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Association of Inhibin B Serum Levels with Parameters of Follicular Response in a Randomized Controlled Trial Comparing GnRH Agonist Versus Antagonist Protocols for Ovarian Hyperstimulation

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***Purpose*:** To study the association of inhibin B with ovarian response to FSH stimulation, applying either GnRH agonist or antagonist.
***Methods*:** In a prospective randomized controlled trial, 46 patients undergoing COH received either triptorelin (group I, $n = 15$) or ganirelix (group II, $n = 31$). Parameters of follicular response and inhibin B serum levels were assessed.
***Results*:** Inhibin B before FSH stimulation was significantly lower in group I than group II. The FSH stimulation phase was significantly longer in group I than group II, and the total FSH dose was significantly higher with a comparable number of retrieved oocytes. Day 1 inhibin B in group I, but not group II, was significantly correlated with the number of large ovarian follicles and retrieved oocytes. In group II, but not group I, inhibin B on day 1 was inversely correlated with the daily and total FSH dose as well as FSH stimulation duration.
***Conclusions*:** The association of inhibin B serum levels with parameters of follicular response in COH is different in patients assigned to GnRH agonist vs. antagonist treatment protocols.

KEY WORDS: COH; GnRH agonist; GnRH antagonist; inhibin B.

INTRODUCTION

Inhibin B is produced in the ovary and has been identified as the main regulator of pituitary follicle stimulating hormone (FSH) secretion. It may also have important paracrine functions influencing folliculogenesis in the ovary itself (1). Two dimeric bioactive forms of inhibin, inhibin A and inhibin B, are known. The main source of inhibin A is the dominant follicle and corpus luteum, whereas inhibin B is predominantly produced in early developing follicles (2). Therefore, early follicular phase inhibin B serum levels have variously been reported as a potential marker of ovarian reserve and possible predictor of ovarian responsiveness during controlled ovarian hyperstimulation (COH) (3–5).

Whereas the response of inhibin B serum levels to FSH stimulation during GnRH agonist application has been studied elsewhere (6,7), controlled data on inhibin B serum levels in GnRH antagonist protocols are lacking. Today, both GnRH agonists and antagonists are used routinely for the prevention of premature LH surges during COH. Various studies (8,9) demonstrated a shorter duration of FSH stimulation and a lower amount of FSH needed for comparable stimulation efficacy when using GnRH antagonists. Therefore, it is of interest to investigate the mechanisms leading to the differences in follicular response

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to FSH stimulation, and also to find suitable predictors of ovarian response when using the different—GnRH agonist vs. antagonist—protocols. The study objective was the association of serum levels of inhibin B at various defined time points with the respective ovarian response to FSH stimulation in a randomized controlled study applying either GnRH agonist or antagonist.

MATERIALS AND METHODS

Subjects and Study Design

Women treated for infertility at our clinical center and participating in a multicenter, open-label, randomized trial assessing the efficacy and safety of the GnRH antagonist ganirelix in COH (10) were included in the study. Patients with indication for IVF or ICSI therapy were randomly assigned in a 1:2 ratio by an interactive voice response system to treatment with either the GnRH agonist triptorelin (Decapeptyl®, long protocol, group I) or the GnRH antagonist ganirelix (Orgalutran®, group II). Patients had to fulfill the following selection criteria: healthy female partners of infertile couples, age at the time of screening ≥18 but <39 years, BMI between 18 and 29 kg/m^2, regular menstrual cycles, and willing to give written informed consent. The study was approved by the ethics committee of our university and by the state medical board.

Treatment Protocol

In the agonist-treated group, pretreatment with triptorelin started between day 21 and 24 of the preceding cycle with a daily dose of 0.1 mg s.c. until and including the day of hCG administration. Ovarian stimulation started after 2 weeks of treatment when pituitary downregulation was established (serum estradiol <200 pmol/L). In the antagonist-treated group, ovarian stimulation was started on day 2 or 3 of menses. From day 6 of ovarian stimulation (day 6), ganirelix was administered at a daily dose of 0.25 mg s.c. until and including the day of hCG administration.

