiration 34 to 36 hours after HCG administration.

The P/E₂ ratio was calculated on the day of HCG administration as follows: P (in nanograms per mL) × 1,000 ÷ E₂ (in picograms per mL). Serum estradiol and progesterone levels were assayed by solid-phase 125-iodine radioimmunoassay (Coat-A-Count; DPC, Los Angeles, CA). The intra-assay and interassay coefficient of variation were <7% and <9% for estradiol and <9% and <10% for progesterone respectively.

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) for Windows version 10.0. Because of the non-Gaussian distribution of continuous variables, the 'median (range)' format was shown and compared by Mann-Whitney U test.

The categorical variables were compared using χ^2 test or Fisher's exact test where appropriate. Binary logistic regression and forward selection method was used to find related factor(s) to pregnancy outcome. Variables with p-value of less than 0.05 on univariate analysis were entered into logistic regression model.

Linear regression analysis and Pearson's correlation coefficient were used to check the linear relationship between continuous variables. P-values of less than 0.05 were considered statistically significant.

Sixteen (22%) of nonpregnant women and eleven (15%) of pregnant women presented with premature luteinization (P/E₂ > 1) in their first cycles of ART (OR = 1.7, 95% CI: 0.7–3.7). The characteristics of the two groups are compared in Table 1. The median age was significantly higher in the nonpregnant compared with the pregnant women.

The results of the first ART cycles in both groups of women are presented in Table 2. There were no significant differences between the two groups in the medians of duration of infertility, causes of infertility, and method of ART.

The median endometrial thickness was significantly lower in the nonpregnant versus the pregnant women ($P = 0.005$). The endometrial pattern, however, was similar in both groups.

Serum LH, estradiol and progesterone levels on the day of HCG administration did not differ significantly between the pregnant and nonpregnant groups. The late follicular P/E₂ ratio didn't have any significant difference between the two groups and the median P/E₂ ratio was similar in both. The serum concentrations of FSH and LH were high in both groups due to administration of HMG, but median serum FSH level on the day of HCG administration was significantly higher in the nonpregnant group against the pregnant group ($P = 0.026$). There were no significant differences between nonpregnant and pregnant women in terms of medians of the required dose of HMG (amount of HMG ampoules), retrieved oocytes, and transferred embryos. A positive correlation was found between age and serum FSH levels ($r = 0.50$, $P < 0.001$), age and the required HMG dose ($r = 0.54$, $p < 0.001$), and the required HMG dose and serum FSH levels ($r = 0.55$, $p < 0.001$). The required HMG dose and serum FSH levels remained positively correlated with controlling the age ($r = 0.40$, $P < 0.001$).

Using logistic regression analysis including cause of infertility, age, progesterone levels, P/E₂ ratio, endometrial thickness, amount of transferred embryos, LH levels and interaction of serum levels of HMG and FSH, only age and endometrial thickness were significantly related to pregnancy outcome.

Table 1. Characteristics of nonpregnant and pregnant women

	Nonpregnant (n = 75)	Pregnant (n = 75)	P-value*
Age (years)	31 (22–46), 31.4**	30 (16–42), 29.6	0.024
< 35	49 (65.3)***	67 (89.3)	< 0.001
≥ 35	26 (34.7)	8 (10.7)	
Duration of Infertility (years)	9 (1–22), 9.3	8 (1–22), 9.9	0.547
Causes of Infertility:			0.148
Male Factor	33 (44)	28 (37.3)	
Female Factor	24 (32)	25 (33.3)	
Both	9 (12)	18 (24)	
Unexplained	9 (12)	4 (5.3)	
Method of ART:			0.423
Rapid ZIFT	51 (68)	55 (73.3)	
ZIFT	9 (12)	8 (10.7)	
ET	10 (13.3)	5 (6.7)	
IVF	0	2 (2.7)	
GIFT	5 (6.7)	5 (6.7)	

* Based on Mann Whitney U test, χ^2 test, or Fisher's exact test where appropriate

** Data are presented as 'median (range), mean'

*** Data are presented as 'number (%)'

The chance of pregnancy in women under 35 was 3.4 times higher than women ≥ 35 years old (95% CI: 1.3–8.3, $p = 0.009$), and the chance of pregnancy were increased by 1.2% per each one millimeter increment in endometrial thickness (95% CI: 1.01–1.4, $P = 0.04$).

DISCUSSION

Gonadotropin-releasing hormone agonists (GnRHa) are being used during controlled ovarian hyperstimulation (COH) to regulate the timing of ovulation. One of their main applications is to prevent early LH elevation. There are quite many studies discussing against or in favor of the importance of premature luteinization. In long GnRHa cycles, premature luteinization, defined as P/E_2 ratio more than 1 on the HCG day, could

adversely affect the clinical outcome of ART (17). The premature luteinization has been said to be related to low ovarian reserve, higher day 3 FSH levels, lower estradiol levels and amount of follicles ≥ 14 mm on the HCG day, less amount of oocytes collected, and embryos achieved, as well as higher HMG requirement as compared to controls (17,19). It can also interfere with the timing of ovulation and thus, on the overall, affect the outcome of treatment. However, there is still some other facts worth to criticize.

