# Day 3 Serum Inhibin B Level of Poor and Good Ovarian Responders in the IVF-Program

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**Abstract**

The aim of this study was to compare day-3 serum inhibin B levels of poor and good ovarian responders in the IVF-program. The study group consisted of 20 poor ovarian responders. The control group composed of 40 good ovarian responders who had similar demographic characteristics. The serum inhibin B levels were analysed by two-site-immunosorbent assay or sandwich ELISA (Enzyme Linked ImmunoSorbent Assay). The mean inhibin B level of the study group was 113.18 ± 57.96 picogram per millilitre and of the control group was 94.05 ± 61.81 picogram per millilitre. There was no statistically significant difference. The results might be useful as base-line data for further study.

**Key word** : Inhibin B, Poor Ovarian Responders, IVF

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A significant number of infertile women who have undergone controlled-ovarian-hyperstimulation cycle for *in vitro* fertilization (IVF) had poor ovarian responses. They had to cancel the cycles with much time and money wastage. Nowadays, methods to predict the ovarian responses include evaluation of day 3 serum follicular stimulating hormone (FSH), estradiol with or without combination with age, and clomiphene citrate challenge test ect[1,2]. None of these tests is ideal. Day 3 serum inhibin B seems to be more useful because it is directly secreted from the growing granulosa cells[3]. However, there

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is some debate. Thus, it is interesting to evaluate the serum inhibin B levels between good and poor ovarian responders in *in vitro* fertilization program.

Inhibin B was first discovered in 1932 by Mc Cullagh, who found a glycoprotein extracted from human testis and identified its properties of inhibiting pituitary FSH synthesis and secretion[4]. Later, other tissues were found to be sources of inhibin B; such as ovary, brain, adrenal gland, kidney, spleen, bone marrow, and placenta etc. Inhibin has specific functional importance in different tissues, but the exact mechanism has not been clearly established[4].

Inhibin is composed of alpha and beta subunits that are linked together by disulfide bonds. The three kinds of inhibin subunits ($\alpha$, $\beta_A$, $\beta_B$) paired to form inhibin A ($\alpha\beta_A$) and inhibin B ($\alpha\beta_B$)[5-7]. In 1960, the assay to detect inhibin was radioimmunoassay (RIA) which was non-specific as it detected not only $\alpha$-subunit of inhibin B but also that of inhibin A. Therefore, the results were usually inaccurate. Nowadays, there is an assay to identify inhibin B more specifically by two-site or sandwich ELISA[4,8].

In female ovaries, inhibin A is synthesized by granulosa cells of mature follicles and corpus luteum, whereas inhibin B is synthesized by granulosa cells of immature growing follicles. Thus, inhibin A reflects the development of dominant follicles while inhibin B reflects development of growing follicle. Inhibin B seems to represent the ability of the follicles to respond to ovarian stimulation in the IVF-program[7,9,10].

The authors designed an analytic study to compare the inhibin B level of poor and good ovarian responders in the IVF program at the Reproductive Medicine Unit, King Chulalongkorn Memorial Hospital.

## MATERIAL AND METHOD

Infertile women in the IVF program at the Reproductive Medicine Unit of King Chulalongkorn Memorial Hospital were retrospectively recruited in this study. After reviewing the demographic data, the authors collected the details of history and associated treatment. The subjects were classified into the study group and control group according to the results of their treatment. The criteria for poor ovarian responders was that less than three follicles with diameters of more than fourteen millimetres on day 8-9 of gonadotropin treatment-IVF cycle, or when requirements for hCG injection were not accomplished following 4-5 additional treatment days as defined by Creus et al[11]. Twenty poor ovarian responders and forty good responders were selected. All subjects had no medical or endocrinological diseases and retained both ovaries. If they had had hormonal treatment in the 3-month interval before the study, they were excluded from the study. Blood was drawn for day 3 hormonal profiles and the serum was stored at -20°C for analyzing the inhibin B levels of the whole subjects in the same session in order to avoid inter/intra-assay variation. The results were analysed by unpaired *t*-test.

