

Gn-RH Agonist Challenge Test for Predicting Ovarian Reserve in Short Protocol Cycles

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OBJECTIVE – To evaluate the role of day three values of estradiol (E₂), follicle stimulating hormone (FSH) and luteinizing hormone (LH) in predicting the number of mature oocytes produced in cycles stimulated with gonadotropin-releasing hormone (GnRH) agonist in a short flare-up protocol. **METHODS** - In this prospective study GnRHa was commenced on the first menstrual day for controlled ovarian hyperstimulation (COH) in 223 cases and day three E₂, LH and FSH levels were measured. We identified the cases from whom, three or fewer mature oocytes were obtained as inadequate responders and based on day three FSH levels in COH cycle, we determined the FSH cut-off level exhibiting inadequate response with receiver operating characteristic (ROC) curve analysis. **RESULTS** - It was found that there was a statistically significant correlation between the number of oocytes and the age of the woman ($r = -0.37, p < 0.01$), basal FSH level in natural cycle ($r = -0.28, p < 0.01$) and day three FSH level in COH ($r = -0.44, p < 0.001$). Day three FSH cut-off level in COH determining three or more mature oocytes available was found with ROC analysis as 9.5 mIU/ml. **CONCLUSION** - Day three FSH level in GnRHa stimulated cycle was found to be the most significant variable in determining response to COH.

Key words: FSH level, poor ovarian reserve, Gn RH agonist challenge test

Introduction

The most important indicator of success in assisted reproductive technologies (ART) is the quality and the number of embryos obtained. Improving the quality of the embryo transferred, increases the pregnancy rate'.

Advanced age of the woman may adversely affect the number of oocytes, but chronological age does not always correlate with biological age. Sometimes younger women produce fewer oocytes while older women produce adequate number of oocytes. Therefore, different tests are being used to determine the ovarian reserve.

High FSH level on day 10 of the cycle after clomiphene citrate challenge test (CCCT)^{2,3,4} and slow increase in E₂ after GnRH-agonist indicate decreased ovarian reserve'. Response to ovarian hyperstimulation in ART or conventional infertility treatment is also accepted as a dynamic test measuring ovarian response". Also high FSH and low inhibin in natural cycle show decreased ovarian reservev",

The aim of this study was to determine E₂, FSH, LH and progesterone levels on day 3 of the flare effect of GnRH-

agonist in predicting the number of oocytes recovered in short flare-up protocol.

Materials and Methods

The study group consisted of 223 cases taken into the treatment programme between May and December 1999. None had previously undergone assisted reproduction treatment.

In these cases the mean age of women was 33.7 ± 4.6 (range 22-42) years, the mean age of their spouses 36.9 ± 5 (range 24-49) years and mean duration of infertility 10 ± 5.3 (range 1-25) years. Causes of infertility in these cases were male factor (21%), male and female factor (34%), tubal factor (15%), ovulation factor (8.5%), peritoneal factor (7.5%), endometriosis (5.5%) and unexplained infertility (8.5%).

In our study, we accepted that the number of oocytes obtained predicted the ovarian reserve and cases with polycystic ovarian syndrome were excluded from the study. All cases with at least one growing follicle were included in the study. Day 3 E₂, FSH, LH, PRL, TSH levels of cases in natural cycles were measured. GnRHa (Triptorelin 0,1 mg/d, Decapeptyl; Erkim, Turkey) was initiated on menstrual day 1 for COH and day 3 E₂, FSH, LH, progesterone levels were measured. Cases with day 3 E₂ value > 120 pg/ml in COH or with an ovarian cyst > 15 mm were not stimulated. Hormone levels were measured with electrochemiluminescence technique (Elecsys 2010 system, Roche Diagnostics, Switzerland). The average inter- and intracoefficients of variation were

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< 5%. HMG (Menegon; Erkim Turkey, or Pergonal; Sereno. Turkey) was initiated on cycle day 3 with three to six ampules according to age and FSH levels of cases and was continued with the step-down protocol.