In both groups, ovarian stimulation was started using a daily fixed dose of recombinant FSH (rFSH, Puregon®, 150 IU s.c.) for the first 5 days of treatment. From day 6 onwards, the daily dose of rFSH could be adjusted and individualized per subject on the basis of the follicular growth as observed by ultrasonography and estradiol serum levels. For ovulation induction, 10,000 IU hCG (Pregnyl®) was administered subcutaneously when at least three follicles ≥17 mm in diameter were measured by ultrasonography. Oocyte retrieval was performed 36 h after hCG injection by transvaginal, ultrasound-guided aspiration. Up to three embryos were transferred to the uterine cavity 2 days after oocyte retrieval, and the luteal phase was supported with transvaginal administration of progesterone (Crinone 8%®).

Hormone Measurements

Morning blood was drawn for basal hormone values in the early follicular phase of the preceding cycle, on day 1, day 6, and at least every other day during ovarian stimulation until the day of hCG injection. Serum estradiol was measured using a competitive enzyme immunoassay (SR1®, Code 162632; BioChem ImmunoSystems GmbH, Freiburg, Germany) with a sensitivity of 18.3 pmol/L. Serum LH was measured by a direct immunoenzymetric assay (SR1®, Code 162032; BioChem ImmunoSystems GmbH, Freiburg, Germany) with a sensitivity of 0.5 IU/L. LH levels below the detection limit were defined as 0.49 IU/L. Serum FSH was measured by a direct immunometric assay (Kryptor, Brahms Diagnostica GmbH, Berlin, Germany) with a detection limit of 0.4 IU/L. Inhibin B was measured using a specific two-site enzyme-linked immunosorbent assay kit (Serotec, Oxford, U.K.) with a detection limit of 15 pg/mL.

Statistical Methods

Differences between study groups were analyzed by *t*-test, Mann–Whitney rank sum test, or by chi-square test, respectively. Two-way analysis of variance for repeated measures was used to test for differences in serum levels of inhibin B, estradiol and follicle development between groups and over time. Correlations between inhibin B serum levels and parameters of ovarian response were calculated with the Pearson product moment. Statistical analysis was performed using the statistical software package Sigmastat 2.03 (Jandel Scientific, Erkrath, Germany). In general, results are given as mean ± SEM. P values <0.05 were considered as statistically significant.

RESULTS

Patient Characteristics

During a recruitment period of 11 months a total of 53 patients were assessed for eligibility at our center,

Table I. Patient Characteristics

	Group I ($n = 15$)	Group II ($n = 31$)	p
Age (years)	31.1 ± 0.8	31.4 ± 0.7	0.78
FSH (IU/L)[a]	6.8 ± 0.4	6.5 ± 0.4	0.68
Inhibin B (pg/ml)[a]	105.9 ± 23.9	111.1 ± 8.7	0.81
Indications for COH (%)			
Andrological (ICSI)	93.3	80.6	
Tubal	6.7	19.4	

[a]Serum levels during early follicular phase of the preceding cycle.

and 46 of them successively treated and analyzed. Six of the initially screened patients did not meet the inclusion criteria and 1 patient did not receive FSH stimulation therapy because of a persisting ovarian cyst during GnRH agonist application. The two study groups (group I, $n = 15$ patients; group II, $n = 31$ patients) were similar regarding patient age, basal FSH, and basal inhibin B serum levels as measured in the early follicular phase of the preceding cycle (Table I). Indications for COH were andrological in 14 and 25 patients, and tubal in 1 and 6 patients, in groups I and II, respectively.

Serum Hormone Concentrations

Inhibin B. Inhibin B serum levels (Fig. 1, panel A) were rising in both groups during the course of ovarian stimulation ($p < 0.001$). On day 1, inhibin B serum levels in group I were significantly lower compared to group II (22.9 ± 5.4 vs. 111.1 ± 8.7 pg/mL, $p < 0.001$). The ratio of day 6/day 1 inhibin B serum levels was significantly higher in group I than group II (43.1 ± 14.3 vs. 9.4 ± 1.5, $p = 0.009$), whereas the ratio of inhibin B on "day of hCG injection"/day 6 was comparable (2.9 ± 0.9 vs. 1.9 ± 0.3, $p = 0.209$).

