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Physiology

Serum Inhibin B Correlates with Successful Ovulation in Infertile Women

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***Purpose*:** To investigate whether inhibin B and activin A serum and follicular fluid levels in infertile women undergoing induction of superovulation correlate with successful ovulation.
***Methods*:** Infertile women ($n = 16$) (30–43 years of age) undergoing induction of superovulation for assisted reproduction were studied. A blood sample was collected before and days 3, 8, and 12 during the induction of superovulation. A follicular fluid sample at the time of ovarian pick up was also collected. Serum and follicular fluid were assayed for inhibin B, activin A, and estradiol.
***Results*:** According to the successful follicular development women were divided in two groups: (A) responders ($n = 10$) and (B) poor responders ($n = 6$). Women of group A showed mean follicular fluid inhibin B levels higher than in group B ($P = 0.001$), while no significant difference for activin A levels was found. During induction of superovulation serum activin A levels did not change in both groups of women, while inhibin B and estradiol levels significantly increase only in responder women ($P < 0.001$). Serum inhibin B and estradiol levels correlated with follicles developed $\geq$10 mm ($P = 0.000$).
***Conclusions*:** Serum inhibin B is an effective marker of follicular development in infertile women undergoing induction of superovulation, and may represent a further marker for ovarian follicular capacity.

KEY WORDS: Activin; follicular fluid; inhibin; IVF; ovulation; serum.

INTRODUCTION

Inhibin B is a dimeric glycoprotein produced by the gonads with a recognized role in inhibiting FSH secretion (1–3), and in fertile-aged women its plasma concentrations are maximal in the follicular–luteal transition phase of the cycle, rising 4 days after the FSH peak, implying that FSH stimulates the production of inhibin B (4).

Among the etiologic factors proposed as cause of infertility (mechanical distortion of tuboovarian anatomy, abnormal serum and follicular fluid hormonal patterns, alterations in peritoneal fluid milieu and in the endometrial environment) on impairment of follicular development has a relevant incidence.

Inhibin B levels continue to increase during the early follicular phase, reaching their highest level at the midfollicular phase of the menstrual cycle. Inhibin B levels and number of oocytes retrieved were significantly lower in patients with endometriosis, suggesting an impaired granulosa cell function (5). In stimulated ovarian cycles, inhibin B levels increase markedly and in parallel with circulating estradiol levels as follicular growth and maturation occurs until administration of hCG. The secretion of large amounts of inhibin B by granulosa cells from preovulatory

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follicles makes it an attractive marker to elucidate the mechanism of infertility (5).

Activin A (βA/βA dimer) is a growth factor mainly secreted throughout pregnancy (6), but highly expressed in various organs and in ovary (7). A lot of data suggest an autocrine/paracrine role of activin A within the ovary, able to modulate the development and luteinization of the follicles and also the production and secretion of ovarian steroid hormones, decreasing progesterone and estradiol secretion, both basal and FSH-stimulated (8). The role of activin A in oocyte maturation is supported by reports of the expression of βA subunit (9) and type II receptors in rat (10) and mouse (11), and by a potent stimulatory effect on the in vitro rat oocyte maturation, being able to increase the signs of nuclear maturation within 48 h of in vitro culture (8). High activin A concentrations are measurable in follicular fluid, but very little changes according to the ovarian follicle maturational events are shown (12).

The aim of this study was to evaluate the levels of inhibin B and activin A in serum and follicular fluid obtained from patients undergoing ovarian superovulation and to correlate these levels with the follicle maturation, the number of oocytes recovered, and estradiol levels.

MATERIALS AND METHODS

Subjects and Sample Collection

A group of infertile women ($n = 16$) (age range 30–43 years) undergoing induction of superovulation treatment participated in the study. They had already attempted from one to three cycles of assisted reproduction.