The incidence of premature luteinization in IVF–ET cycles with long GnRHa greatly differs between studies. From our literature search (2–12,17,18,22), the incidence of premature luteinization varied from 13% (11) to 71% (7). The incidence of premature luteinization in this study was found to be only 18% (22% in the nonpregnant and 15% in the pregnant women).

Table 2. Results of the first ART cycles in nonpregnant and pregnant women

	Nonpregnant (n = 75)	Pregnant (n = 75)	P-value*
FSH on the HCG day (mIU/mL)	12.4 (1–48), 16.2**	10 (1.1–49), 12.4	0.026
LH on the HCG day (mIU/mL)	2.8 (1–22), 3.6	2.1 (1–26), 3.3	0.104
E ₂ on the HCG day (pg/mL)	2120 (107–4000), 2312	2079 (142–4000), 2285	0.850
P on the HCG day (ng/mL)	1 (0.1–8.9), 1.5	1 (0.2–3.7), 1.1	0.299
P < 2 ng/mL	61 (81.3)***	66 (89.2)	0.177
P > 2 ng/mL	14 (18.7)	8 (10.8)	
P/E ₂ ratio	0.5 (0.03–10.3), 0.9	0.5 (0.08–4.7), 0.7	0.338
P/E ₂ ≤ 1	57 (78)	63 (85)	0.270
P/E ₂ > 1	16 (22)	11 (15)	
Endometrial Thickness (mm)	10 (6–17), 10.5	12 (8–15), 11.4	0.005
Endometrial Patterns:			0.892
Triple Line	44 (61.2)	46 (64.8)	
Intermediate	14 (19.4)	12 (16.9)	
Solid (echogen)	14 (9.4)	13 (18.3)	
Number of retrieved oocytes	8 (2–30), 9.3	9 (2–21), 9.5	0.638
Number of transferred embryos	5 (1–9), 4.6	5 (2–10), 4.9	0.211
Number of HMG ampoules	32 (16–72), 36.7	30 (16–84), 32.6	
Required HMG dose (IU)	4800 (2400–10800), 5505	4500 (2400–12600), 4890	0.107

* Based on Mann Whitney U test, x² test, or Fisher's exact test where appropriate

** Data are presented as 'median (range), mean'

*** Data are presented as 'number (%)'

Younis and colleagues suggested that the main reason for this large discrepancy lies in the study methodology and design, mainly in the definition of premature luteinization as well as patient selection (17).

Most of other studies have used an absolute progesterone level on the HCG day as an indication

for premature luteinization, with different cutoff points ranging from 0.8 to 2 ng/mL. This was done without controlling for the ovarian response in the cohort of patients studied. Although Younis and colleagues have mentioned that there has been significantly higher clinical pregnancy rates achieved in the group without premature

luteinization compared with those with premature luteinization (17), we observed that even in those cases with premature luteinization there will still be acceptable chances of pregnancy, and there are not significant differences between the two groups in regard to the required HMG dose, amount of retrieved oocytes, and transferred embryos. The statistics show insignificant differences between the two groups in respect to the late follicular serum progesterone levels, which in turn, it reveals similarities of ovarian reserves between the two groups. As Younis and colleagues have stated (17), it can be assumed that the pathogenesis of premature luteinization is not necessarily an LH-dependent event. Our finding that serum LH levels on the HCG day did not differ between those with or without premature luteinization also supports this assumption. Furthermore, we just noticed a significant higher serum FSH levels on the HCG day in the nonpregnant group. This finding sustains the Hofmann and colleagues (20) and Younis and colleagues (17) hypothesis that the cause is related to low ovarian reserve and presumably is associated with the dysmature oocytes usually present in this setting, not the P/E₂ ratio on the HCG administration day.

The increase in serum HCG levels, resulted from the HCG content of the available HMG preparations used for ovarian hyperstimulation, may be responsible for premature rise of serum progesterone (21).

Although this accumulation in serum HCG is most likely not related to the amount of administered HMG, it's related to inter-individuals differences in clearance of HCG, or to the differences in the amount of HCG between batches of HMG (11,21). On the other hand, Younis and colleagues (17) and Hofmann and colleagues (23) have mentioned that premature luteinization may not solely be the result of an early LH elevation, since the long GnRHa treatment cannot abolish this phenomenon in the majority of cycles. This fact strengthens our belief that an early LH elevation is not solely responsible for this phenomenon and that other explanations should be investigated.

Taken together, the low incidence of premature luteinization, its insignificant effect on the outcome

of ART, and the minimal effect of long GnRHa cycles on its elimination, it does not seem necessary to consider long GnRHa cycles for all the candidates of COH. Additional studies are required to demonstrate the actual incidence of premature luteinization and the subsequent chance of pregnancy in absence of GnRHa. In case of similar results, it may be possible to exclude long GnRHa cycles from routine COH protocols to reduce the time for such therapeutic cycles and save money for patients. In conclusion, the present report shows that premature luteinization defined as late follicular progesterone/estradiol ratio more than 1 in a long GnRHa cycle seems not to adversely affect the clinical outcome.

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