

Citation: Corson, S.L., Gutmann, J. , Batzer, F.R., Wallace, H., Klein N., Soules, M.R. Inhibin-B as a Test of Ovarian Reserve for Infertile Women. African Journal of Medical Sciences, 2020, 5 (4) ajmsc.info

Inhibin-B as a Test of Ovarian Reserve for Infertile Women

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Abstract

Background: As demographic studies document postponement of pregnancy during the peak reproductive years and the desire by many women to reproduce in their later years, evaluation of ovarian reserve becomes more important as a screening test before embarking on arduous and expensive programmes of infertility therapy. The clomiphene citrate challenge test has become one of the more popular and widely accepted methods of testing for ovarian reserve (Navot *et al.*, 1987; Scott and Hofmann, 1995; Hansen *et al.*, 1996; Hofmann *et al.*, 1996).

Objective: To evaluate the inhibin-B as a test of ovarian reserve for infertile women, and to compare the standard clomiphene citrate challenge test with inhibin-B serum concentrations.

Materials and methods: The inhibin-B test was performed in a group of 106 women at risk for compromised ovarian function on cycle days 3 and 10 as a negative predictor of pregnancy. Mean duration of follow-up was 8.25 months in 95 patients with 30 pregnancies recorded (plus one biochemical). Inhibin-B concentrations on cycle days 3 and 10 were correlated only with each other and not with serum oestradiol, follicle stimulating hormone (FSH) and/or pregnancy rates.

Results: Pregnancy occurred in 34.5% (10/29) of all patients with inhibin-B values ≥ 45 pg/ml on cycle day 3 and in 31.8% (21/66) of those with values < 45 pg/ml. For FSH > 11 mIU/ml on either day, pregnancy rate was 13.6% versus 38.4% for FSH of ≤ 9 mIU/ml ($P = 0.03$).

Conclusion: The clomiphene citrate challenge test is useful as an indication of ovarian reserve but failed to find clinical value for inhibin-B testing.

Keywords: Inhibin-B test, Ovarian reserve, Clomiphene citrate challenge test, Infertility.

Introduction

Development of specific two-site enzyme-linked immunoassays (ELISA) to measure both inhibin-A and inhibin-B allows investigators to track the changes of these two heterodimers in serum during the menstrual cycle (Groome *et al.*, 1994). Inhibin-A is low in the follicular phase, rising at ovulation to its eventual maximum in the midluteal phase. Inhibin-B, however, has an early follicular phase elevation followed by a decrease before another brief peak just after the

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luteinizing hormone (LH) surge and subsequent lower values in the luteal phase (Groome *et al.*, 1994). This pattern suggests that inhibin-B as a granulosa cell product plays a role in follicular development with the possibility that serum concentrations reflect follicular function and oocyte number. Another study suggested that decreased inhibin-B secretion was a reflection of a diminished ovarian follicular pool in older women (Klein *et al.*, 1996).

Seifer *et al.* studied 156 women who underwent 178 cycles of assisted reproductive technology and measured day 3 inhibin-B concentrations in an effort to determine if this hormone was predictive of a subsequent poor response to ovulation induction (Seifer *et al.*, 1997). Follicle stimulating hormone (FSH) and oestradiol concentrations were also measured in their study. Using a threshold value for inhibin-B of >45 pg/ml (conversion factor to SI units: 1), women whose serum concentration was less than this value demonstrated a decreased oestrogen response to stimulation, a reduced number of oocytes retrieved, three times the cancellation rate and only a 28% clinical pregnancy rate per initiated cycle compared with women having a 'normal' value. The current study was designed to examine further a possible role for inhibin-B as a predictor of the ability to achieve pregnancy in a group of infertile patients, who also had a standard day 3 and day 10 clomiphene citrate challenge test.

Materials and methods

Subjects: With institutional review board approval, female patients seen at the Women's Institute in Philadelphia from November 1996 to April 1997 who were seeking pregnancy and were aged >35 years or had a history of oligo-ovulation or a poor prior response to ovarian stimulation, had blood drawn on cycle day 3 for oestradiol and FSH concentrations. An aliquot was saved and frozen at -20°C for later determination at the University of Washington (Seattle) for FSH and inhibin-B concentrations. Patients then received clomiphene citrate 100 mg per day from day 5 through to day 9. Blood samples were drawn on day 10 for repeat determination of FSH, oestradiol, and inhibin-B. Patients with FSH values >15 mIU/ml (conversion factor to SI units: 1) on cycle day 3 were referred to an egg donor programme and are not included in this report. All the other patients had a complete diagnostic work-up and appropriate therapy for infertility according to the findings. The therapies employed included ovulation induction, intrauterine insemination, and hysteroscopic and/or laparoscopic repair of uterine or pelvic pathology.