.With the diameter of follicles >18 mm and Ezlevels per follicle >150 pg/ml, 10000 IU hCG (Pregnyl; Organon, Istanbul, Turkey) was given. Oocyte aspiration was performed under transvaginal ultrasound guidance 34-36 hours after hCG administration. Sperm was prepared by swim-up or percoll gradient technique.

For embryo culture 10% maternal serum containing human tubal fluid (Irvine Scientific) was used.

Statistical analysis was performed with Unistat 5.0 statistical program. Data were analysed in three categories ~

1. The relation between the age of the patient, hormone levels in natural cycle (FSH LH, E_z , PRL, ISH) and day 3 hormone levels in COH cycle (E_z , FSH, LH, progesterone), total duration in days and total number of ampules of hMG, E, and progesterone levels on the day of hCG, the number of oocytes retrieved and the number of embryos produced were analysed with Pearson's correlation test.
2. In ART, variable definitions are being used for cases with poor response and the most important of these is the number of oocytes produced. In our study, we defined the cases from whom three or fewer oocytes were obtained as poor responding group. Based on day 3 FSH levels in ovulation induction, FSH cut-off value predicting poor response was determined by Receiver Operating Curve analysis.
3. Cases were divided into two groups according to the FSH threshold value and cycle characteristics of two groups were compared with Student's t-test,

RESULTS

Table I gives the characteristics of women in the study. Hundred and twenty nine IVF cycles, 63 ICSI cycles, 6 GIFT cycles and 2 ZIFT cycles were performed in 223 cases. In 16 cycles no oocytes were retrieved and in seven cycles because of absence of follicular development, cycles were canceled. The relation among the age, hormone levels and the number of oocytes is presented in Table II. Significant correlation was detected between the number of oocytes and the age of the woman ($r = -0.37$, $p < 0.01$), between the number of oocytes and basal FSH in natural cycle ($r =$

-0.28 , $p < 0.001$), between the number of oocytes and day 3 FSH in COH ($r = -0.44$, $P < 0.01$) and between Ezon the day of hCG and day 3 FSH in COH ($r = -0.43$, $p < 0.01$). A positive statistically significant correlation was observed between day 3 FSH in COH and the age of the woman ($r = 0.26$, $p < 0.01$) and between day 3 FSH in COH and day 3 LH ($r = 0.55$, $p < 0.01$).

Threshold value of day 3 FSH in COH predicting three or more oocytes recovered was found as 9.5 mIU/ml by receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, PPV and NPV of this value were determined as 65%, 73%, 58% and 76% respectively. The cases were divided into two groups according to this cut-off value; the group with FSH level 9.5 mIU/ml or less was named as group A, the group with FSH level above 9.5 mIU/ml as group B. Hormone levels and stimulation characteristics of these groups were compared (Table III).

Age of the woman (in group A 32.9 ± 4.6 years, in group B 35.2 ± 4 years; $p < 0.001$), basal FSH (in group A 6.7 ± 3.2 mIU/ml, in group B 9.9 ± 6.9 mIU/ml; $p < 0.001$), day 3 FSH in COH (in group A 6.3 ± 1.9 mIU/ml, in group B 16 ± 6 mIU/ml; $p < 0.001$), day 3 LH in COH (in group A 8.6 ± 5.8 mIU/ml, in group B 16 ± 12 mIU/ml; $p < 0.001$) were found to be significantly higher in elevated FSH group.

Infertility duration, basal LH, ISH, PRL, day 3 E_z and progesterone on the day of hCG, duration (in days) and the number of ampules of hMG were not different in the two groups. E, on the day of hCG (in group A 1195 ± 645 pg/ml, in group B 709 ± 414 pg/ml; $p < 0.001$), the number of oocytes (in group A 8.7 ± 4.5 , in group B 4.5 ± 3.8 ; $p < 0.001$) and the number of embryos (in group A 2.5 ± 2.3 , in group B 1.8 ± 1.3 ; $p = 0.014$) were found to be significantly lower in elevated FSH group.