The reasons for assisted reproduction were tubal obstruction, an abnormal semen profile, or unexplained infertility. The oligoasthenoteratospermia was defined by the Kruger cirteria: concentration $< 5 \times 10^6$; progressive motility $< 20\%$; normal morphology $< 20\%$. There was no evidence of endocrine disorders of the pituitary and/or ovary (hyperprolactivinemia, hypogonadotropic hypogonadism, or polycystic ovary syndrome defined as plasma LH elevated in the early follicular phase of a recent cycle to > 12 mU/mL and oligomenorrhea) in any subject, nor was there any evidence of premature ovarian failure or premature menopause. All subjects had normal adrenal and thyroid function. Before enrolled in the study, every woman underwent clinical and psychological examination, transvaginal ultrasound, and hormonal evaluation (baseline levels of estradiol, androstenedione, progesterone, testosterone, and cortisol; LH release after Gn-RH stimulation, and androgen plasma levels following dexamethasone suppression).

Women received Decapeptyl 3.75 (Ipsen Italia, Roma, Italy) subcutaneously in a single 3.75-mg dose for pituitary desensitization on day 14 of the preceding cycle to prevent receipt of therapy after spontaneous fertilization (13–15). On the 15th day of the preceding cycle, medroxyprogesterone acetate (10 mg/day) was given for 10 days to induce menstruation according the method of Meldrum *et al.* (14). Once menstrual bleeding began, vaginal ultrasonography was performed to rule out the presence of ovarian cysts or larger follicular residue.

On the second day of menstruation, human recombinant FSH in the form of follitropin-α (Gonal-F; Serono Pharma, Geneva, CH) was given in one daily subcutaneous dose of 300 IU, independent of the ultrasound finding and the results of the previous in vitro fertilization cycles, to stimulate development of multiple follicles.

Sixteen women were divided, according to the successful follicular development in two groups:

- Group A: responders ($n = 10$; women with sonographically documented follicular maturation)
- Group B: poor responders ($n = 6$; women without sonographically documented follicular maturation).

Transvaginal ovarian ultrasound scan and serum E2 levels were used for monitoring the follicular maturation. Follicular size was measured from the fifth day of FSH stimulation, every second day, and daily when the follicle diameter was > 15 mm. Blood samples for evaluating estradiol levels were collected before (basal), days 3, 8, 12, and ovarian pick-up during the IVF cycle. The blood was centrifuged to separate the serum and quickly assayed.

The criteria for defining an adequate follicular maturation were 1) the presence of at least three follicles >15 mm in diameter, 2) a leading follicle >17 mm in diameter, 3) estradiol levels >200 pg/mL per large follicle at the hCG (10000 IU) administration. Oocytes and follicular fluid were collected 35 h after the treatment by using transvaginal ultrasonography-guided follicle puncture; the follicular fluid sample collected was centrifuged and stored at -20°C until assay was performed.

Inhibin B Assay

Inhibin B concentrations were measured using a two-site enzyme immunoassay as previously described (4). Briefly, standards and samples were diluted as appropriate and mixed with an equal volume of 6% SDS. After 3 min at 100°C, tubes were cooled before adding freshly prepared hydrogen peroxide solution. Plates were incubated at room temperature, overnight. After washing with enzyme immunoassay (EIA) wash buffer, 50-μL alkaline phosphatase-conjugated Fab mouse anti-human inhibin α subunit was added, and plates were incubated for 3 h. Plates were washed and bound alkaline phosphatase was quantitated adding 50 μL of substrate solution. After 1 h of incubation, 50-μL amplifier solution was added and the plates were read at 492 nm on an automated ELISA plate reader (BRIO: Basic Radim Immunoassay Operator, Radim spa, Pomezia, Italy).

The assay detection limit for inhibin B was less than 5 pg/mL. Within- and between-plate coefficient of variation were 6.0 and 8.0%, respectively. Cross-reactions for the assay with the various proteins of the inhibin-related family were less than 0.1%.