Laboratory determinations: Oestradiol determinations were done with an automated immunomagnetic separation assay (Bayer, Tarrytown, N.Y., USA) with intra- and inter-assay coefficients of variation (CV) between 2 and 8% depending on concentration. FSH determinations in Philadelphia were performed with a similar automated technique with intra-assay and inter-assay CV of 4.0 and 4.6%, respectively. FSH and oestradiol determinations were done in batches. FSH determinations in Seattle were performed with a solid phase, two-site fluoroimmuno-metric (Delfia, Wallace, Inc., Gaithersburg, MA, USA) assay in batches with CV of 2.3 and 4.6% (Wallace Inc.). The inhibin-B assay was an ELISA with a monoclonal antibody directed against the Beta B subunit. Ninety-six well plates coated with antibody were kindly provided by Nigel Groome (Oxford Brookes University, Oxford, UK) and the human

recombinant inhibin-B standard was graciously donated by Teresa Woodruff (Northwestern University, Chicago, IL, USA). The assay detection limit was <15 pg/ml; activin A and B, follistatin, and purified human pro- α C have <0.1% cross-reactivity, and recombinant inhibin-A has a 0.05% cross-reactivity in this assay system. The intra- and inter-assay CV were <10%. All samples were assayed in duplicate in serial assays using a polynomial least squares analysis programme. The lower limit of detectability was 15.6 pg/ml for each of three assays. No samples were measured on different assays and then analyzed. Fig. (1) shows standard curve of the assay. **Statistics:** Statistical methods employed to analyze results included Pearson correlation coefficient and χ^2 .

Results

Serum samples from 106 infertile women, mean age 37.1 years (SD 4.1, range 24.9–46.3), were analyzed for oestradiol, FSH and inhibin-B obtained on day 3 and day 10 of a menstrual cycle in which a clomiphene citrate challenge test was performed. The duration of follow-up was 8.25 months (SD = 3.96) in 95 patients; 11 were lost to follow-up. There were 23 deliveries, four ongoing pregnancies, three spontaneous abortions and one biochemical pregnancy. There were eight categories of infertility considered: male factor, tubal factor, ovulatory disturbance, endometriosis, cervical factor, uterine factor, unexplained infertility, and male factor treated with donor insemination. Each patient was assigned a single primary diagnosis.

Table (I): Patient population demographics and pregnancy rates

Diagnosis (<i>n</i>)	Age (years) ^a	% ART	% surgery	% pregnant
Male (<i>n</i> = 26)	36.4 ± 3.2	38.5	11.5	19.2
Tubal (<i>n</i> = 25)	36.0 ± 4.2	48.0	52.0	32.0
Ovulatory (<i>n</i> = 5)	35.0 ± 5.1	20.0	20.0	40.0
Endometriosis (<i>n</i> = 9)	34.5 ± 2.9	66.7	55.6	22.2
Unexplained (<i>n</i> = 18)	39.9 ± 3.6	27.8	11.1	61.0
Uterine (<i>n</i> = 4)	38.7 ± 3.8	25.0	50.0	50.0
Cervical (<i>n</i> = 1)	35.5	0	0	0
No initial female				
Factors, AID (<i>n</i> = 5)	40.3 ± 1.5	0	0	20.0
Oocyte donor (<i>n</i> = 2)	25.3 ± 0.6	100	0	0
Total (<i>n</i> = 95)	36.7 ± 4.3	45.3	27.4	32.6

^aValues = mean ±SD. ART = Assisted reproductive technique

AID = Artificial insemination by donor

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Table I shows the distribution of subjects by diagnosis and pregnancy rate. In some cases, such as endometriosis, patients may have been treated with both surgery and assisted reproductive techniques. There was no statistical difference in pregnancy rate according to age among diagnostic categories and therapeutic modalities employed. Of those treated either with office therapy or surgery, 14/53 conceived while 17/42 conceived with assisted reproductive techniques, either as in-vitro fertilization (IVF) or gamete intra-Fallopian transfer. In this selected group of women studied, most of whom were between 35 and 40, neither FSH nor inhibin-B was related to age.