Discussion

In this study it was demonstrated that FSH value on day 3 of the flare effect of GnRHa predicts the number of oocytes available for retrieval. Statistically significant correlation was found between the number of oocytes and day 3 FSH in COH ($r = -0.44$, $p < 0.01$), the age of woman ($r = -0.37$, $p < 0.01$) and basal FSH in natural cycle ($r = -0.28$, $p < 0.001$). FSH level in GnRHa stimulated cycle is superior to basal FSH screening and to the age of the woman in predicting response to COH.

Muasher et al" suggested that day 3 FSH predicted the good responding group to COH and showed a decrease in pregnancy rate with increasing FSH levels. Because day 3 FSH in natural cycle is easily applied and inexpensive, it is one of the most commonly used tests

Table I. Characteristics of cases

Age of women (years)	: 33.7 ± 4,6 (22-42) ^a
Age of spouses (years)	: 36.9 ± 5 (24-49) ^a
Infertility duration (years)	10 ± 5.3 (1-25) ^a
Causes of infertility	21% Male factor
	34% Male + female factor
	15% Tubal factor
	8.5% Ovulatory factor
	7.5% Peritoneal factor
	5.5% Endometriosis
	8.5% Unexplained infertility

a Range

Table II. The relation among the age of woman, hormone values and the number of oocytes in 233 cases (Pearson correlation test)

Variable		Pearson coefficient	P value
Age of women		- 0.37	p<0.01
Natural cycle	E ₂	0.037	NSa
	LH	- 0.063	NS
	FSH	-0.282	p<0.01
Day 3 hormone post-GnRHa			
	E ₂	0.147	p<0.05
	LH	- 0.131	NS
	FSH	- 0.444	p<0.01
	Progesterone	- 0.057	NS
Total duration of HMG (days)		- 0.012	NS
Total number of ampules of HMG		0.043	NS

a NS : Nonsignificant

Table III. Comparison of hormone values and stimulation characteristics between low (< 9.5 mIU/ml) and high (≥9.5 mIU/ml) FSH groups-

	Group A (FSH<9.5)	Group B (FSH>9.5)	P value
Number	133	90	
Age (years)	32.9 ± 4.6	35.2 ± 4.1	0.000
Infertility duration (years)	10.3 ± 5.1	10.1 ± 5.5	NS
HMG days	8.5 ± 3.9	8.4 ± 2.4	NS
HMG ampules	31.5 ± 14	34.2 ± 10	NS
Natural cycle			
E ₂ (pg/ml)	55.4 ± 49	51.4 ± 35	NS
FSH (mIU/ml)	6.7 ± 3.2	9.9 ± 6.9	0.000
LH (miU / ml)	6 ± 4.8	6.3 ± 4	NS
Prolactin (ngl ml)	14.8 ± 8.5	14.3 ± 8.4	NS
TSH (mIU/ml)	1.6 ± 1.6	1.6 ± 1.1	NS
COH cycle			
E ₂ post-GnRHa (pg/ml)	51.7 ± 49	53 ± 49	NS
FSH post-GnRHa (miU/ml)	6.3 ± 1.9	16 ± 6	0.000
LH post-GnRHa (miU/ml)	8.6 ± 5.8	16 ± 12.2	0.000
Progesterone post-GnRHa (ngl ml)	0.9 ± 0.9	1.2 ± 2.8	NS
E ₂ on hCG day (pg/ml)	1195 ± 645	709 ± 414	0.000
Progesterone on hCG day (ng/ml)	0.6 ± 0.3	0.6 ± 0.6	NS
Oocyte number	8.7 ± 4.5	4.5 ± 3.8	0.000
Embryo number	2.5 ± 2.3	1.8 ± 1.3	0.014

NS - Nonsignificant

P < 0.05 = significant

to determine ovarian reserve in clinical practice. FSH is produced by the feedback effect on hypothalamus and hypophysis of E, and inhibin which are secreted by the cohort of follicles in the ovary. For this reason basal FSH level does not reflect ovarian reserve directly. Furthermore, FSH level measured a long time before the entry into an ART programme does not predict the number of follicles available in that cycle. Even in the same case response to COH may be different from one cycle to another". Therefore every cycle should be evaluated at the beginning of stimulation and the chance of success should be assessed and patients should be counseled accordingly.