Activin A Assay

Activin A concentrations were measured using a two-site enzyme immunoassay as previously described (16). Briefly, standards and samples were diluted as appropriate and mixed with an equal volume of 6% SDS. After 3 min at 100°C, tubes were cooled before adding freshly prepared hydrogen peroxide solution. After an additional time of incubation at room temperature, duplicate aliquots of denatured and oxidized samples/standards were transferred to antibody-coated microtiter plates. In activin A plates 25-μL biotinylated monoclonal antibody to the βA subunit was added, then plates were incubated at room temperature, overnight.

After washing with enzyme immunoassay (EIA) wash buffer, 50-μL streptavidin alkaline phosphatase was added, and plate was incubated for 1 h. Plate was washed and bound alkaline phosphatase was quantified according to the supplier's instructions.

The activin A plates was read at 492 nm on an automated ELISA plate reader (BRIO: Basic Radim Immunoassay Operator, Radim spa, Pomezia, Italy).

The limit of detection for activin A was 10 pg/mL, and intra- and interassay coefficients of variations (CVs) were 3.0 and 9.0%, respectively. Cross-reactions for the assay with the various proteins of the inhibin-related family were less than 0.5%.

Estradiol Assay

Serum concentrations of estradiol were measured by specific commercially available radioimmunoassay (RIA) kits (Radim; Pomezia, Rome, Italy). The sensitivity for estradiol was 10 pg/mL and the intra- and interassay CVs were 2.1 and 3.5%.

Statistical Analysis

Potential differences in each data set were analyzed by using the Student *t*-test. All data are presented as means ± SD when appropriate. A P value <0.05 was considered statistically significant.

RESULTS

According to the clinical response, Table I shows the two groups of patients divided: group A women with sonographically documented follicular

Table I. Data on Patients with Sonographically Documented Follicular Maturation During Recombinant Follicle-Stimulating Hormone Stimulation and Patients Without Sonographically Documented Follicular Maturation

Variable	Subjects		
	Responders ($n = 10$)	Poorresponders ($n = 6$)	P value
Mean (± SD) age	34.8 ± 2.8	36.8 ± 5.6	ns
Mean (± SD) gravidity	0.33 ± 0.5	0	ns
Mean (± SD) no. of ampules of gonadotropin administred	45.7 ± 7.1	41 ± 9.9	ns
Mean (± SD) no. of oocytes retrieved per patient	7.7 ± 5.0	1.5 ± 1.4	0.02
Mean (± SD) no. of oocytes > 17 mm in diameter retrieved per patient	2.2 ± 1.4	0	0.001
Fertilization rate (%)	40	0	0.01
Mean (± SD) no. of embryos transferred	2.6 ± 1.6	0	0.002
Clinical pregnancy rate per initiated cycle (%)	10 (1/10)	0	0.001
Cancellation rate per initiated cycle (%)	10 (1/10)	100	0.001
Spontaneous abortion rate per clinical pregnancy (%)	100 (1/1)	0	0.001

Table II. Serum Concentration of Inhibin B, Activin A, and Estradiol in Women Undergoing Recombinant Follicle-Stimulating Hormone Stimulation with (Group A) and Without (Group B) Sonographically Documented Follicular Maturation

	Inhibin B (pg/mL)	Activin A (pg/mL)	Estradiol (pg/mL)
Group A			
Basal	119.8 ± 9.8	0.23 ± 0.15	17.3 ± 8.5
Day 3 of FSH treatment	104.6 ± 9.6	0.22 ± 0.06	233.3 ± 5.7
Day 8 of FSH treatment	669.4 ± 294.7	0.20 ± 0.04	593.4 ± 176.04
Day 12 of FSH treatment	719.1 ± 180.6	0.27 ± 0.04	1494.0 ± 246.3
Oocyte pick up	685.2 ± 128.9	0.46 ± 0.12	1442.8 ± 258.3
Embryo transfer	746.2 ± 122.7	0.32 ± 0.03	1474.1 ± 206.1
Pregnancy test	722.0 ± 146.1	0.39 ± 0.15	1410.5 ± 318.0
Group B			
Basal	128.2 ± 49.3	0.17 ± 0.09	46.5 ± 5.2
Day 3 of FSH treatment	76.6 ± 58.4	0.20 ± 0.04	212.2 ± 217.7
Day 8 of FSH treatment	163.2 ± 60.5	0.23 ± 0.07	446.1 ± 312.5
Day 12 of FSH treatment	179.1 ± 119.8	0.26 ± 0.10	485.2 ± 116.3

maturation (responders); group B women without sonographically documented follicular maturation (poor responders). Table II shows the mean ± SD of serum concentration of inhibin B, activin A, and estradiol in responders and poor responders throughout the therapeutical protocol. Basal serum inhibin B, activin A, and estradiol levels did not differ between group A, and group B.