An inhibin-B threshold value of 45 pg/ml was considered the normal lower limit based on previous investigations (Seifer *et al.*, 1997; Hofmann *et al.*, 1998). Counting only those 95 patients available to follow-up, inhibin-B values of ≤ 45 pg/ml were found in 66 women on day 3 and in 61 on day 10.

Table (II): Pregnancy rate for inhibin-B and FSH combinations

Hormones	Day 3 (%)	Day 10 (%)
Inhibin B >45 pg/ml	10/26 (38.5)	12/30 (40.0)
FSH <10 mIU/ml		
Inhibin B >45 pg/ml	0/3 (0)	2/4 (50.0)
FSH ≥ 10 mIU/ml		
Inhibin B 45 pg/ml	19/38 (50.0)	14/44 (31.8)
FSH <10 mIU/ml		
Inhibin B <45 pg/ml	2/28 (7.1)	3/17 (17.6)
FSH ≥ 10 mIU/ml		
Total	31/95	31/95

Table II shows the pregnancy rate according to day 3 and day 10 inhibin-B concentrations using 45 pg/ml as threshold value, and by day 3 and day 10 FSH concentrations, using 10 mIU/ml as a threshold value.

FSH concentrations >10 mIU/ml occurred in 31 & 21 patients on day 3 and day 10 respectively. FSH elevation on either day was associated with reduced reproductive performance. The statistical significance of this was demonstrated only when the thresholds for FSH were either >11 mIU/ml or ≤ 9 mIU/ml. For FSH >11 mIU/ml, pregnancy rate was 13.5 compared with 8.4% for FSH of nine or less ($P = 0.03$).

The correlation between FSH values obtained from the same sample with aliquots measured in both laboratories was good with the correlation coefficient being 0.87 for day 3 samples and 0.96 for day 10 samples.

Day 3 oestradiol concentrations >80 pg/ml (SI Unit conversion factor = 3.67) were found in four patients with FSH >10 mIU/ml on either or both day 3 and day 10, but this number was too small

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to permit analysis of the possible inverse relationship to pregnancy. Day 3 FSH concentrations were not related to oestradiol concentrations.

Table III shows the correlation and *P* values between inhibin-B, oestradiol and FSH on cycle days 3 and 10. No significant correlations were found.

Table III : Correlations between inhibin-B versus FSH and oestradiol on cycle days 3 and 10

Days	Day 3 inhibin	Day 10 inhibin-B
Day 3 oestradiol	$r = 0.16 (P = 0.10)$	
Day 3 FSH	$r = 0.11 (P = 0.26)$	
Day 10 oestradiol		$r = 0.08 (P = 0.41)$
Day 10 FSH		$r = 0.12 (P = 0.24)$

No statistically significant correlations were found.

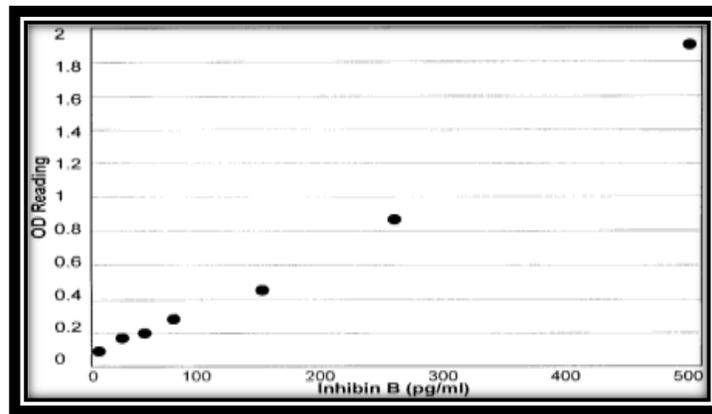


Fig. (1): Standard curve for inhibin-B assay.

Discussion

The clomiphene citrate challenge test has been well accepted as a prognostic indicator of reproductive performance having more value as a negative predictor than as a positive one, with elevated FSH concentrations thought to be related to diminution both in oocyte quality and quantity. Inhibin-B, as a product of the granulosa cells, might also be an indirect indicator of the numbers of pre- and small antral follicles early in the follicular phase. The finding that inhibin-B had a cyclical variation gave hope that this potential might be realized. In the study by Seifer *et al.* a low day 3 serum inhibin-B concentration was predictive of poor response to ovulation induction and of decreased success during IVF cycles (Seifer *et al.*, 1997). Inhibin-B concentrations were found to increase within follicular fluid as growth during the menstrual

cycle proceeded with a mean of 19.2 ng/ml in 4 mm follicles and 409 ng/ml in 13 mm follicles, but with a decline in 17 mm follicles to 275 ng/ml.

