



## EVALUATION OF SERUM INHIBIN B AS A PREDICTIVE VALUE OF OVARIAN RESPONSE FOLLOWING OVULATION INDUCTION PROGRAM IN INTRACYTOPLASMIC INSEMINATION (ICSI)

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### ABSTRACT

Measurement of serum inhibin B as a predictive evaluation of early ovarian response following ovulation induction program in Intracytoplasmic insemination (ICSI). The study was included 20 patients who were involved in program of *in vitro* fertilization (Intra cytoplasmic sperm injection ICSI). They were treated by long protocol of gonadotropin-releasing hormone agonist (GnRH-a) started at day 21 of the previous cycle and then restarted at day 3 of the menstrual cycle, the use of rFSH as stimulating therapy. This part was divided according to the ovarian response to Poor group (follicle $\leq$ 4), Normal group (follicles 5-15) and High group (follicles $>$ 15). Serial hormonal profile tests inhibin B, Estradiol (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 revealed that there was no significant change ( $P>0.05$ ) in the level of inhibin B between day 3 and 7 of the cycle in poor group, while there was a significant difference ( $P<0.05$ ) in the level of inhibin B between day 3 and 7 of the cycle in normal group and there was also a significant difference ( $P<0.05$ ) in the level of inhibin B between day 3 and 7 of the cycle in high group. There was no significant correlation ( $P>0.05$ ) between the outcome of pregnancy and ovarian response. It was concluded that serum inhibin B obtained at day 7 (day 5 of gonadotropin (Gn) therapy) offers an early and accurate prediction of ovarian response to gonadotropin (Gn) stimulation in ICSI, also inhibin B may be a more sensitive index than serum E2 in predicting early ovarian response of women in different ages, and cycle pregnancy rate does not correlate with the ovarian response.

**KEYWORDS:** inhibin B, rFSH, ICSI.

### INTRODUCTION

With the development of ART, various treatment protocols for ovarian stimulation have been used in fertility treatment, which are controlled ovarian hyper stimulation (COH) for in vitro fertilization (IVF). These protocols are aimed at the development of multiple follicles instead of one dominant follicle and there by result in a non-physiological condition<sup>[1]</sup>. However, women may react differently to the various type and dose of ovarian stimulation therapy, resulting in different ovarian responses<sup>[2]</sup>. A patient's ovarian response to stimulation drug 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<sup>[3]</sup>. Traditional criteria used to predict ovarian response to ovarian stimulation drug include the patient's age, baseline serum concentration of hormones such as Follicle stimulate hormone (FSH), Luteinizing hormone (LH), Estradiol (E2), FSH to LH ratio<sup>[4]</sup>. Also inhibin B was suggested as an early sensitive predictor of ovarian reserve<sup>[5]</sup>.

Inhibins are dimeric polypeptides produced by granulosa cells and composed of  $\alpha$ -subunit along with a  $\beta$ A-subunit (inhibin A) or a  $\beta$ 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<sup>[6]</sup>. Many studies were done to investigate whether there is a correlation between the increase in inhibin B concentration and the early ovarian response either poor response or the risk of ovarian hyper stimulation syndrome (OHSS) and the pregnancy outcome. Also to delineate whether inhibin B is more sensitive than E2 in predicting ovarian response to gonadotropin (Gn) stimulation<sup>[7]</sup>. Therefore, this study was designed to identify prospectively the values of dynamic measurements of inhibin B following ovarian stimulation therapy by gonadotropin (recombinant FSH) in predicting early ovarian response and pregnancy outcome in ICSI.

### MATERIALS AND METHODS

This study was conducted in Department of Reproductive Endocrinology and Infertility at Selcuk University- Konya –Turkey from 5/8/2011-5/10/2011. Twenty patients were involved in program of *in vitro* fertilization (intra cytoplasmic sperm injection ICSI). All the females involved in this study were examined generally and gynecological after thorough history taking<sup>[8]</sup>. Assessment of male infertility by the physician is based on a patient's history, physical examination, and seminal analysis and before being admitted into any IVF program, the couple would probably be required to have a complete medical evaluation. They would most likely undergo all the routine

