

Measurement of serum Inhibin B as a predictive evaluation of ovarian response following ovulation induction program in intrauterin insemination

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Abstract:

Background:

The evaluation of ovarian reserve has been, and still, the focus of substantial clinical research. The assessment of ovarian reserve is valuable for determining stimulation protocols, and because of limited predictive value of age alone, or other passive hormonal analysis in estimating response to the exogenous stimulation, Dynamic research was done on serum inhibin B.

Objective:

Measurement of serum inhibin B as a predictive evaluation of early ovarian response following ovulation induction program in intrauterine insemination(IUI).

Materials and Methods:

Thirty couples were involved in IUI program. They divided into two groups: group (1) 15 women were treated by Gonadotropin(Gn) recombinant follicle -stimulating hormone (rFSH). While group (2), 15 women were treated by Clomiphene citrate (CC). Treatment started at day 3 of the menstrual cycle. Serial hormonal profile tests namely; inhibin B, Estadiol (E2), Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) were done at days 3, 7 of the menstrual cycle and the day of human chorionic gonadotropin hormone (hCG) injection with recurrent ultrasonography (U/S) to confirm the number and development of the follicles.

Results:

There was a significant difference in the level of inhibin B ($P < 0.05$) between day3 and 7 of the cycle with the mean of the follicle number 1.67 ± 0.18 in group(1), while in group (2) there was a non significant difference ($P > 0.05$) in the level of inhibin B between day 3 and 7 of the cycle with the mean of the follicle number 2.14 ± 0.17 . Percentage of pregnancy rate in IUI in this study was 19.23%.Percentage of pregnancy rate in group (1) was 25% ,while in group (2) was 14.28% ,but there is a significant difference ($P < 0.05$) in the pregnancy rate between the two groups.

Conclusion:

It was concluded that serum inhibin B obtained at day 7(day 5 of (Gn) therapy) offers an early and accurate prediction of ovarian response to (Gn) stimulation while there was no significant change in the level of inhibin B between day 3 and 7 of the cycle in patients treated by CC .

Key Words: Inhibin B, Gonadotropin, Clomiphene Citrate, IUI.

Introduction

The hormonal control of ovarian function by gonadotropins plays a key role in the physiologic process of follicular growth and differentiation, that is selection of a single dominant follicle followed by a mono ovulation⁽¹⁾. A patient's ovarian response to stimulation medicine is mainly determined by her ovarian reserve, which comprises the quantity and functional capacity of follicles. Pre-evaluation of ovarian reserve and prediction of ovarian response would provide a valuable means of assisting clinicians in selecting appropriate dose of therapeutic agents for each patient⁽²⁾. Traditional criteria used to predict ovarian response to ovarian stimulation drug include the patient's age, baseline serum concentration of hormones such as (FSH), (LH), Estradiol (E₂), FSH to LH ratio⁽³⁾. Inhibin B was suggested as an early sensitive predictor of ovarian reserve⁽⁴⁾.

Inhibins are dimeric polypeptides produced by granulosa cells and composed of an α -subunit along with a β A-subunit (inhibin A) or a β B-subunit (inhibin B). Inhibin B concentrations rise across the luteal-follicular transition and peak in the mid follicular phase, suggesting that they are secreted by the developing cohort of follicles, and may mark the number or quality of developing follicles at the baseline⁽⁵⁾. With the better understanding of the control, synthesis and secretion of the inhibins and their potential endocrine role in the menstrual cycle, attention has turned to the possibility of this family of peptides to provide a more direct index of ovarian reserve^(6,7).

Therefore, this study was designed to identify prospectively the values of basal and dynamic measurements of inhibin B following ovarian stimulation therapy (recombinant FSH or CC) in predicting early ovarian response and pregnancy outcome in IUI.

Materials and Methods

All the females age involved in this study were (19 -39) years old with regular menstrual cycle excluding poly cystic ovarian syndrome (PCOS), FSH<15(mIU/mL), and normal prolactin level. They are examined generally and gynecological after thorough history taking⁽⁸⁾. Assessment of male infertility by the physician is based on a patient's history, physical examination, and semen analysis⁽⁹⁾.

