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Original Articles

Follicular fluid anti-Müllerian hormone, inhibin-A, progesterone, and estradiol level differences in patients under controlled ovarian stimulation

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Summary

Purpose of Investigation: The aim of this study is to determine the differentiation between follicular fluid (FF) anti-Müllerian hormone (AMH), inhibin-A, progesterone (P), and estradiol (E2) levels in patients with poor response, normal response, and high response after controlled ovarian stimulation and the relationship between this differentiation and pregnancy outcome. *Materials and Methods:* The prospective study included 64 patients who applied for IVF treatment, during a two-month period, in Gazi University Infertility Center. Patients were evaluated in three groups: poor-responders (n=18), normo-responders (n=26), and high-responders (n=20), for their controlled ovarian stimulation response. Quantitative measurements of AMH, inhibin-A, P, and E2 were made by ELISA. *Results:* The FF, AMH, inhibin-A, E2, and P levels were all found to be similar in the groups. The pregnancy rates were 11.0% in the poor-responders, 27.0% in the normo-responders, and 16.7% in the high-responder group. Although higher inhibin-A, AMH, P, and E2 hormones were all detected in the non-pregnant, as compared to the pregnant patients, no statistically significant relationship was found. *Conclusion:* In this study, inhibin-A, P, E2, and AMH hormone FF levels were not found to be correlated with the ovulation induction response nor effective for predicting the pregnancy outcome.

Key words: Follicular fluid; AMH; inhibin-A; Progesterone; Estradiol; IVF outcome; Controlled ovarian hyperstimulation.

Introduction

Beginning in the early 1930s, simultaneous with ovarian reserve declines, reproductive performance begins to decline [1]. It is especially important to assess the ovarian reserve in in-vitro fertilization, intracytoplasmic sperm injection (IVF-ICSI) cycles, where the success of treatment depends on multiple follicular development. Women with reduced ovarian reserves have fewer oocytes collected, reduced embryo development, and increased miscarriage rates. A specific marker, directly indicating the functional viability of the oocyte and predicting pregnancy outcome in conventional IVF cycles, is still awaited.

Since oocytes have to communicate with the environment via follicular fluid (FF) and blood plasma, the FF microenvironment is integral to the growing oocyte and changes in follicular dynamics are reflected in FF composition [2]. The oocyte and surrounding follicular cells play interdependent roles during follicular development: regulating growth, selection, and ovulation of a competent oocyte through the signaling of paracrine factors and gonadotropins [2, 3]. The concentration of steroid hormones or biochemical markers may predict implantation and pregnancy, which suggests a link between hormonal regulation and oocyte competence [4].

Given this background, the present study was directed toward determining how FF concentrations of the anti-Müllerian hormone (AMH), which is exclusively produced by preantral and early antral follicles in the adult female and exuded into the FF [5, 6], estradiol (E2), and progesterone (P), which are the main hormones in the menstrual cycle, and inhibin-A, in which cytokine has been in the follicular fluid and correlated with oocyte maturation [7, 8], effect the oocyte's response to controlled treatment.

Materials and Methods

The prospective study included 64 patients who applied for IVF treatment in Gazi University Faculty of Medicine Obstetrics and Gynecology Infertility Center during a two-month period. Patients were evaluated in three groups: poor-responders (number of retrieved oocytes <5, n=18), normo-responders (number of retrieved oocytes 5–15, n=26), and high-responders (number of retrieved oocytes >15, n=20) for their controlled ovarian stimulation response. The inclusion criteria were: age between 18–45 years, regular menstrual cycles (21–35 days), had no co-existing endocrine disorders (thyroid disorders, polycystic ovarian syndrome, adrenal

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and pituitary gland diseases, diabetes mellitus), and no prior ovarian surgery. The approval of the local ethics committee from "Ankara University Clinical Research Ethics Committee" was obtained before the study began.

FF was obtained from the dominant ovarian follicles, cleared by centrifugation, transferred into sterile tubes, and stored at -70°C until analysis. The FF concentrations of inhibin-A were analyzed using enzyme-linked immunosorbent assay kits with values presented in nanogram concentration per milliliter. All of the immunodiagnostic kits were processed according to the manufacturer's instructions.

Inhibin-A levels were measured with intra-assay and inter-assay coefficients of variation of 6.1% and 7.8%, respectively.

A commercial kit of enzyme-linked immunosorbent assay was used as the AMH assay; values were presented in nanogram concentration per milliliter. Inter- and intra-assay coefficients of variation were 5.6% and 3.6%, respectively.

