ABSTRACT

Introduction: We aimed to compare the follicular fluid and serum levels of inhibin-B on various days of the menstrual cycle in women undergoing in vitro fertilization (IVF) cycles and to determine their correlation with ovarian response and pregnancy.

Materials and Methods: This prospective study was performed on 70 patients undergoing controlled ovarian hyperstimulation and followed by the IVF Department between February 2009-June 2009. Age, body mass index (BMI), basal levels of follicle-stimulating hormone (FSH), estradiol (E$_2$), luteinizing hormone (LH), ovarian volume, and number of antral follicles were compared between the good responders and poor responders. Blood samples of inhibin-B were collected before the treatment, on the day of human chorionic gonadotropin (hCG) administration, and on the day of oocyte pick-up. Follicular fluid samples were obtained from dominant follicles during oocyte retrieval. These parameters and characteristics of the cycles were compared between the good responders and poor responders.

Results: The correlations of collected oocytes with basal inhibin-B, number of mature oocytes and number of transferred embryos were found to be significant. Among all the parameters, inhibin-B levels on the third day and on the day of hCG administration had the highest sensitivity and specificity. Age, antral follicle count and ovarian volume followed these parameters.

Conclusion: This study demonstrated that serum, but not follicular fluid, levels of inhibin-B on the third day and on the day of hCG administration are effective markers of follicular development and ovarian response in women undergoing IVF cycles.

Key words: Inhibin-B, Follicular fluid, In vitro fertilization, Ovarian response

Accepted: August 06, 2011

ÖZET

Serum ya da Folliküler Sıvı İnhibin-B Seviyesi: Hangisi IVF Hastalarındaki Follikül Gelişiminin Saptatmadığı Dahası Etkili Bir Belirteçtir?

Giriş: In vitro fertilizasyon (IVF) planlanan hastaların menstrüel sikluslarının farklı günlerinde folliküler sıvı ve serum inhibin-B seviyelerini tespit ederek, bu parametrelerin over cevabını ve gebelikteki iliskilerini ortaya koymayı amaçladık.

INTRODUCTION

Before entering an in vitro fertilization (IVF) program, evaluation of ovarian reserve gives information about the reproductive potential of women, including the process of follicular depletion and oocyte quality, and can permit the physician to decide the optimal infertility treatment for the patient\[1,2\].

Several studies have been done to show the predictive effects of basal follicle-stimulating hormone (FSH) and estradiol (E\(_2\)) level, anti-Müllerian hormone (AMH), follicular blood flow, ovarian volume, patient’s age, and body mass index (BMI), and furthermore, of the clomiphene citrate challenge test (CCCT), the exogenous FSH ovarian reserve test (EFORT) and the gonadotropin agonist stimulation test (GAST) to show the ovarian reserve\[3-6\]. However, none of these parameters was a direct determinant of follicular development, ovarian response and pregnancy in women undergoing IVF cycles.

Inhibin-B is produced mainly by granulosa cells of the small developing follicles and selectively inhibits the pituitary FSH secretion; measurement of its serum concentration may offer a direct assessment of ovarian response \[7-9\]. It was demonstrated that serum inhibin-B is believed to be of predictive value in monitoring ovarian stimulation treatment for IVF\[10,11\].

The relationship between inhibin-B concentrations in follicular fluid and serum on various days of the menstrual cycle has not yet been explored.

The purpose of this study was to demonstrate the correlation of follicular fluid levels of inhibin-B with serum inhibin-B concentrations on day 3, on the day of human chorionic gonadotropin (hCG) administration and on the day of ovarian pick-up (OPU) and the relation with ovarian response and pregnancy.

MATERIALS and METHODS

The medical Ethics Committee of Dr. Zekai Tahir Burak Women’s Health Teaching and Research Hospital, Ankara, Turkey approved this work, and all participating patients provided written informed consent. This was a prospective controlled study with a total of 70 women undergoing IVF treatment between February 2009 and June 2009.

Sixty-one (87.1%) patients were admitted to our hospital with primary infertility and 9 (12.9%) with secondary infertility. Patients were treated with either IVF or intracytoplasmic sperm injection (ICSI). At the end of the treatment, patients were divided into two groups: Group A: women with a collected number of oocytes > 5 (good responders) and Group B: women with a collected number of oocytes ≤ 5 (poor responders). Cycles of six patients were excluded, and pregnancy was achieved in 27 (38.6%) cases. In these patients, the reasons for infertility included the following diagnoses: tubal factor (11%), endometriosis (3%), male factor (39%), and unexplained infertility (44%).

To evaluate the basal hormonal status, FSH, E\(_2\) and luteinizing hormone (LH) were measured by enzyme linked immunosorbent assay (ELISA, Serotec Ltd, Oxford, UK) on the third day of the menstrual cycle. On the same day, transvaginal ultrasonographic measurement of the ovarian volume and number of antral follicles were detected with a ALOKA SSD-1000 machine with a 5 mHz ultrasound probe. All follicles between 2 and 10 mm in both ovaries were counted and included in the analysis.