### Method for measuring serum inhibin B level

The Oxford Bio-Innovation Inhibin B Immunoassay kit (MCA 1312, Serotec, Oxford Bio-Innovation Ltd, UK) was chosen for measuring serum Inhibin B levels. The kit was a solid phase sandwich ELISA (Enzyme Linked ImmunoSorbent Assay). According to the principle of two monoclonal antibodies, the first antibody coated the microtitre plate was specific for beta-B subunit of inhibin and the second one attached to the other end of the inhibin was specific for alpha subunit. The latter antibody coupled to alkaline phosphatase which would react with the sensitive amplified substrate included in the kit. The reaction resulted in a red-reactive product with a colour intensity that was directly proportional to the concentration of inhibin B presented in the sample.

The red colour intensities were measured by spectrophotometer (Automated microplate leader, Model Elx800, Bio-tek instruments Inc, USA). Translation of the photo-absorbance to inhibin B concentration was performed by calibrating with the standard curve.

This method was specific for inhibin B without cross-reaction to inhibin A or other subunits.

The inhibin B standard was prepared by extracting a mixture of inhibin forms from human follicular fluid. The concentration was determined by calibration against recombinant 32 Kd inhibin B.

Sensitivity of the assay was <15.0. Intra- and inter-assay variation CV were 4.1 and 7.8 respectively. Accuracy range was 76.5 – 91.7 per cent and $\bar{X}$ was 82.8 per cent.

### Method for measuring other serum hormone levels

Other hormones were measured by standard fluorescent immunoassay.

### Stimulation regimen

Ovarian stimulation was carried out with recombinant FSH under pituitary suppression with gonadotrophin-releasing hormone (GnRH) agonist according to the protocol previously reported(11). Gonadotrophin stimulation of the ovaries was started when serum estradiol concentrations declined to <50 picogram per millilitre (pg/ml) and vaginal sonographic scan showed an absence of follicles >10 millimetres (mm) diameter. The dosage and type of the recombinant FSH used was individually justified and adjusted according to the ovarian responses. The criteria for human chorionic gonadotrophin (hCG) administration were the presence of two or more follicles >18 mm in diameter with ≥5 follicles measuring ≥14 mm in association with a consistent rise in serum estradiol concentration. Oocyte aspiration was performed with vaginal ultrasonographic guidance 36 hours after hCG administration. Up to four embryos per patient were replaced and the luteal phase was supported with additional doses of hCG. The cycle was cancelled when there were <2 follicles with a diameter ≥14 mm after 8-9 days of gonadotrophin therapy or when requirements for hCG injection were not accomplished following 4-5 additional treatment days.

## RESULTS

The mean ages of both groups were similar. Interestingly, the most common causes of infertility in good responders were male factors, followed by multifactorial causes, whereas, the common causes of infertility in poor responders were endometriosis and unexplained causes.

Day 3 serum hormonal profiles of both groups were compared. There were no statistical differences of serum inhibin B, FSH, LH and estradiol in both groups.

**Table 1. Day 3- hormonal profiles.**

| | Poor responders | Good responders | P value |
|---|---|---|---|
| Inhibin (pg/ml) | 94.05 ± 61.81 | 113.18 ± 57.96 | 0.220 |
| FSH (IU/L) | 10.30 ± 7.21 | 6.61 ± 2.48 | 0.056 |
| Estradiol (pg/ml) | 58.06 ± 37.63 | 51.20 ± 31.65 | 0.607 |
| LH (IU/L) | 3.66 ± 1.86 | 4.23 ± 1.70 | 0.061 |

**Table 2. Percentage of causes of infertility.**

| Causes of infertility | Poor responders | Good responders |
|---|---|---|
| Male factor | 30.0* | 52.5 |
| Tubal factor | 15.0* | 7.5 |
| Endometriosis stage I/II | 20.0* | 12.5 |
| Oligo-ovulation | - | - |
| Unexplained | 10.0 | 5.0 |

* $p<0.05$

**Table 3. Stimulation responses.**

| | Poor responders | Good responders |
|---|---|---|
| Number of follicles | 0.40 ± 0.88 | 12.58 ± 5.68 |
| Number of days of stimulation | 8.85 ± 3.19 | 11.53 ± 1.46 |
| Ampoules of gonadotropins | 28.40 ± 10.51 | 37.65 ± 10.27 |

Data shown as Mean ± SD

## DISCUSSION

From the study, it was found that there were no statistical differences of inhibin B levels between poor and good ovarian responders. According to the knowledge that inhibin B was synthesized and secreted by granulosa cells, inhibin B should be a more accurate marker for ovarian secretory capacity and reserve than FSH. Nevertheless, the ovarian responses seemed to be related to many other factors besides ovarian reserve. These confounding factors included doses and types of gonadotrophin, appropriate protocols and procedures, timing of stimulation, oocyte qualities and their micro-environments etc. Most peri-menopausal women had wide ranges of inhibin B fluctuation. They responded well in some cycles, but poorly in others. Thus, they may have been classified in the wrong groups, resulting in classification bias. This bias had an important effect on the interpretation of the results because the women had different stimulation outcomes in each cycle. Some responded well in some cycles, but poorly in others. However, the studies of Hall,[12] Corson[13] and Creus[11] reported similar results, but Seifer[14], Danforth[15] and Pellicer[16] found different results.