Estradiol, LH, and FSH. Estradiol serum levels (Fig. 1, panel B) were rising significantly during ovarian stimulation in both groups ($p < 0.001$). Estradiol serum levels were significantly lower in group I than in group II on day 1 (I: 29.9 ± 8.9, II: 73.6 ± 8.9 pmol/L, $p = 0.001$) and day 6 (I: 594.7 ± 236.3, II: 961.6 ± 122.7 pmol/L, $p = 0.004$), whereas on the day of hCG injection, estradiol serum levels were higher in group I without reaching statistical significance (Table II). LH serum levels were significantly different between groups on day 1 (I: 1.43 ± 0.90 vs. II: 3.25 ± 0.28 IU/L, $p < 0.001$) and remained low during the further course of ovarian stimulation without statistical difference between groups (Fig. 1, panel C). FSH serum

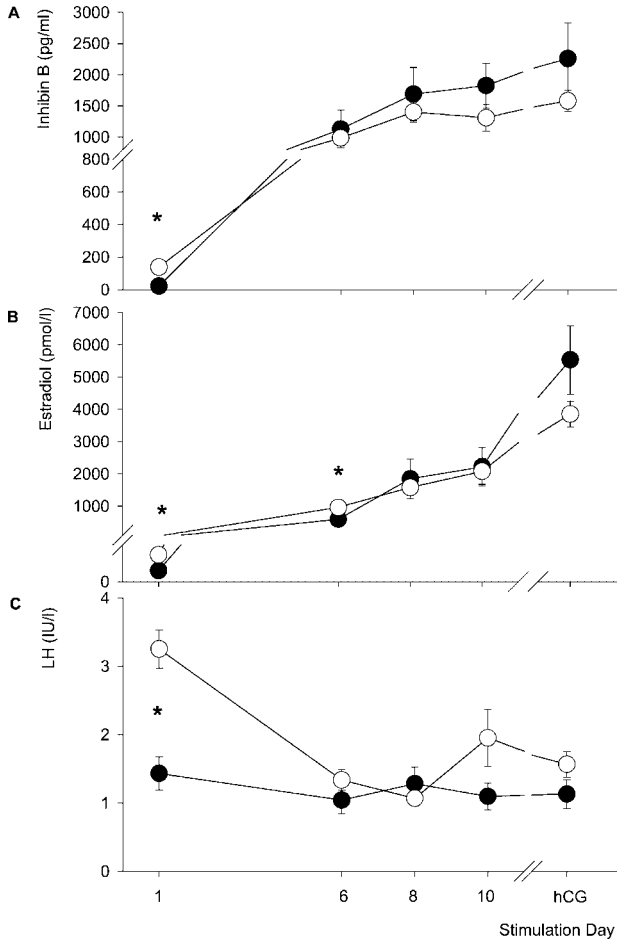

Fig. 1. Serum levels (mean ± SEM) of inhibin B (pg/ml, panel A), estradiol (pmol/L, panel B) and LH (mIU/mL, panel C) during ovarian stimulation and on the day of hCG injection. ●, group I (GnRH agonist protocol); ○, group II (GnRH antagonist protocol). *$p < 0.05$ between groups.

levels on day 1 were significantly higher in group II compared to group I (6.0 ± 0.3 vs. 2.6 ± 0.5 IU/L, $p < 0.001$).

Measures of Ovarian Response and Clinical Outcome

The number of antral follicles on day 1 was similar in both groups (Table II). The duration of rFSH treatment was significantly longer in group I compared to group II, and the total rFSH dose was significantly higher (Table II). The FSH dose per day was not significantly different between the two groups (Table II). Follicle growth was delayed in group I as compared to group II (Fig. 2), with significant differences between follicles of 11–14 mm on day 6 (2.1 ± 0.6 vs. 3.7 ± 0.5,

Table II. Measures of Ovarian Response and Clinical Outcome

	Group I	Group II	*p*
Number of antral follicles[a]	13.2 ± 1.4	13.3 ± 1.2	0.97
Stimulation days	10.4 ± 0.4	8.9 ± 0.3	0.003
FSH dose (IU)			
Total	2410 ± 225	1829.0 ± 123.5	0.024
Daily	199.5 ± 7.1	226.2 ± 13.6	0.060
Number of follicles[b]			
≥ 11 mm diameter	11.8 ± 1.6	10.9 ± 0.9	0.60
>17 mm diameter	5.5 ± 0.6	4.6 ± 0.3	0.20
Estradiol serum level[b]	5534 ± 1059	3854 ± 399	0.14
Retrieved oocytes	13.2 ± 1.4	9.8 ± 1.0	0.05
Transferred embryos	2.7 ± 0.1	2.5 ± 0.1	0.57
Clinical pregnancy rate	25.8%	25.0%	

[a]On stimulation day 1.
[b]On the day of hCG injection.