The study by Hofmann *et al.* correlated inhibin-B concentrations with results of CCCT in 19 patients with normal ovarian reserve testing and in 15 whose CCCT was abnormal (Hofmann *et al.*, 1998). Inhibin-B concentrations were higher on day 10 than day 3 for all patients, and women with normal CCCT results had higher inhibin-B concentrations on both days than those with diminished ovarian reserve. There was a negative correlation between FSH and inhibin-B concentrations on day 3 ($r = -0.37$) and on day 10 ($r = -0.41$). A positive correlation was found between day 10 oestradiol and inhibin-B ($r = 0.67$). As documented in Table III, no such correlations were found in our sample of 106 women.

Klein *et al.* found that women aged 40–45 compared with younger women had lower serum inhibin-B concentrations both in the early follicular phase and on the day of oocyte retrieval in a natural cycle, but no differences in follicular fluid concentrations could be found when the older patients were compared with women aged 20–25 years (Klein *et al.*, 1996).

Although not the major focus of this study, it was found that the CCCT was once again validated as having prognostic significance in evaluation of women aged >35 years and in those with a poor stimulation history. The correlation between laboratories for FSH results was surprisingly good, considering that different methods of analysis were employed. The differences were well within the limits described for aliquot sampling by multiple laboratories (Hershlag *et al.*, 1992). It was not possible to find any value in inhibin-B testing either on day 3 or day 10 at the usual normal concentration threshold value of 45 pg/ml. Because these results are discordant with those reported by Seifer *et al.*, corroborating studies are necessary (Seifer *et al.*, 1997). A plausible explanation for the discordance between this study and prior studies in relation to inhibin-B as an indicator of advanced reproductive age is assay methodology. There is no international assay standard for inhibin-B yet, so comparisons of results between laboratories remains a problem. The studies by Seifer *et al.* (1997) and Hofmann *et al.* (1998) used as their assay standard an immunopurified preparation of human follicular fluid. The standard contains at least four forms of inhibin (33, 36, 55 and 66 kDa) that are biologically and immunologically active (Robertson *et al.*, 1996). This study used a recombinant inhibin-B standard as a single molecular form that represents the single most common and biologically active form (33 kDa) of inhibin-B. There are two schools of thought as to which standard is more relevant and there is no consensus yet. Furthermore, it should be recognized that the inhibin-B assay as performed with either standard is technically challenging and not readily available.

A recent study (Hall *et al.*, 1999) dealt with inhibin B serum measurements in 78 patients achieving pregnancy in assisted reproduction treatment in up to three cycles matched to 78 patients who were unsuccessful. It was concluded that the results did not support the use of day 3 inhibin-B as a predictive marker of IVF outcome. There was extensive overlap in baseline inhibin-B concentrations between pregnant and non-pregnant subjects, and inhibin-B alone failed to predict pregnancy. Additionally, another study (Schipper *et al.*, 1998) found a lack of correlation between maximum early follicular phase serum FSH concentrations and inhibin-B concentrations when measurements were performed on samples drawn daily and assayed with the Serotec follicular fluid standard.

One could argue that the group tested represented a heterogeneous population with infertility

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based on varied pathologies; but this is exactly what a screening test is all about—something applicable to the general population. FSH concentrations, whether solely as day 3 determinations or with a full clomiphene citrate challenge, have clinical relevance, whereas inhibin-B may or may not. Pregnancy as an endpoint is associated with many confounding variables—semen quality, tubal function and others besides ovarian function. Therefore, any screening test for ovarian function or oocyte quality has more value as a negative predictor than as a positive one, but establishment of an absolute threshold value above or below which pregnancy will not occur is probably not a useful clinical exercise. Instead, quoting pregnancy rates with any specific therapy according to (in this case) FSH concentrations seems to be a more useful approach. For the same reason calculations have not been included of predictive value or receiver operating curves since patients with FSH values of ≥ 15 mIU/ml were excluded from the data set, and the inhibin-B data showed no statistical correlation with pregnancy initiation. Until the inhibin-B assay is standardized and more readily available it would be prudent for clinicians to continue to assess ovarian reserve with measurements of FSH and oestradiol before and after clomiphene citrate stimulation.

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