E2 levels were measured by immunoassay values presented in the nanogram concentration per milliliter. Intra-assay and inter-assay coefficients of variation were 2.3% and 2.4%, respectively.

P levels were measured by immunoassay with values presented in nanogram concentration per milliliter. Intra-assay and inter-assay coefficients of variation were 3.2% and 3.4%, respectively.

All patients underwent appropriate pituitary suppression protocols; long gonadotropin releasing hormone (GnRH) agonist down-regulation protocol, microdose flare up GnRH agonist protocol, and GnRH antagonist protocol with hCG induction of the oocyte maturation 36 hours before oocyte collection.

GnRH agonist long protocol: one mg daily leuprolide (1 mg) was given for at least 14 days via a subcutaneous route.

Gonadotropin treatment was begun if the serum estradiol level was lower than 50 pg/ml at the mid-luteal phase (on the 21st day) of the cycle, prior to beginning gonadotropin and if menstrual bleeding had occurred. Leuprolide acetate treatment was decreased to a dose of 0.5 mg/day and continued with the same dose during the gonadotropin treatment.

Microdose flare-up protocol: oral contraceptive treatment consisting of ethinyl estradiol 0.03 mg + levonorgestrel 0.150 mg daily was given between days 1 and 21 of the prior cycle of gonadotropin treatment. Leuprolide acetate40-microgram bid from a subcutaneous route was begun two days after the oral contraceptive drug was stopped. Gonadotropin stimulation was begun at a suitable dose the day after leuprolide acetate was begun. Pituitary suppression was continued with the same dose during gonadotropin stimulation until the day of hCG.

GnRH antagonist protocol: gonadotropin stimulation was begun at the third day of the cycle with the appropriate dose, if the cystic lesion was not found with ultrasonography, without pituitary suppression treatment. Cetrorelix 0.25 mg/day, via subcutaneous route, was begun when the dominant follicle reached a 14-mm diameter and was given with gonadotropins until the day of hCG. Gonadotropin treatment dose was ordered due to the ovarian response.

Statistical analyses were conducted using SPSS 11.5. Descriptive statistics were shown as mean ± standard deviation or median (minimum – maximum). Categorical variables were shown as number of cases and percentages. The difference in the means between groups was evaluated with Student's *t*-test and a one-way ANOVA analysis. The difference of medians was evaluated using a Mann-Whitney *U* test and a chi square test. Factors either having a statistically significant effect on pregnancy at univariate analysis or thought to have an effect on pregnancy were evaluated with a multivariate logistic regression analysis. The statistical significance level was accepted as $p < 0.05$.

Table 1. — *Characteristics of patients*.

	Poor responder n=18	Normo-responder n=26	High-responder n=20	*p*-value
Age (years)	35.0±5.4	30.2±5.1	30.1±5.8	0.010
BMI (kg/m2)	27.6±1.3	26.1±0.9	26.4±1.4	0.085
Day 3 FSH (IU/L)	7.5±4.5	5.7±1.7	5.6±1.2	0.146
Day 3 LH (IU/L)	5.9±3.9	5.3±2.8	4.8±1.8	0.694
Day 3 E2 (pg/mL)	50.6±18.0	52.2±35.4	80.7±120.5	0.401
İnfertility duration (months)	65.7±53.0	57.3±50.0	70.4±64.6	0.131
GnRH use (days)	7.5±6.4	10.5±8.3	12.6±6.3	0.122
Ovulation induction total FSH dose (IU)	1826.4± 936.6	2032.8± 580.0	1966.6± 671.4	0.657
hCG day E2 (pg/mL)	920.2± 530.4	1719.0± 1217.1	2969.2± 1080.6	0.008
hCG day LH (IU/L)	6.2±3.8	2.7±0.9	2.3±1.3	0.004
hCG day progesterone (ng/mL)	0.6±0.2	1.3±0.8	1.0±0.4	0.112
Total oocyte number	1.8±0.7	10.1±3.2	21.0±5.6	0.000

Table 2. — *Comparison ofinhibin-A, AMH, E2, and P levels in groups.*

	Poor responder n=18	Normo-responder n=26	High-responder n=20	*p*-value
Inhibin-A (ng/ml)	341.3±195.8	428.3±247.3	439.3±229.8	0.085
AMH (ng/ml)	1.9±5.1	1.5±2.0	1.3±1.0	0.796
E2 (ng/ml)	476.3±311.4	311.4±261.5	451.6±254.4	0.366
P (ng/ml)	11010.3± 8463.3	8897.5± 5354.2	10335.7± 5702.3	0.143

Values are given as mean ± SD.