$p = 0.047$, panel A), between follicles of 15–17 mm on day 8 (0.9 ± 0.4 vs. 2.3 ± 0.4, $p = 0.022$, panel B) and between follicles >17 mm on day 8 (0.1 ± 0.36 vs. 1.1 ± 0.16, $p = 0.002$, panel C) and day 10 (2.2 ± 0.6 vs. 3.4 ± 0.4, $p = 0.035$, panel C). On the day of hCG injection, the number of the respective follicles (Fig. 2) as well as the number of retrieved oocytes (Table II) was not different between groups. Treatment resulted in a comparable number of transferred embryos and clinical pregnancy rate (Table II).

Correlation of Inhibin B Serum Levels with Parameters of Ovarian Response

Inhibin B serum levels on days 1 and 6 were significantly and positively correlated with the number of follicles >17 mm on the day of hCG injection and the number of retrieved oocytes in group I, but not in group II (Fig. 3, panel A). Only in group I, serum levels of inhibin B on day 6 were significantly correlated with estradiol serum levels on the day of hCG injection ($r = 0.77$, $p = 0.001$, data not shown). Significant negative correlations were found between both day 1 and day 6 inhibin B serum levels and the total and daily FSH dose in group II, but not in group I (Fig. 3, panel B). In group II, inhibin B serum levels on day 1 and day 6 were significantly correlated with the duration of FSH stimulation (day 1 inhibin B: correlation coefficient $r = -0.46$, $p = 0.015$; day 6 inhibin B: $r = -0.48$, $p = 0.009$), whereas in group I only inhibin B on day 6 was correlated with the number of stimulation days (day 1: $r = -0.33$, $p = 0.276$; day 6: $r = -0.63$, $p = 0.020$).

DISCUSSION

The main objective of this study was to analyze the association of inhibin B serum levels with ovarian response in COH using GnRH agonist versus antagonist protocols. From the physiological and clinical point of view, the beginning of the stimulation cycle is of special interest, as it provides the clinician with relevant information at an early point of treatment. For various reasons, individualized stimulation protocols using minimal but effective FSH doses have come to the focus of COH research (11). In the search for possible predictors of individual ovarian response, early follicular phase inhibin B is a

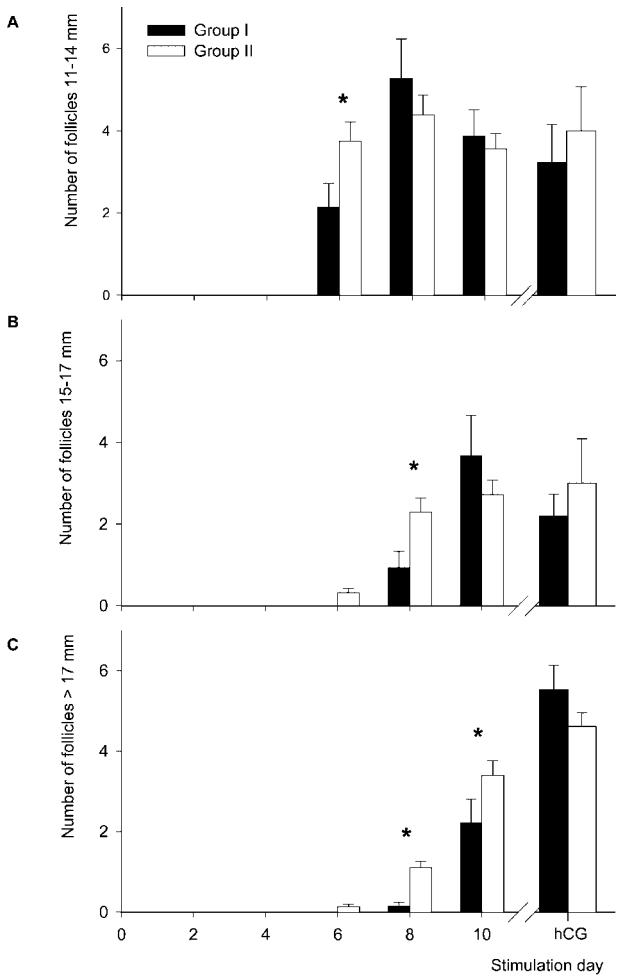

Fig. 2. Number (mean ± SEM) of small (11–14 mm diameter, panel A), medium (15–17 mm diameter, panel B), and large growing follicles (>17 mm, panel C) measured during ovarian stimulation and on the day of hCG injection. On stimulation day 1 only follicles <11 mm were present. $^*p < 0.05$ between groups.