Most of the studies related to basal FSH were performed in elderly patient group. Therefore, although basal FSH has a high predictive value in advanced age group, in general infertile group, its predictive value is low ¹².

In the current study, all cases with at least one follicle produced were included without age distinction.

The variation of basal FSH in different cycles and the fact that some poor responders have a normal day 3 FSH led to the proposal of ovarian performance predictive test. CCCT proposed by Navot et al² was evaluated by several groups. They confirmed the excellent predictive value of CCCT for diminished ovarian reserve and poor pregnancy rate in IVF³⁴. But a recent study in women over forty years suggests that a CCCT is no more useful than basal day 3 FSH levels in predicting IVF outcome".

One of the stimulation tests determining ovarian reserve is GnRHa stimulation test. Muasher et al⁷ in their study measured FSH and LH levels before and one hour after GnRHa administration and showed that the number of oocytes and pregnancy rate decreased in high FSH/LH group. He suggested that FSH and LH values after stimulation had no more prognostic value than basal values. Flare-up effect of GnRHa reaches peak level on the third day. Therefore, we assessed day 3 hormones in our study. Padilla et al¹¹ and Winslow et al¹² evaluated the FSH, LH and Ezlevels using a stimulation test with leuprolide acetate and suggested that E₂ response was the best prognostic indicator of IVF outcome. For this, blood sample was taken every day.

GnRHa stimulation test is not standardized, so it is not being used commonly in clinical practice. Some authors state that it is an invasive and expensive test". In our center, we prefer the short protocol in cases with diminished ovarian reserve. GnRHa is initiated on the first day of cycle and cycle day 3 hormone levels are obtained routinely. So we are using GnRHa test in an easy and non-invasive way.

We found the cut-off value of day 3 FSH level post GnRHa in COH determining three or more oocytes available for retrieval as 9.5 mIU/ml by ROC curve analysis. In the literature threshold values for basal FSH are presented. Scott et al¹³ observed that in patients with an FSH and FSH level of >15mIU/ml on cycle day 3, fewer pregnancies occurred and when the FSH level was >25 mIU/ml, no pregnancies occurred. Gurgan et al¹⁶ suggested the basal FSH level predicting poor hormonal response to COH and cycle cancellation in cycles stimulated with GnRha in short flare-up protocol as 13mIU/ml by using ROC curve analysis. Sensitivity, specificity, PPV and NPV of that value were found as 52%, 91%, 63% and 87% respectively. In our study sensitivity, specificity, PPV and NPV of day 3 FSH cut-off value in COH were determined as 65%, 73%, 58% and 76%, respectively.

De Koning et al¹⁷ demonstrated that FSH and LH pulse amplitude increased, pulse frequency was unchanged and response to GnRH increased in patient group with imminent ovarian failure. In concordance with this, in the present study we found day 3 FSH levels in GnRHa stimulated cycles are higher in cases with diminished ovarian reserve.

San Roman et al¹⁸ demonstrated that in cases given GnRHa in short flare-up protocol, LH in follicular phase increased and this also elevated the serum levels of progesterone and testosterone. This was explained as a direct effect of GnRHa on enhancing LH secretion or stimulation of the corpus luteum from the prior cycle. In our study, there was no difference in day 3 E₂ and progesterone levels in COH between low and high FSH groups because oral contraceptives had given to the majority of cases before GnRHa cycle and stimulation was not initiated in cases with E₂ levels >120 pg/ml.

Elevated day 3 FSH level in COH demonstrates that a good response to COH will not be achieved if E₂ level on the day of hCG, the number of oocytes and the number of embryos were found significantly lower.

FSH level on day 3 of flare effect of GnRHa was found to be more important than the age of woman and basal FSH concentration in determining the response to COH or GnRHa challenge test like CCCT, permits identifying low responders. When FSH level is high in treatment cycle, the patients can be counseled regarding the lower chances for pregnancy. In stimulation protocol higher gonadotropin dosage should be given to provide adequate follicular and oocyte production, and when FSH level is excessive, treatment cycle should be cancelled.

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