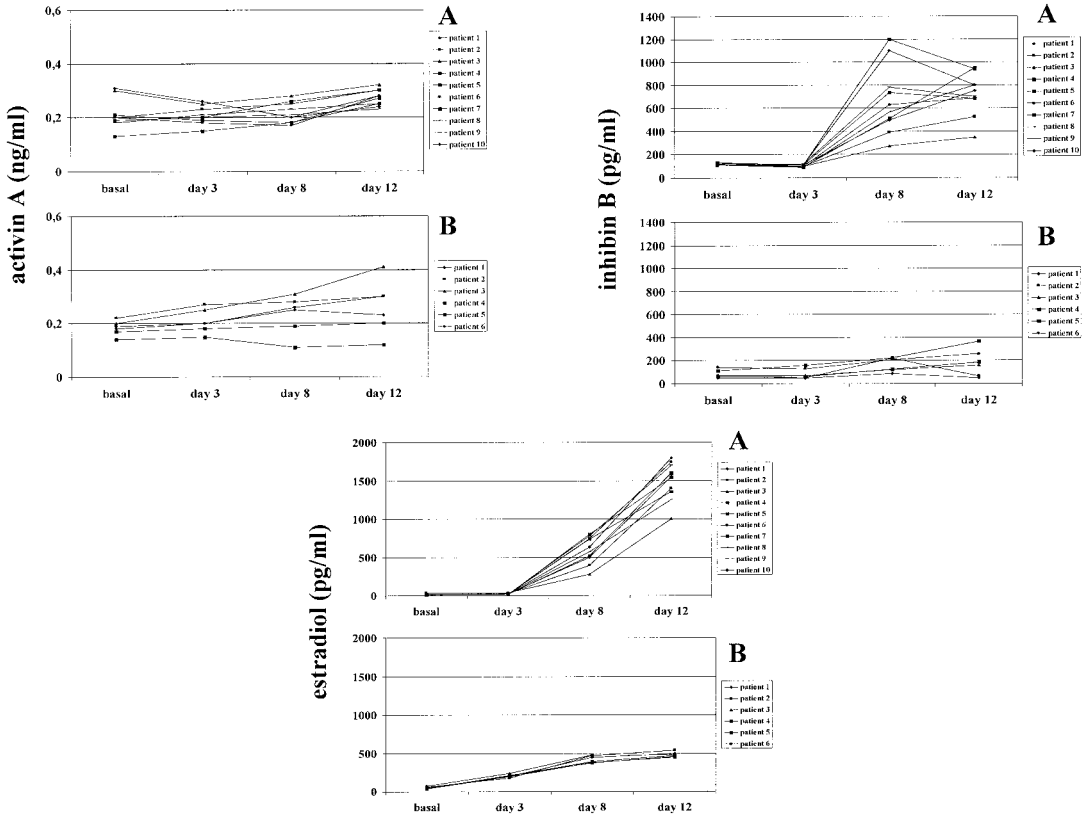

Fig. 1. Activin A, inhibin B, and estradiol levels in responders (A) and poor responders (B) during a cycle of IVF treatment. Blood samples were collected the day 0 (basal), the day 3, the day 8, and the day 12.