Seifer[14] studied women with mean ages less than those in the present study (34.8-35.4 years old compared with 36.7-37.0 years old). He had set 45.00 pg/ml of serum inhibin B as the cut off value of day 3 serum inhibin B, whereas Hall[12] chose 86.00 pg/ml and the present study had mean serum inhibin B of the study group of 94.05 pg/ml. This might account for the dissimilar results.

The study of Pellicer[16] used immunoreactive α-inhibin which cross-reacted with a number of similar proteins other than inhibin B such as pro α-C, inhibin A and activin. This might account for the different results.

Importantly, the limitation of time and funds delineated the present study to be a descriptive design with a small sample size. However, the data obtained might be useful as preliminary results for further studies.

In conclusion, there was no definite statistical differrence of mean serum inhibin B between poor and good ovarian responders. A number of limitations and confounding factors of this descriptive study interfered with the conclusions.

Nowadays, inhibin B assay is still technically challenging without an international assay standard and the cost is quite high. Further work is warranted in this field.

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# การทำนายการตอบสนองของรังไข่ต่อการกระตุ้นไข่ ด้วยการใช้ระดับค่าซีรั่มอินฮิบิน บี ในกระบวนการปฏิสนธินอกร่างกาย

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คู่สมรสที่มีบุตรยาก ซึ่งฝ่ายสตรีได้รับการรักษาด้วยการกระตุ้นไข่ เพื่อดำเนินการทำเด็กหลอดแก้วต่อ มีสตรีจำนวนหนึ่ง ตอบสนองต่อการกระตุ้นไม่ดี และต้องยกเลิกการกระตุ้นรอบนั้น ผู้วิจัยจึงได้ทำการศึกษา ระดับของ inhibin B ในซีรั่ม ในกลุ่มที่ตอบสนองต่อการกระตุ้นดี 40 ราย เทียบกับ กลุ่มที่ตอบสนองไม่ดี 20 ราย ในสตรีที่เข้ารับการตรวจรักษา ที่หน่วยเวชศาสตร์การเจริญพันธุ์ ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย สำหรับเกณฑ์ของการตอบสนองไม่ดีต่อการกระตุ้นไข่กำหนดตาม Stadtmauer คือ ได้จำนวน follicles น้อยกว่า 2 ใบ ที่มีขนาดใหญ่กว่า 18 มิลลิเมตร หรือระดับเอสตราไดออลในซีรั่มน้อยกว่า 1,850 พิโคโมลต่อลิตร ในวันที่ 8-9 ของการกระตุ้น นำค่าinhibin B ที่เจาะในช่วงวันที่ 3 ของรอบประจำเดือนจากประชากรทั้งสองกลุ่มมาตรวจวิเคราะห์ด้วย two-site-enzyme-linked immunosorbent assay (ELISA) และนำผลที่ได้ มาคำนวณทางสถิติ ได้ค่าเฉลี่ยและส่วนเบี่ยงเบนมาตรฐาน ของกลุ่มศึกษาเท่ากับ 94.05 ± 61.18 พิโคกรัมต่อมิลลิลิตร และ กลุ่มควบคุมเท่ากับ 113.18 ± 57.96 พิโคกรัมต่อมิลลิลิตร ซึ่งไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ จากผลการวิจัยนี้ อาจจะกล่าวในเบื้องต้นได้ว่า ค่าinhibin B ในซีรั่ม ในช่วงวันที่ 3 ของรอบประจำเดือน ในทั้งสองกลุ่มไม่แตกต่างกัน ข้อมูลที่ได้นี้อาจใช้เป็นพื้นฐานสำหรับศึกษาเพิ่มเติมต่อไป

**คำสำคัญ** : อินฮิบิน บี, การตอบสนองไม่ดีต่อการกระตุ้นไข่

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