Patients underwent a baseline transvaginal ultrasound evaluation. When normal ovarian morphology was noted, an oral contraceptive pill consisting of 0.15 mg of desogestrel and 0.03 mg ethinyl estradiol (Desolett, Organon, Oss, the Netherlands) was given for 21 days. All cycles were stimulated using the long...
gonadotropin-releasing hormone (GnRH)-a protocol with the same preparation (Leuprolide acetate-Lucerin, Abbott, France). GnRH-a was instituted in the last 4 days of the oral contraceptive pill administration. After the withdrawal of oral contraceptive pills, GnRH-a was continued, along with gonadotropin stimulation, and stopped on the day of hCG administration.

Gonadotropin treatment was started with recombinant FSH (rFSH) (50 IU, Puregon, Organon, Oss, the Netherlands or Gonal F, Serono Laboratories, Aubonne, Switzerland) applied at various dosages in the range of 150-450 IU/day, adjusted for the age, basal FSH value and BMI for each patient.

Cycles were cancelled if the ovarian response was poor, i.e., less than three follicles with a diameter > 14 mm after 8 days of stimulation or serum E₂ level < 300 pg/mL after 14 days of stimulation.

Ovulation was triggered with 10,000 IU of hCG (5000 IU, Pregnyl, Organon, Oss, the Netherlands) when at least three follicles were > 18 mm. hCG was not administered due to increased risk of ovarian hyperstimulation syndrome (OHSS), when 12 follicles were > 15 mm and/or E₂ plasma level was > 4000 pg/mL.

Follicular fluid was collected 36 hours after the treatment by using transvaginal ultrasonography-guided OPU; the follicular fluid sample collected was centrifuged and stored at -20˚C until assay was performed. One embryologist detected the oocyte maturation by using the same microscope (.....).

Blood samples for evaluating inhibin-B levels were collected at the following time points: baseline period (on day 3), the day of administration of hCG and the day of OPU (serum and follicular fluid). Samples were centrifuged for 10 minutes (min) at a rate of 3500 cycles/min. Serum samples were stored in a freezer at -80˚C in 1 mL polypropylene tubes. Inhibin-B was measured by ELISA (Serotec Ltd., Oxford, UK). The limit of detection is 15 pg/mL and intra and interassay coefficients of variation (CV) were < 6% and < 13% over the working range of 15-1000 pg/mL.

The data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA). Student’s t-test, the Levene test for equality of variances and chi-square tests were used. All data were presented as means ± SD when appropriate. A p value < 0.05 was considered statistically significant.

RESULTS

There was a significant difference between the good and poor responder groups in age, inhibin-B levels on the third day, inhibin-B levels on the day of hCG administration, and number of ampules used, as seen in Table 1.

In serum basal FSH, day 3 E₂ and E₂ on the day of hCG administration, there was no significant difference between the two groups (Table 1).

The correlations of collected oocytes with infertility duration, number of ampules used, number of collected oocytes, number of mature oocytes, number of transferred embryos, and number of fertilized oocytes are shown in Table 2. The correlation of collected oocytes with basal inhibin-B, mature oocytes and number of transferred embryos was found to be significant. On the other hand, the correlation of the
number of collected oocytes with basal $E_2$ levels and antral follicle number was not significant (Table 2).

We found the patient's age to be significantly correlated with infertility duration, ovarian volume, number of ampules used, and number of follicles. Day 3 FSH level had a good correlation with basal $E_2$ levels and cleavage rate, but did not correlate with the other basal parameters and had less correlation with the number of collected oocytes and fertilization rate (Table 2).

Day 3 inhibin-B had a significant correlation with inhibin-B on the day of hCG administration, number of collected oocytes, number of mature oocytes, and infertility duration, but had less correlation with age and fertilization rate (Table 2). The correlation of day 3 inhibin-B with inhibin-B on the day of hCG administration is important, since it shows the ovarian response.

The clinical pregnancy rate of the study patients was found as 38.6%, as seen in Table 3.

### DISCUSSION

Inhibin is secreted mainly from the granulosa cells of the ovarian follicles and serves as a protein marker of granulosa-cell function, which modulates the follicular development\(^\text{[13]}\).

Follicular growth during controlled ovarian stimulation may be monitored by serum inhibin-B levels, but we hypothesized that the follicular fluid levels of inhibin-B may be a stronger indicator for predicting the outcome of IVF. In the present study, we found that serum but not follicular fluid levels of inhibin-B on the third day and on the day of hCG administration had the highest sensitivity and specificity. The patient's age, number of antral follicles and ovarian volume followed these parameters with respect to ovarian response in patients undergoing an IVF program.

