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

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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, inbibin-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 retrived <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 completly 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 apparantly due to reduced number of ovarin 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 folliculaor phase FSH nor estradiol concontrations, 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 amoung 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).

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According to these contraversial 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, testostrone, androstendione, 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 elliposoid (length× width× depth× π/6) by transvaginal ultrasound (6.5 MHZ, Dynamic immaging) on day 3. If no follicular cysts larger than 12mm in diameter was detected, Busereline was reduced to 200 μg/day and gonadotropin (Gonal F, Serono, Swiss) was started i.m. daily, depending on age (150 IU in $\geq$35 years old and 225 IU in <35 years old). Whenever follicular cysts bigger than 12mm were seen, estradiol was checked and if it was $\leq$ 50 pg/ml, then the administration of gonadotropin was started. The dose of the gonadotropin was changed according to the follicular growth. When more than 2 follicles bigger than or equal to 18mm were seen, HCG (Pregnyle, Organon, Germany) 10000 IU were injected to induce final oocyte maturation and 36 hours later, ovum was picked up. After 3 days if fertilization occured, embryo was transfered. Poor response was defined when fewer than four follicules at retrival were collected. Pregnancy was diagnosed by increasing concentrations of ß-HCG 2 weeks after embryo transfer and the subsequent demonstration of an intrauterine gestational sac by transvaginal scan 2 weeks later.

Hormone analyses

FSH and stradiol concentrations were measured at diagnostic laboratory of the Fatemieh Hospital using routine procedures. To detect Inhibin-B, all of collected sera immediately were freozen and stored at –80°C until assay. Inhibin-B concentration in serum was measured by Inhibin-B assay kit purchased from Serotec (Oxford, UK), using specific two-site enzyme immunoassay. Briefly, standards and samples were diluted as appropriate and mixed with a half volume of distilled water containing 10% sodium dodecyl sulphate (SDS). After 3 min at 100°C, tubes were cooled before adding freshly prepared hydrogen peroxide solution. After additional incubation at room temperature, duplicate aliquots of denatured and oxidized samples and standards were transferred to antibody-coated microtitre plates, that were incubated at room temperature, overnight. After washing with enzyme immunoassay (EIA) wash buffer [0.1 mol/l Tris–HCl, 0.15 mol/l NaCl, 10% (w/v) bovine serum albumin, 5% (v/v) Triton X-100, and 0.1% (w/v) sodium azide, pH 7.5], 50 µl alkaline phosphatase-conjugated mouse anti-human inhibin-α subunit antibody was used. The plates were then incubated for 3 hours. Plates were washed and bound alkaline phosphatase was quantified using a commercially available enzyme immunoassay amplification system (ELISA), which was used according to the supplier's instructions. The inhibin-B plates were read at 490 nm 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 commericially available software package SPSS version 11. Student's t-test and χ^2 were used for analysis and results were reported as mean ± SD. $p<0.05$ was considered as significant level.

Results

Amoung 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 casuses 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 characteristitcs, pretreatment hormonal profiles and results of two groups are shown

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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, E_2 and inhibin-B levels, total ovarian volume, number of ampoulses 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 $\leq$70 pg/ml (14 patients) and inhibin-B concentrations >70 pg/ml (46 patients) (11). Demographic characteristitcs, 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 $\leq$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

	Poor responders (n=12)	**Normal responders (n=48)**	**p**
Age (yr)	30.41 ± 5.51	29.20 ± 3.78	0.37
Primary infertility (%)	91.7	85.4	0.49
FSH on day 3 (min/ml)	6.51 ± 2.72	7.81 ± 7.96	0.58
Estradiol on day 3 (pg/ml)	79.60 ± 53.88	66.64 ± 56.20	0.47
Inhibin-B on day 3 (pg/ml)	115.89 ± 87.13	166.90 ± 141.81	0.24
Total ovarian volume (ml)	6.81 ± 3.12	7.99 ± 4.05	0.35
Number of HMG ampoules	32.04 ± 19.15	32.70 ± 12.23	0.88
Number of retrieved oocytes	1.25 ± 1.1	11.75 ± 5.71	0.000
Number of embryos transferred	0.25 ± 0.45	5.5 ± 7.54	0.02
Fertilization rate (%of oocytes)	29.1	58	0.01
Clinical pregnancy rate (%)	0.00	25	0.04

Table II. Demographic characteristics, pretreatment hormonal profiles and results in two groups, subdivided by day 3 inhibin-B serum concentrations.

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

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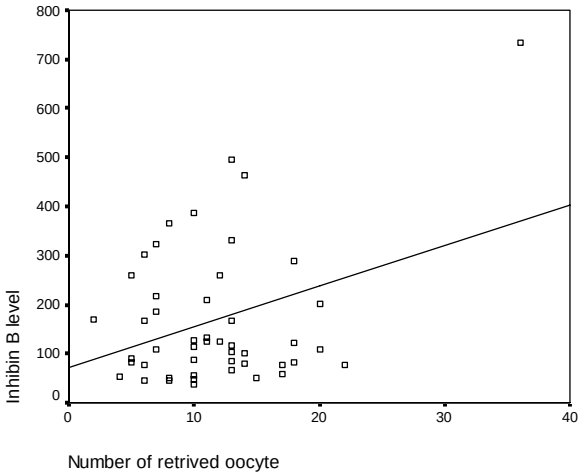


Figure 1. Correlation between the number of oocytes retrieved and serum inhibin-B measured on days 3 of FSH treatment.

Disscusion

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 leveles between the two groups, but poor responders had significantly lower day 3 inhibin-B leveles (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, base line FSH, E_2 and inhibin-B were not significantly different between pregnant and non pregnant patients. In the present study the mean inhibin-B 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 ($p= 0.24$). In addition, there were no statistically significant differences in day 3 serum FSH, estradiol and ovarian volume between the two groups. A combination of FSH higher than the median value (6.51 mIU/ml) and inhibin-B lower than the median value (115.8 pg/ml) was not seen in poor responders. A cut off of serum inhibin-B conceantrations between normal and poor responders was calculated (cut- offs 45-70-100-120-150 pg/ml), but these cut-offs were not statistically significant.

There was a positive correlation between inhibin-B level on day 3 and retrived 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 positevly 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.

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