Day 3 serum inhibin-B level is not predictive of ovarian assisted reproductive technologies outcome

Marzieh Farimani1, M.D., Iraj Amiri2, Ph.D., Sedigheh Hoseini1, M.D.
1 Department of Gynecology & Obstetrics, Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.
2 Department of Anatomy, Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.
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Abstract
Background: The ability of the ovary to respond to exogenous gonadotrophin stimulation and development of several follicles is essential in assisted reproductive technology. Neither age and regularity of menses nor follicular phase FSH and estradiol concentrations are reliable predictors of ovarian response. Day 3 serum inhibin-B level, during induction ovulation, has been proposed as a predictor of ovarian response.

Objective: To determine day 3 serum inhibin-B as a predictor of ovarian response to induction ovulation in IVF/ICSI cycles.

Materials and Methods: Seventy one infertile patients under 40 years old were enrolled in this study. All women have both ovaries, basal FSH level under 15 mIU/ml, and no evidence of endocrine disorders. Day 3 FSH, estradiol, inhibin-B concentrations and ovarian volume were measured before treatment. All patients underwent standard long GnRH agonist protocol. The number of oocytes retrieved, fertilization rate, clinical pregnancy rate, days of stimulation and number of HMG ampoules were determined. The patients were divided into two groups, normal responders and poor responders (number of oocytes retrieved <4).

Results: The mean inhibin-B level in normal responders was 166.9 ± 141 pg/ml versus 115.8 ± 87 pg/ml in poor responders, which the difference was not statistically significant (p=0.24). We could not find a cut off between normal and poor responders.

Conclusion: The use of day 3 inhibin-B level as a predictive marker of ovarian response in IVF/ICSI cycles is not reliable.

Key words: Inhibin-B, IVF, Ovarian reserve, Poor responder

Introduction

Assisted reproduction and development of in-vitro fertilization (IVF) techniques have revolutionized the treatment of infertility. Because IVF technique is expensive and not completely successful, many researches attempted to determine predictive factors for more successful outcome. Prognostic assessment of ovarian reserve has relied upon indirect markers of ovarian function, such as age (1-3), Follicle Stimulating Hormone (FSH) at baseline (4-7), and estradiol concentrations (8,9). The reduction of ovarian function or “reserve” is apparently due to reduced number of ovarian premordial follicles, from over 250,000 at menarche to very few at the end of reproductive age. Age and regularity of menses alone are unreliable predictors of ovarian reserve. Neither follicularaor phase FSH nor estradiol concentrations, could finally indicate that ovarian function is normal and unimpaired (1-3). Ovarian volume has also been proposed as a predictor of ovarian response but there were still a substantial number of pregnancies among the women with very small ovarian volumes (10,11). In theory, the direct products of granulosa cells might better reflect ovarian secretory capacity and follicle number. Inhibin-B is one of these products which regulates FSH secretion by negative feedback (11-17). The aging of ovary is accompanied by a decrease in inhibin-B secretion (18,19). Early follicular phase serum inhibin-B may be a suitable marker of ovarian follicle reserve and fertility potential (20-24). However, several studies found no or limited clinical value in measuring basal early follicular inhibin-B regarding to ART outcome (25-33).
According to these controversial data and in order to test the hypothesis that baseline inhibin-B concentration would serve as improved marker of IVF outcome, we examined baseline inhibin-B on day 3, prior to ovarian stimulation and compared it with standard markers of ART outcome.