It is very important to help couples in making decisions after a cycle of negative outcome since both IVF and ICSI are becoming rather expensive and stressful treatments. Prognostic parameters, such as the woman's age, basal FSH and antral follicle count, may not be enough to satisfy the couples for further management options\(^\text{[14]}\).

High-quality embryos lead to a high pregnancy ratio; thus, the quality of the embryo is an important predictor in IVF treatment\(^\text{[15]}\). Nevertheless, a marker from follicular fluid that directly indicates embryo quality has not yet been discovered. Several studies

| Table 2. Correlation of collected oocytes with the parameters investigated |
|-----------------------------|-----------|-------------|
|                             | r         | p           |
| Age (years)                 | -0.179    | 0.137       |
| BMI (kg/m$^2$)              | 0.11      | 0.32        |
| Infertility duration (years)| -0.182    | 0.131       |
| FSH on day 3 (mIU/mL)       | -0.213    | 0.077       |
| $E_2$ on day of hCG (ng/mL) | 0.126     | 0.29        |
| Inhibin-B on day 3 (ng/mL)  | 0.497     | ≤ 0.001     |
| Inhibin-B on day of hCG (ng/mL) | 0.205 | 0.089       |
| Inhibin-B on OPU day (ng/mL) | 0.086 | 0.47        |
| Follicular fluid inhibin-B  | 0.074     | 0.54        |
| on OPU day (ng/mL)          |           |             |
| Mature oocytes              | 0.88      | ≤ 0.001     |
| Transferred embryos         | 0.39      | ≤ 0.002     |
| Fertilization rate (%)      | 0.014     | 0.915       |
| Cleavage rate (%)           | -0.057    | 0.66        |

<table>
<thead>
<tr>
<th>Table 3. Clinical pregnancy rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
reported lower follicular fluid levels of inhibin-B associated with higher quality of oocytes and high fertilization and pregnancy outcomes, but the reverse has also been reported\[11,16-18\]. In our study, follicular fluid levels of inhibin-B were not significantly different in the good versus poor responder groups.

In the serum, basal inhibin-B levels from the early follicular phase have been reported to be a predictor of follicular quality during controlled ovarian stimulation in IVF treatment\[19\]. Serum inhibin-B levels on the OPU day were not statistically different.

Previous studies reported significant positive relationships between follicular fluid inhibin-B levels and IVF outcomes, like number of follicles, number of oocytes retrieved and embryo scores\[20-22\]. However, we demonstrated no correlation between follicular fluid inhibin-B levels and oocyte quality and fertilizing capacity. Our results suggest that the role of follicular fluid inhibin-B level as a marker for ovarian response will need to be confirmed with later investigations.

Chang and his colleagues investigated the correlation between inhibin-B and E\(_2\) levels in 233 follicular fluid samples of 156 IVF patients with the quality of embryo development and demonstrated that inhibin levels were significantly correlated with embryo scores on days 2 and 3; in contrast, both inhibin-B and E\(_2\) levels were inversely related to age. They also showed that follicular fluid inhibin-B levels were inversely associated with serum FSH levels on day 3, which was believed to reflect the ovarian reserve\[23\].

Several studies have suggested that basal serum inhibin-B level is a strong marker of ovarian reserve and follicular development in women undergoing IVF treatment\[11,13,24\]. A correlation between serum inhibin-B levels and the number of antral follicles supports the notion that serum inhibin-B levels quantitatively reflect the follicle pool. However, although it was shown that serum inhibin-B levels during IVF management may reflect embryo and oocyte quality as well as ovarian response, it is not clear whether peripheral inhibin-B exclusively reflects the follicle pool or whether it has role as a qualitative marker.

Similar to our results, Jörg and colleagues compared the inhibin-B levels on the day of hCG administration in 15 pregnant and 16 nonpregnant IVF patients with classical predictive factors such as E\(_2\) and oocyte numbers and found that inhibin-B on hCG day seems to be the better prognostic factor for the outcome of IVF\[24\].

In our study, basal FSH levels had a strong correlation with basal E\(_2\) levels and cleavage rate, but did not correlate with the other basal parameters and had a lower correlation with the number of collected oocytes and fertilization rate.

When we compared the means of the parameters based on the oocytes collected, a significant difference was found between age, inhibin-B on day 3, inhibin-B on the day of hCG administration, and the number of ampules used. Basal serum FSH levels were not significantly different between the two groups.

Finally, indicators that had a significant correlation with the collected oocytes were day 3 inhibin-B, mature oocytes and number of embryos transferred, and when we evaluated the parameters that could be used as a basal indicator, serum levels of inhibin-B had the highest correlation coefficient.

In conclusion, an important point of practical importance of our study is that inhibin-B levels in follicular fluid are not necessary markers for IVF. Serum levels of inhibin-B on the third day and on the day of hCG administration give more information about the follicular development in women undergoing IVF treatment when compared with the other prognostic parameters.

REFERENCES