steps of an infertility assessment, usually performed by their own primary physician, in order to rule out the possibility that procedures other than IVF might better address their needs<sup>[9]</sup>. All patients were 19-39 years old, regular menstrual cycle excluding polycystic ovarian syndrome (PCOS), FSH<15(mIU/mL) and normal prolactin level, they entered long protocol of COH (controlled ovarian hyper stimulation). GnRHa is administered daily 10IU of Lupron from the mid-luteal phase of the previous cycle then stimulation done by using rFSH daily (Puregon 225 IU), with decreasing the dose of GnRH. Further titration of Puregon dose is based on follicular response observed from E2 and vaginal ultrasound scans started from day 7 of the menstrual cycle. When there are three follicles over 16 mm and the leading follicle is over 18 mm and if the endometrium is over 7 mm, ovulation is triggered by( hCG 6500IU) Ovitrelle<sup>[9]</sup>. Hormonal analysis (Inhibin B, FSH, LH and E2) were done for all patients at days (3, 7 of the cycle, and the day of hCG injection) by using ELISA. Oocyte retrieval is usually timed at 34-36 hours following hCG injection and is carried out via ultrasound guidance. It is done under general or local anaesthesia for transvaginal aspiration<sup>[10]</sup>. The required ICSI dishes had also been prepared and incubated in CO2 incubatore (5%CO2at 37°C) until used for injection by the micromanipulator <sup>[11]</sup>. Assessment of Embryo Quality (Grading and Staging), the embryos

should then be carefully evaluated to select those with the highest implantation potential <sup>[12]</sup>. Embryo transfer is the single most important step in IVF. Its need confidence and skill to perform a good transfer. It's performed under direct ultrasound guidance to ensure proper placement in the uterine cavity. This practice, properly conducted, will significantly enhance embryo implantation and pregnancy rates. All embryo transfer was preferred when the woman has a full bladder. Patient receives 50 mg progesterone IM daily for 2weeks. The woman should have a quantitative B-hCG blood pregnancy test, about 11 days after the egg retrieval <sup>[10]</sup>. Serum levels of Inhibin B (pg/mL), FSH (mIU/mL), LH (mIU/mL) and E2 (pg/mL) were measured by using ELISA at days (3,7 of the cycle) and the day of hCG injection).

**Statistical Analysis**

Statistical analysis was performed by using SPSS (Statistical Package of Social Science; version 17.0 LED Technology, USA and Microsoft Excel Work Sheet 2007. Analysis of variance (ANOVA) was performed and the results were expressed as mean ± standard error. Paired sample t- test was applied to compare the differences between means. The differences were considered statistically significant if the P value is lower than 0.05 (P> 0.05).

**RESULTS**

**TABLE 1:** Level of the hormones at days (3 ,7 and day of hCG injection) in Poor response group(number of follicles≤4).

Hormone	CD 3	CD 7	Day – hCG injection	LSD	P value
Inhibin B	45.40±25.80 A	131.30±19.70 A	83.45±15.25 A	101.42	0.131 NS
FSH	3.39±0.23 B	17.83±3.32 A	8.94±2.99 B	26.07	0.063 NS
LH	2.00±0.57 A	0.90±0.71 A	0.45±0.35 A	1.43	0.280 NS
E2	48.05±47.05 B	680.88±306.24 A	616.96±362.46 A	606.22	0.336 NS

NS – P> 0.05 non-significant difference

**TABLE 2:** Level of the hormones at day (3, 7 and day of hCG injection) in Normal response group (number of follicles 5-15).

Hormone	CD 3	CD7	Day-hCG injection	LSD	P value
Inhibin B	23.60±3.68 C	545.57±85.41 A	278.69±65.36 B	127.03	<0.001**
FSH	2.64±0.47 C	11.65±1.97 A	9.31±1.16 B	3.34	<0.001**
LH	1.85±0.57 A	1.39±0.29 A	0.53±0.20 B	1.23	0.062 NS
E2	17.78±7.12 C	474.72±96.31 B	854.95±128.79 A	312.29	<0.001**