Materials and Methods

From April 2004 until June 2005, seventy one women undergoing IVF/ICSI treatment were included in this study in the Fatemieh Infertility Research Center. Our inclusion criteria were 1) age under 40 years old, 2) basal FSH level under 15 mIU/mL 3) presence of both ovaries, 4) no evidence of endocrine disorders (normal levels of thyroid-stimulating hormone, testosterone, androstenedione, and prolactine), 5) no evidence of ovarian cyst bigger than 2cm in diameter, and 6) written informed consent. All patients received oral contraceptive pills (LD) from the 5th day of their previous menstrual cycle then administration of GnRH agonist (Suprefact, Hoechst, Germany), 500 µg/day, was begun on the 19th day. After menstruation, ovarian volumes were calculated as the volume of an ellipsoid (length×width×depth×π/6) by transvaginal ultrasound (6.5 MHZ, Dynamic imaging) on day 3. If no follicular cysts larger than 12mm in diameter was detected, Busereline was reduced to 200 µg/day and gonadotropin was started. The dose of the gonadotropin was changed on an automated EIA plate reader (BRIO: Basic Radium Immunoassay Operator, Radim spa, Pomezia, Italy). The assay detection limit for inhibin-B was less than 10 pg/ml. Within and between plate coefficients of variation were <5.0 and 9.0% respectively. Cross-reactions for each assay with the various inhibin-related proteins were <0.5%.

Statistical analysis

Statistical analysis was performed using the commercially available software package SPSS version 11. Student’s t-test and χ² were used for analysis and results were reported as mean ± SD. p<0.05 was considered as significant level.

Results

Among 71 patients, 11 cases were excluded from the study because of discontinuation of the cycle. The mean age of the women was 29 years (range 20-39). The causes of infertility were: male factor infertility (40%), tubal infertility (13.3%), ovulatory infertility (18.3%), compound infertility (25%) and unexplained infertility (3.3%).

According to the number of retrieved oocytes, the patients were divided into two groups; 12 poor responders (less than 4 oocyte), and 48 normal responders (≥4 oocytes).

Demographic characteristics, pretreatment hormonal profiles and results of two groups are shown...
on table I. Total pregnancy rate was 20%. There were no statistically significant differences in mean of age, mean of duration of infertility, type of infertility, mean of day 3 FSH, E2 and inhibin-B levels, total ovarian volume, number of ampoules and duration of the ovarian stimulation in two groups. As expected, fertilization rate and clinical pregnancy rate are significantly reduced in poor responders. There was no significant differences in the mean of inhibin-B levels between two groups.

According to inhibin-B levels, the patients were divided into two groups; inhibin-B concentrations ≤70 pg/ml (14 patients) and inhibin-B concentrations >70 pg/ml (46 patients) (11). Demographic characteristics, pretreatment hormonal profiles and results of the two groups are shown on table II. There were no statistically significant differences in FSH and estradiol on day 3 between two groups. However, the number of retrieved oocytes in the patients with inhibin-B ≤70 pg/ml was lower than this number in the other group but this difference was not statistically significant. Correlation coefficients were determined between the number of oocytes retrieved and serum inhibin-B levels on day 3 administration, and significant correlations (r=0.358) was found between them (fig.1).

**Table I.** Demographic characteristics, pretreatment hormonal profiles and results in two groups of normal responders and poor responders