Results

The comparison of demographics, endocrinologic variables, and stimulation characteristics of these three groups are summarized in Table 1. Baseline characteristics such as body mass index, infertility duration, and third-day E2, LH and FSH levels, and total FSH dose were similar among all three groups. However, age was higher ($p = 0.01$) in the poor-responder group than the other groups. hCG day E2 was different at a statistically significant level ($p = 0.008$), which was highest in the high-responder group and lowest in the poor-responder group.

hCG day P was similar in all groups. FF, AMH, inhibin-A, E2, and P levels between groups were all found to be similar (Table 2).

Of the 64 patients, 14 (21.9%) were pregnant, and 50 (78.1%) were not pregnant. Although the pregnancy rates were 11.1% in the poor-responders, 27.0% in the normo-responders, and 16.7% in the high-responder group, there was no statistically significant difference among them. In addition, inhibin-A, AMH, E2, and P hormone levels were detected at higher rates in non-pregnant than pregnant patients; however, no statistically significant relationship was

Table 3. — *Comparison ofinhibin-A, AMH, E2, and P levels in pregnant and non-pregnant women.*

	Pregnant n=14	Not pregnant n=50	*p*-value
Inhibin-A (ng/ml)	319.4±156.5	419.0±217.2	0.120
AMH(ng/ml)	1.5±1.5	1.6±3.3	0.545
E2 (ng/ml)	440.5±233.0	509.2±269.1	0.387
P (ng/ml)	8854.0±6480.7	10140.4±6000.0	0.486

Values are given as mean ± SD.

found (Table 3).

According to a multivariate logistic regression analysis in the study population, E2, P, inhibin-A, and AMH are not found to be predictive for obtaining clinical pregnancy or correlated with patient' response to ovulation induction.

Discussion

Since ovarian proteins facilitate both intraovarian paracrin effects as well as endocrine feedback effects, FF properties play an important role [9]. In the present study, FF concentrations of inhibin-A, AMH, E2, and P in patients undergoing IVF treatment were not found to be correlated with the ovulation induction response nor were they effective for predicting the pregnancy outcome.

In COH, the low basal AMH level is associated with antral follicle number, total dose of gonadotropins used, the number of mature follicles on hCG day, the duration of the gonadotropin administration, hCG day E2 level, and the number of oocyte collected [10-13]. Nonetheless, several studies seem to have conflicting results, as FF and AMH positively related with fertilization rates and pregnancy outcome [14-16]. In the present study, although lower FF AMH levels were related with clinical pregnancy and a high response to COH, no statistically significant relation was found.

A recent study demonstrated that serum AMH differs with COH, declines its minimum level on hCG day, increases on OPU day, and the strongest correlation with AMH level and COH outcomes were found by measuring the baseline AMH, instead of other times [17]. This change may occur in FF content and that could make the FF AMH level useless as a predictor of COH outcome.

Some studies have suggested that FF levels of E2 and P are an indicator of oocyte maturity [18, 19]. However, a more recent study concluded that E2 and P levels are associated with follicular size, not oocyte maturation/ability to fertilize [20]. In the present study, E2 and P levels in FF were not different in groups nor were they shown to predict the stimulation response and pregnancy outcome. A previous study demonstrated a negative correlation between FF AMH and E2 level [21]. However, the statement was not confirmed in the present study, that the association of low FF AMH with higher clinical pregnancy rates and high response are not supported by the observation of higher levels of FF E2.

In one study, the cytokine inhibin-A demonstrated that an elevated FF level is associated with better ovarian response and a high pregnancy rate [22]. In contrast, higher levels of inhibin-A and B were found to be associated with oocyte presence and function but not with fertilization rates or predicting assisted reproduction outcome [7, 23]. In the present study, the FF inhibin-A was found to be higher in non-pregnant patients.

Conclusion

For COH cycles, FF levels of inhibin-A, AMH, E2, and P did not correlate with pregnancy outcome and stimulation response in the present population. Under these results, FF hormone levels are not good markers for predicting ovarian response to COH. Some unknown control mechanisms may exist between these substances and oocyte fertilization capacity, although further studies with larger numbers are required to confirm this observation.

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