\*\*P<0.001 highly significant differences

**TABLE 3:** Level of the hormones at day (3, 7 and day of hCG injection) in High group(number of follicles>15)\*

Hormone	CD 3	CD 7	Day-hCG injection	LSD	P value
Inhibin B	25.76±3.84 C	1500.70±220.37 A	1011.80±253.18 B	723.96	0.005*
FSH	2.38±0.63 B	8.10±1.68 A	4.07±1.18 B	3.11	0.043*
LH	0.96±0.46 A	0.72±0.39 A	0.30±0.20 A	1.01	0.482
E2	10.71±7.72 B	2415.30±816.29 A	2846.00±372.82 A	1045.87	0.017*

P<0.05 significant difference

**TABLE 4:** Comparison of various variables among three groups (poor, normal and high)

Parameters	Poor response (n= 2)	Normal response (n= 14)	High response (n= 3)	P- value
Age(years)	35.00±3.00	32.43±1.14	28.33±1.66	0.201
BMI(Kg/m <sup>2</sup> )	29.88±0.58	26.04±0.60	27.30±3.56	0.246
Follicles	4.00±0.00	8.71±0.51	16.00±1.00	<0.001**
Fertilized egg	3.00±0.00	6.35±0.46	12.00±1.52	<0.001**
Embryo	2.00±0.00	4.92±0.37	10.66±1.76	<0.001**
Day of rFSH stimulation	10.00±2.00	8.57±0.46	9.00±0.57	0.559
Dose of rFSH	3050.0±250.0	2141.10±159.33	1891.70±254.26	0.093

**TABLE 5:** Comparison of pregnancy outcome among the three groups

Groups	Pregnancy		Non-pregnancy		Total	
	No.	%	No.	%	No.	%
Poor response (n= 2)	1	12.5	1	9.09	2	10.52
Normal response (n= 14)	6	75	8	72.72	14	73.68
High response (n= 3)	1	12.5	2	18.18	3	15.78
Total	8	100	11	100	19	100

P&gt;0.05(P-value =0.930)

P-value was 0.930

**TABLE 6:** Comparison between the pregnancy out come and various variables

	Age(years)	Follicles	Fertilized egg	Embryos
Non Pregnancy (n= 11)	32.45±1.09	9.00±1.08	6.36±0.78	5.09±0.73
Pregnancy (n= 8)	31.50±1.88	9.88±1.44	7.62±1.26	6.12±1.25
P	0.647	0.628	0.386	0.461

**TABLE 7:** Person correlation between the hormonal levels at days (3, 7 and day of hCG injection) and various variables in ICSI.

	Inhibin B(3)	Inhibin B (7)	Inhibin day hCG	FSH 3	FSH7	FSH day hCG	LH3	LH7	LH day hCG	E2(3)	E2(7)	E2 day hCG
Age	r .29	-.45	-.40	.55*	.18	-.02	.25	-.009	.01	.23	-.39	-.35
	P .22	.04*	.08	.01	.46	.91	.28	.97	.95	.34	.09	.13
Follicle no.	r -.31	.88**	.77**	-.23	-.06	-.27	-.14	-.08	-.19	-.25	.72**	.84**
	P .18	.001	.001	.33	.78	.26	.55	.71	.42	.29	.001	.001
Dose of R	r .18	-.21	-.06	.25	.58**	.27	.54*	.23	-.005	.38	-.03	-.02
	P .45	.38	.77	.28	.009	.25	.01	.33	.98	.10	.88	.91
Fertilized no	r -.27	.87**	.79**	-.22	.03	-.16	-.05	.01	-.15	-.19	.77**	.86**
	P .25	.001	.001	.34	.88	.50	.83	.95	.52	.41	.001	.001
Embryo no	r -.14	.83**	.83**	-.16	-.01	-.13	.02	.08	-.05	-.18	.81**	.87**
	P .56	.001**	.001**	.49	.96	.59	.92	.71	.83	.45	.001**	.001**