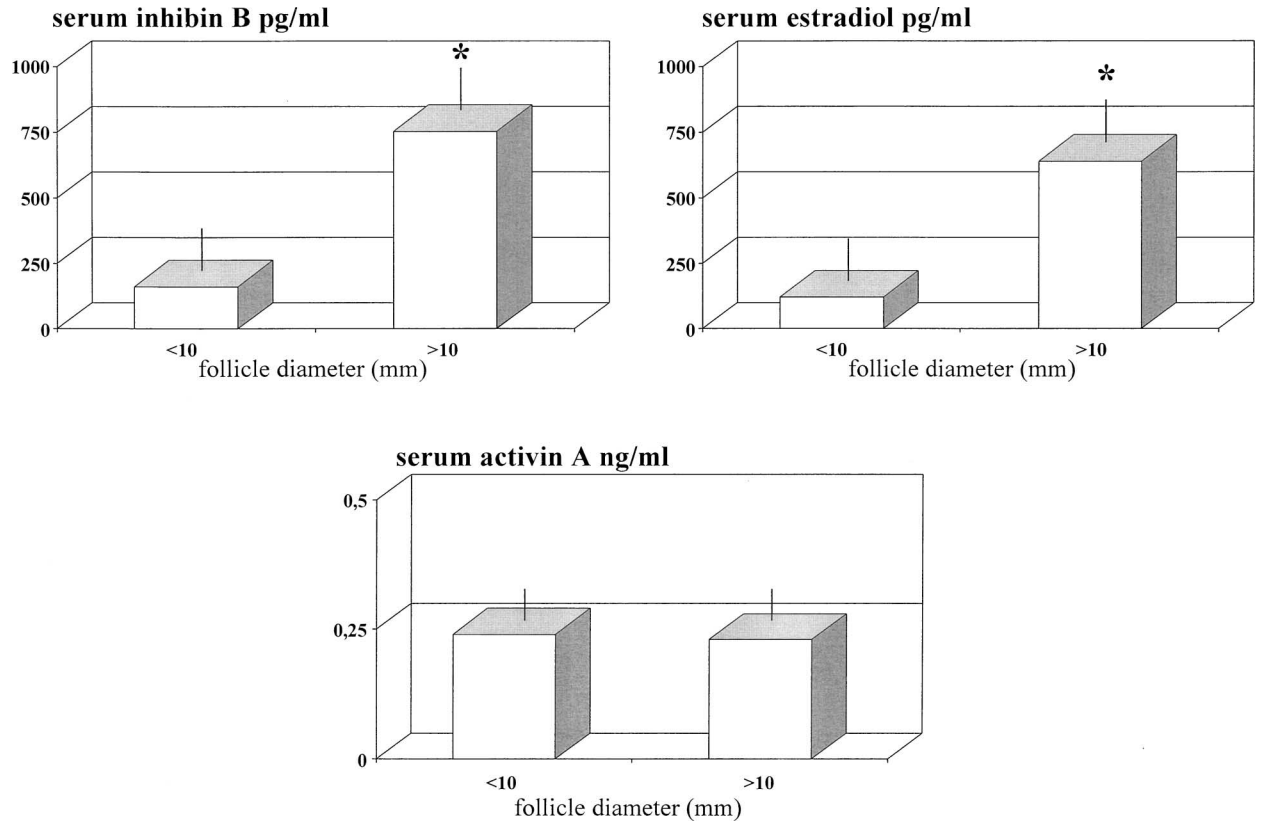

Fig. 2. Serum levels of inhibin B and estradiol on day 7 of recombinant FSH treatment comparison with follicle size ($^* p = 0.000$).

In responders women a significant increase of inhibin B and estradiol levels ($P < 0.001$) was shown while not in poor responders; activin A serum levels did not change in both groups of women (Fig. 1).

The follicle size $\geq$10 mm diameter correlated to higher concentrations of inhibin B and estradiol serum levels ($P = 0.000$) on day 7 of recombinant FSH treatment (Fig. 2).

The concentrations of inhibin B in follicular fluid in responder patients were higher than in poor responders ($P = 0.001$), while there is not significant difference for activin A levels (Fig. 3).