Group (1) treated by Gonal- F(R) 75 IU on alternate day⁽¹⁰⁾, while group (2) treated by CC tablet containing 50 mg/ (1 \times 2)/ daily from day 3 to day7 of the menstrual cycle⁽¹¹⁾. The ultrasonography study was performed at day 3 of the cycle to detect antral follicle number and diameter in the two groups, and any uterine abnormalities⁽¹²⁾.

Diameter of the follicle (s) was monitored by serial examinations of vaginal ultrasound. At least one or two mature (dominant) follicles were recognized about 17 – 20 mm, supplemented by the ovulatory hormone Ovitrelle^(R) (Merck/Serono) (hCG) 250 μ g, 6500IU was used to induce ovulation. *In vitro* Activation Technique: Simple layer procedure was performed for the semen samples preparation that involved in this study. Then IUI was done by IUI catheter after 34 -36hr⁽¹³⁾

Hormonal assays, serum levels of Inhibin B (pg/mL), FSH (mIU/mL), LH (mIU/mL) and E₂ (pg/mL) were measured by using ELISA (enzyme-linked immunosorbent assay) at days (3,7 of the cycle and the day of hCG injection).

Results

The basal level of inhibin B in group (1) at day 3 of the menstrual cycle mean \pm SE was 40.94 \pm 1.99, at day 7 of the cycle, the mean \pm SE of inhibin B was 50.88 \pm 1.87. At day of hCG injection, the mean \pm SE was 40.35 \pm 3.11. There is statistically significant difference (P<0.05) among the three values as shown in Figure (1).

The basal level of inhibin B mean \pm SE in group (2) at day 3 of the menstrual cycle was 39.69 \pm 3.41, at day 7 of the cycle, it was 36.71 \pm 3.61, while at day of hCG injection, the mean \pm SE was 31 \pm 4.25. There is statistically no significant difference (P>0.05) among the three values as shown in figure (2).

The basal level of E₂ in group (1) at day 3 of the menstrual cycle mean \pm SE was 42.53 \pm 3.20, at day 7 of the cycle, the mean \pm SE was 97.18 \pm 13.90. At day of hCG injection, the mean \pm SE was 203.52 \pm 28.47. There is statistically highly significant difference P<0.001 among the three values as shown in figure (3)

The level of E₂ in group (2) at day 3 of the menstrual cycle mean \pm SE was 50.493.97 \pm . At day 7 of the cycle, the mean \pm SE was 118.24 \pm 15.45. While at day of hCG injection, the mean \pm SE was 342.46 \pm 64.30. There is statistically highly significant difference P<0.001 among the three values as shown in figure (4).

Regarding the clinical characteristic of patients in two groups there were significant difference in follicle number as shown in Table (1).