## DISCUSSION

The recruitment of an optimal number of follicles during an IVF treatment is fundamental to the success of a treatment cycle. Poor ovarian response, with a small number of oocytes collected and evens a smaller number of embryos available for transfer, reduces the success rate [13]. On the other hand, an excessive ovarian response to stimulation increases the risk for (OHSS) [14]. The availability of a screening test to predict the ovarian response would provide a valuable means of assisting clinicians in selecting appropriate Gn dose for stimulation. In IVF stimulation maximal effect is reached with FSH dosages 225 IU [15]. There was no significant difference (p>0.05) in the level of inhibin B among days ( 3, 7 of the cycle and day of hCG injection) in Poor group. In Normal group, there was a high significant difference ( p<0.001) in the level of inhibin B among days(3,7 of the cycle and day of hCG injection), while in High group ; there was a

significant difference (p<0.05) in the level of inhibin B among days (3, 7 of the cycle and day of hCG injection). Our results agree with Mio and Hung [16] who reported that serum inhibin B concentration on day 5 of rFSH (day7 of menstruation) stimulation was a high significant difference among different ovarian response . Also agree with Hlena [17] who found that number of follicles less than 10 mm correlate positively with inhibin B serum concentration, it's also in total agreement with Eldar-Geva *et al* [18], they found that inhibin B measured 2–6 days after starting COS was highly correlated with the number of oocytes retrieved and with poor ovarian response . While decrease in the level of serum inhibin B at day of hCG injection could be explained by the fact found by Byung C.J *et al* [19], they demonstrated that the number of immature as well as mature oocytes retrieved in stimulated IVF cycles is independently correlated with serum AMH and inhibin B levels measured on ovulation triggering, So inhibin B is better predictor than E2 in detection of early

ovarian response between poor and normal response, and this is in agreement with results obtained by Eldar-Geva *et al*<sup>[18]</sup>, they reported that serum inhibin B levels measured early during FSH administration for IVF may be of value in predicting the number of oocytes to be retrieved in normal and low responders. There is no significant difference ( $p > 0.05$ ) in the level of FSH and LH among the three groups at days (7 and day of hCG injection), this is in agreement with the same results found by Mio and Hung<sup>[16]</sup>. There was a high significant difference ( $p < 0.001$ ) between the three groups in the number of follicles, fertilized oocytes and embryo numbers, these results confirm those results found by other authors (Mio and Hung and Peñarrubia J, *et al*<sup>[16,20]</sup>) who proposed that the clinical response to Gn treatment would be a more direct way to assess ovarian reserve and a better predictor of fertility.

Total pregnancy rate in this study was 42% with the use of a single embryo transfer (mostly blastocyst), this result agrees with Christine C. and Catherine R.<sup>[21]</sup>, who reported that single blastocyst transfer leads to improve pregnancy rate. Also agrees with other researchers (Scott RT *et al* and Martin JS *et al*<sup>[22,23]</sup>), who reported that FSH less than 15 IU/L were twice as likely to conceive than women with FSH value between 15 and 24.9 mIU/ml. There was no significant difference ( $P > 0.05$ ) in the pregnancy outcome among the three groups (poor 10.52%, normal 73.68%, high group 15.78% (P-value was 0.930) this result agrees with the result found by Mio and Hung, Toner JP. *et al* and Simon C. *et al*<sup>[16,24,25]</sup> reported that although there were significant differences in the numbers of follicles, oocytes, fertilized oocytes, and embryos among poor, Normal, and High groups, the cycle pregnancy rates were not different. There was no significant difference ( $P > 0.05$ ) between the pregnancy outcome and all the variables (age, number of follicles, fertilized eggs, embryos). This result was consistent with Mio and Hung<sup>[16]</sup> who found that the success of IVF pregnancy depends on several factors such as patient's age, embryo quality, uterine receptivity, and a perfect dialogue between good-quality embryos and receptive endometrium. So the correlation between ovarian response and IVF pregnancy outcome remains controversial<sup>[24]</sup>. There was a significant negative correlation ( $r = -0.45$ ,  $p$  value 0.045) between the age and serum inhibin B level day 7, means with increasing the age, ovarian response to stimulation will decrease. In view of this we can conclude that serum inhibin B is a better predictor than E2 for ovarian response, and our results are confirm previous results reported by other authors<sup>[18,16]</sup>. It was concluded from this study, that serum inhibin B obtained at day 5 of Gn therapy offer's an early and accurate prediction of ovarian response to Gn stimulation in ICSI group. Serum inhibin B obtained at day 5 of Gn therapy predict poor ovarian response and OHSS, and it's a more sensitive index than E2 in early detection of ovarian response in different age groups. Cycle pregnancy rate does not correlate with the ovarian response.

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