DISCUSSION

The present study provided insights into the relationship between inhibin B and the follicle development in infertile women undergoing induction of superovulation. A relationship between ovarian inhibin B and follicle number and the development after r-FSH stimulation was shown in healthy women, and was suggested as an indicator of the ovarian reserve (17).

As reported in the results mean inhibin B basal levels between normal and poor responders are not significant because the large standard deviation and the issue can only be addressed greatly increasing the study population. In addition the focus of the paper is the inhibin B values of day 3 where there was a significantly difference between the two groups. However, it is known already that women with diminished ovarian reserve have lower day 3 and day 10 inhibin B levels than women with normal ovarian reserve (18), thus supporting the concept of qualitative and quantitative ovarian reserve being closely related.

The marked stimulation of inhibin B by r-FSH early in treatment of infertile women suggests that inhibin B may be a useful predictor in monitoring ovarian hyperstimulation treatment for IVF. This observation provides an in vivo evidence for ovarian inhibin B as target of pituitary FSH.

Our present observation agrees with previous results showing in infertile women increased serum

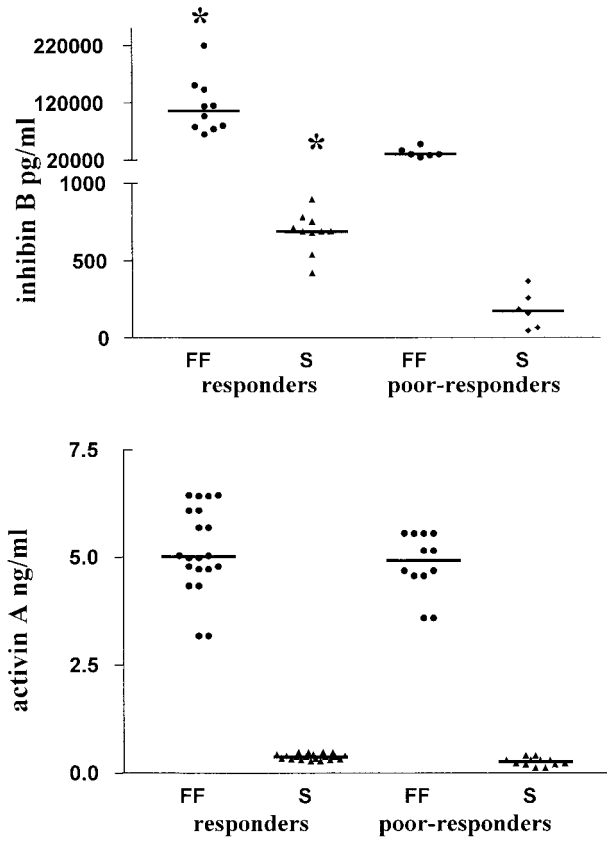

Fig. 3. Inhibin B, activin A levels in follicular fluid (FF) and serum (S) in responders and poor responders to IVF treatment ($^*p = 0.001$).

inhibin B levels during gonadotropin treatment, attributed to production by follicles. Early follicular phase antral follicle number positively correlates with total and mature oocyte numbers after gonadotropins stimulation for in vitro fertilization and is linked to inhibin B, androgen, and insulin in predicting ovarian follicle recruitment by gonadotropins (19).

Also the follicular fluid inhibin B levels resulted lower in poor responderrs women, reflecting the ovarian effect of gonatropins. Infertile women with a reduced ovarian activity do not respond to the exogenous and maturation of gonadotropins. The concentration of inhibin A in the follicular fluid is higher than inhibin B, considering that the inhibin-producing cells in patients are luteinized after hormone stimulation (20). At the contrary the determination of inhibin A in serum would not provide the same information, because the relationship between inhibin A and B in serum does not reflect their production rate by follicular cells (20).

In conclusion, our present study showed that inhibin B and estradiol levels increase in the serum and in the follicular fluid of women that respond to the treatment of superovulation for IVF, correlated with the size of the follicle. Therefore, inhibin B may be a marker of follicle development and that may represent an additional marker for the monitoring of ovarian responsiveness in assisted reproduction.

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