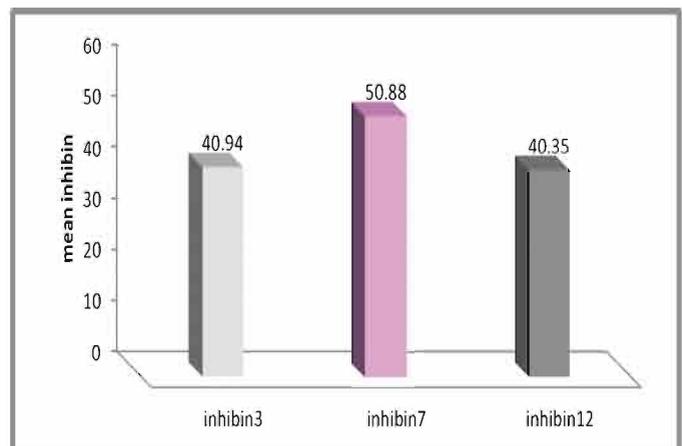


Figure (1)inhibin B level changes in group (1) after treatment by rFSH

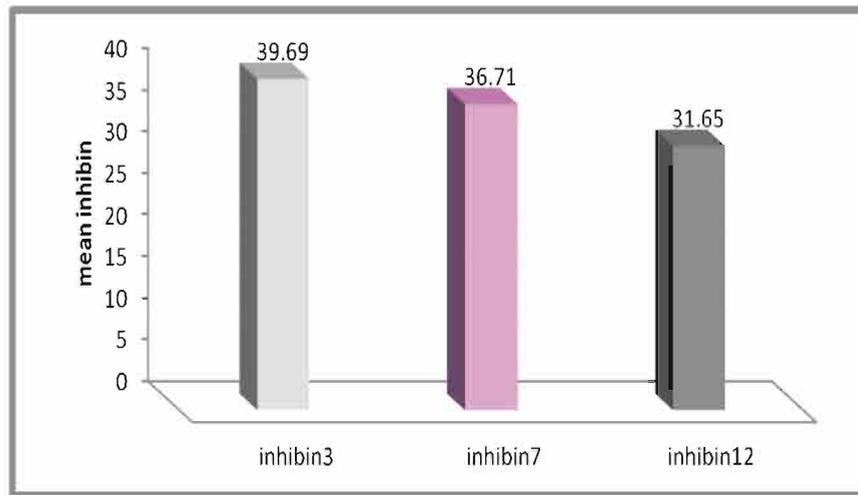


Figure (2)inhibin B level changes in group (2) after treatment by (cc)

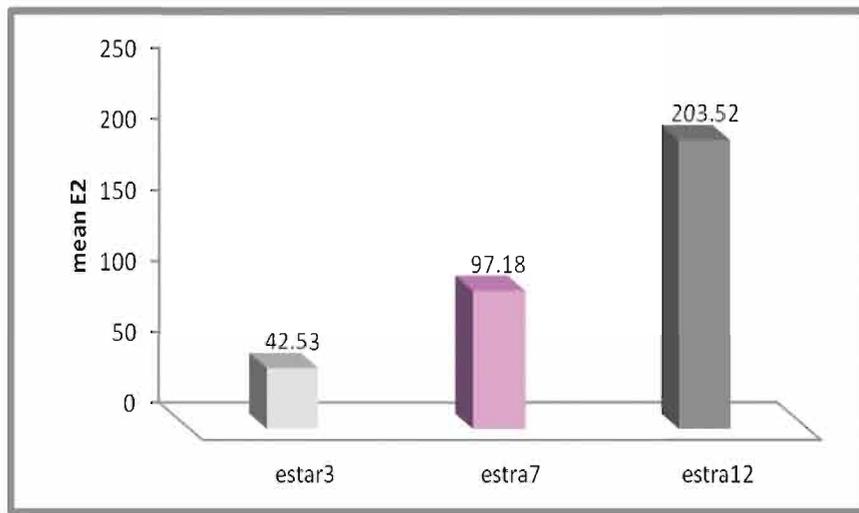


Figure (3) E2 level changes in group (1) after treatment by rFSH

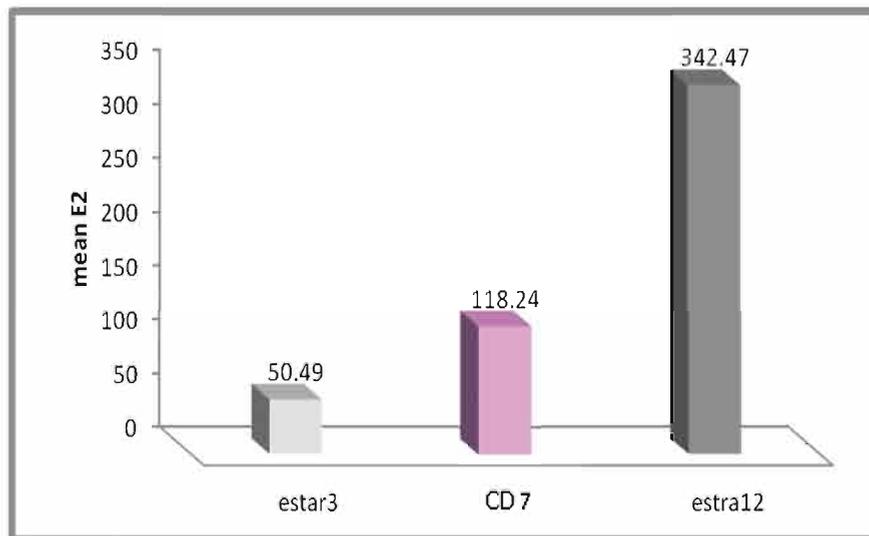


Figure (4) E2 level changes in group (2) After treatment by (CC)

Table (1) Comparison clinical characteristic of patients in the two groups(1) patients treated by rFSH ,(2) patients treated by CC.