<table>
<thead>
<tr>
<th></th>
<th>Poor responders (n=12)</th>
<th>Normal responders (n=48)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>30.41 ± 5.51</td>
<td>29.20 ± 3.78</td>
<td>0.37</td>
</tr>
<tr>
<td>Primary infertility (%)</td>
<td>91.7</td>
<td>85.4</td>
<td>0.49</td>
</tr>
<tr>
<td>FSH on day 3 (min/ml)</td>
<td>6.51 ± 2.72</td>
<td>7.81 ± 7.96</td>
<td>0.58</td>
</tr>
<tr>
<td>Estradiol on day 3 (pg/ml)</td>
<td>79.60 ± 53.88</td>
<td>66.64 ± 56.20</td>
<td>0.47</td>
</tr>
<tr>
<td>Inhibin-B on day 3 (pg/ml)</td>
<td>115.89 ± 87.13</td>
<td>166.90 ± 141.81</td>
<td>0.24</td>
</tr>
<tr>
<td>Total ovarian volume (ml)</td>
<td>6.81 ± 3.12</td>
<td>7.99 ± 4.05</td>
<td>0.35</td>
</tr>
<tr>
<td>Number of HMG ampoules</td>
<td>32.04 ± 19.15</td>
<td>32.70 ± 12.23</td>
<td>0.88</td>
</tr>
<tr>
<td>Number of retrieved oocytes</td>
<td>1.25 ± 1.1</td>
<td>11.75 ± 5.71</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>0.25 ± 0.45</td>
<td>5.5 ± 7.54</td>
<td>0.01</td>
</tr>
<tr>
<td>Fertilization rate (%of oocytes)</td>
<td>29.1</td>
<td>58</td>
<td>0.04</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>0.00</td>
<td>25</td>
<td>0.04</td>
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**Table II.** Demographic characteristics, pretreatment hormonal profiles and results in two groups, subdivided by day 3 inhibin-B serum concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Inhibin-B ≤ 70 pg/ml (n=14)</th>
<th>Inhibin-B &gt; 70 pg/ml (n=46)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>27.78 ± 4.8</td>
<td>29.95 ± 3.84</td>
<td>0.08</td>
</tr>
<tr>
<td>FSH on day 3 (min/ml)</td>
<td>7.03 ± 2.18</td>
<td>7.71 ± 8.18</td>
<td>0.76</td>
</tr>
<tr>
<td>Estradiol on day 3 (pg/ml)</td>
<td>61.12 ± 31.21</td>
<td>71.71 ± 61.12</td>
<td>0.53</td>
</tr>
<tr>
<td>Total ovarian volume (ml)</td>
<td>7.25 ± 3.84</td>
<td>7.91 ± 3.93</td>
<td>0.58</td>
</tr>
<tr>
<td>Number of HMG ampoules</td>
<td>33.42 ± 11.6</td>
<td>32.31 ± 14.38</td>
<td>0.79</td>
</tr>
<tr>
<td>Number of retrieved oocytes</td>
<td>7.46 ± 5.25</td>
<td>10.26 ± 6.95</td>
<td>0.2</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>3.71 ± 3.04</td>
<td>5.5 ± 4.41</td>
<td>0.16</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>28.6</td>
<td>17.4</td>
<td>0.28</td>
</tr>
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</table>
**Discussion**

The most useful predictive information for an infertile couple should be obtained before beginning assisted reproductive techniques. Basal inhibin concentrations have been evaluated previously as predictive markers for pregnancy in IVF cycles. In two (12,24) of three (13) previous studies, higher inhibin-B on day 2–3 was associated with a greater number of oocytes retrieved in IVF cycles (24) and with subsequent pregnancy (12) which was better predictor of the response to exogenous gonadotrophins than age (24) and was equivalent to day 3 FSH. Seifer et al (20) demonstrated that, pregnancy rates were higher in patients with day 3 inhibin-B concentrations of over 45 pg/ml than this rate in patients with lower values. In 1999, they compared 109 normal responders with 47 poor responders and they did not find any significant difference in day 3 FSH levels between the two groups, but poor responders had significantly lower day 3 inhibin-B levels (21). The study of Hall et al (27) did not support the use of day 3 inhibin-B as a predictive marker of IVF outcome. In addition, basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. Fertil Steril 1991; 55: 784–791.

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There was a positive correlation between inhibin-B level on day 3 and retrieved oocyte number but there was not any correlation between inhibin-B level on day 3 and ART outcome. There was tremendous overlap in baseline inhibin-B concentrations between pregnant and non-pregnant subjects, and inhibin-B alone failed to predict pregnancy. The results of this study confirm Hall et al findings (27) and are contraversal with Tsuchia et al (12) and Seifer et al (20) results.

**Conclusion**

The present study shows that inhibin-B concentration on day 3 positively correlate with the number of oocytes retrieved during ART. Our results do not support the use of day 3 inhibin-B as a predictive marker of IVF outcome.

**Acknowledgment**

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