Characteristics	Group 1	Group 2		LSD	P value
Age	29.50 ±1.64A	29.07± 1.78A		6.89	0.129 NS
BMI	27.02± 1.00A	28.95± 0.53A		4.37	0.161NS
Antral follicle number	7.83± 0.53A	9.07 ±0.70A		3.37	0.198 NS
Follicle number	1.67+ 0.18B	2.14± 0.17B		2.45	0.007*
Endometrial thickness	8.99± 0.17A	8.81 ±0.18A		3.24	0.201NS

NS-non significant

*-significant (P<0.05)

Table(2): Comparison between the pregnancy outcome and (hormonal levels of inhibin B, E₂ , and various variables) in group(1) .

Parameter	Pregnancy	No	Mean	SEM	P value
Age	Yes	3	26.00	3.51	0.236
	No	9	30.67	1.80	
Antral follicle	Yes	3	9.33	1.33	0.107
	No	9	7.33	0.50	
Follicle number	Yes	3	2.00	0.57	0.329
	No	9	1.56	0.17	
Endometrial thickness	yes	3	8.9333	0.26	0.856
	No	9	9.0111	0.22	
inhibinCD3	Yes	3	47.9933	1.87	0.006*
	No	9	38.5911	1.40	
inhibinCDV	Yes	3	55.8967	1.37	0.127
	No	9	49.2078	2.21	
Inhibin B day hCG injection	Yes	3	42.7000	4.13	0.685
	No	9	39.5767	4.00	
E2-CD3	Yes	3	47.6533	5.19	0.382
	No	9	40.8322	3.88	
E2-CD7	Yes	3	96.4733	32.99	0.978
	No	9	97.4233	16.23	
E2-Day of hCG injection	Yes	3	244.73	71.31	0.430
	No	9	189.79	31.11	

Group(1)- patients treated by rFSH

• P<0.05 significant difference

Table(3): Comparison between the pregnancy outcome and (hormonal levels of inhibin B , E₂ , and various variables) in group(2)

Parameter	Pregnancy	N	Mean	SEM	P value
Age	Yes	2	34.50	2.50	0.227
	No	12	28.17	1.93	
Duration of infertility	Yes	2	5.00	1.00	0.761
	No	12	4.58	0.51	
Antral follicle	Yes	2	10.00	2.00	0.612
	No	12	8.92	0.78	
Follicle number	Yes	2	2.50	0.50	0.433
	No	12	2.08	0.19	
Endometrial thickness	Yes	2	9.25	0.55	0.345
	No	12	8.74	0.19	
Inhibin B- CD3	Yes	2	36.69	5.76	0.735
	No	12	40.19	3.92	
InhibinB- CD7	Yes	2	34.24	3.34	0.792
	No	12	37.12	4.20	
InhibinB-day hCG	Yes	2	24.58	10.67	0.519
	No	12	32.82	4.72	
E2-CD3	Yes	2	53.53	3.97	0.769
	No	12	49.99	4.61	
E2-CD7	Yes	2	182.07	68.21	0.092
	No	12	107.60	13.66	
E2-Day hCG	Yes	2	704.64	326.36	0.014*
	No	12	282.10	42.06	

Group(2)- patients treated by CC.

* - P<0.05 significant difference

Discussion

In group (1),

There was a significant difference ($P < 0.05$) in the level of inhibin B among day 3, 7 of the cycle and day of hCG injection. This result is in agreement with the those authors^(5,14) reported that inhibin B is the predominant inhibin in the small antral follicles rising in the early to mid follicular phase, then falling throughout the late follicular phase and remaining low for the duration of the luteal phase.

In this study, it was detected that E₂ at day of hCG injection is more sensitive index of ovarian response to Gn stimulation, this result agrees with the other authors⁽¹⁵⁾ who reported that, during ART cycle, plasma E₂ measurements are routinely used to calibrate the Gn doses in conjunction with data obtained by ultrasound, and its directly related to follicular size and that the contribution of mature

follicles to E₂ output may be estimated at about 200pg/ml. While there is a better correlation of inhibin B during the mid-follicular phase, when granulosa cell function is strongly dependent on FSH⁽¹⁶⁾.

There was significant difference ($P < 0.05$) of LH level among day 3, day 7 and the day of hCG injection, this is due to the fact that sign of LH measurements during Gn stimulation are usually restricted to the detection of the endogenous LH surge, specially required for women undergoing IUI⁽¹⁵⁾.

2-Group(2) received clomiphene citrate

There was no significant difference ($P > 0.05$) in the level of inhibin

B among day 3, day 7 of the cycle and the day of hCG injection, also no significant difference ($P>0.05$) in the level of FSH among day 3, 7 and the day of hCG injection due to the fact that clomiphene citrates long retention within tissues, so physiologic response of the developing follicles is assumed to overcome the influence of CC on the hypothalamic-pituitary axis and reduce FSH levels to normal range by cycle day 10 as shown in figure (2-4)⁽¹⁷⁾. Our results agree with the facts⁽¹⁸⁾ reported that the rate of follicular growth and number of follicles in patient who received CC from cycle days 5- 9 was significantly better than the group who received CC from cycle day 2-6.

Pregnancy outcome in IUI

There was only significant correlation between basal inhibin B level at day 3 of the cycle and pregnancy rate in group (1), this result agrees with that found by those authors⁽⁴⁾, reported that inhibin B serve as a better and direct measure of ovarian reserve. There was a significant correlation between the pregnancy outcome and serum E_2 level at day of hCG injection in group(2), this is in agreement with those authors⁽¹⁵⁾ reported that E_2 is more sensitive index of ovarian response to Gn stimulation during ART cycle.

Percentage of pregnancy rate in IUI in this study was 19.23%. Percentage of pregnancy rate in group (1) was 25% ,while in group (2) was 14.28% with the fact that using swim up *in vitro* activation techniques of the semen samples, gets safely separation of spermatozoa based on their motility and morphology and gained significantly higher percentage of motility⁽¹⁹⁾, but there is significant difference ($P<0.05$) in the pregnancy rate between the two groups, this is agree with those authors⁽²⁰⁾ found that in IUI with the use of rFSH get higher pregnancy rate.

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The object of ovulation induction is to restore the ovulatory state and restore fertility potential. An assessment of ovarian reserve and consequent fertility potential is becoming an integral part of the work-up in these modern days of a desire for pregnancy in the more advanced fertile age groups. Overweight and frank obesity may have a devastating effect on fertility potential, both for conception and the prevalence of miscarriage. Biochemical measures of ovarian reserve are intended to probe and to reflect the biology of the aging ovary, the one component of the reproductive system most closely related to decreased fecundity. Inhibin B and AMH are glycoprotein hormones produced by small ovarian follicles and are therefore direct measures of the follicular pool. Decreased inhibin B secretion lowers the level of central negative feedback, resulting in increased pituitary FSH secretion and in higher late luteal and early follicular FSH concentrations (an indirect measure). The CCCT involves measurements of serum FSH before (cycle day 3) and after (cycle day 10) treatment with clomiphene citrate (100 mg daily, cycle days 5-9). Inhibin as an oocyte meiotic inhibitor. *Mol Cell Endocrinol* 1989; 62: 307-11. Google Scholar. 63 Woodruff, TK, Lyon, RJ, Hansen, SE, Rice, GC, Mather, JP. Measurement of exogenous and endogenous inhibin in sheep serum using a new and extremely sensitive bioassay for inhibin based on inhibition of ovine pituitary FSH secretion in vitro. *J Endocrinol* 1986; 110: 341-52. CrossRef Google Scholar PubMed. 109 Robertson, DM, Tsonis, CG, McLachlan, RI. Plasma inhibin levels during gonadotrophin-induced ovarian hyperstimulation for IVF: a new index of follicular function? *Lancet* 1986; i: 1233-34. CrossRef Google Scholar. 140 Hughes, EG, Robertson, DM, Handelsman, DJ, Hayward, S, Healy, DL, de Kretser, DM. A prolonged, secondary elevation of serum FSH follows the primary surge and lasts until the following morning (estrus) (15). The anesthetic pentobarbital prevents completely the pre-ovulatory primary LH and FSH surges and the expected ovulation, as well as seriously attenuating the secondary rise in serum FSH (13, 15). One of us has recently shown that an ovulatory dose of LH injected into pentobarbital-treated rats on the day of proestrus induces a synchronized endogenous "secondary" secretion of FSH that is maximal at 0400 on the day of estrus (16). No endogenous LH is secreted,