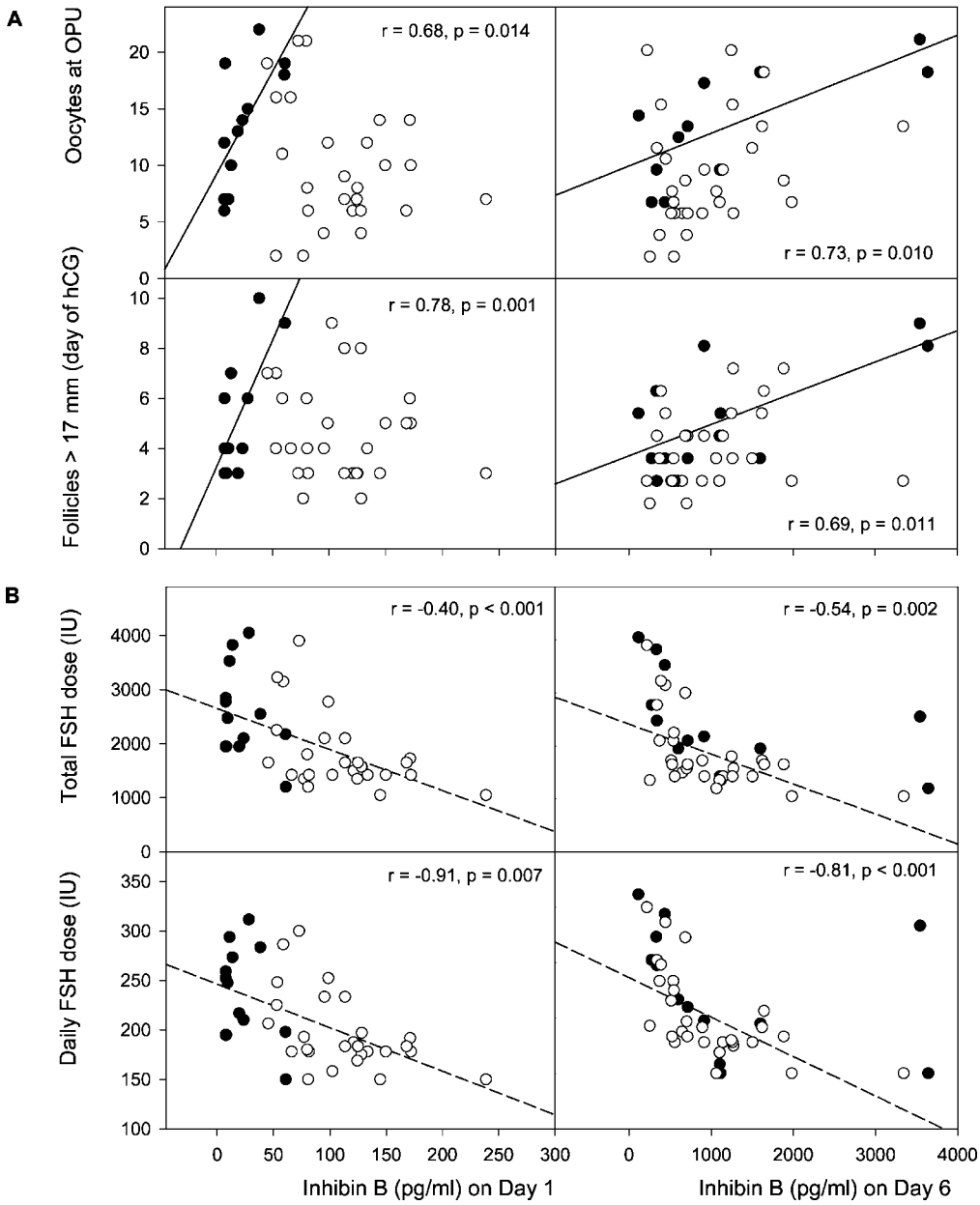

Fig. 3. Correlations between serum inhibin B levels on days 1 and 6 and follicles >17 mm on day of hCG administration (panel A), retrieved oocytes at OPU (ovum pickup) (panel A) and the total and daily FSH dose (panel B). ●, group I (GnRH agonist protocol); ○, group II (GnRH antagonist protocol). Correlation coefficients (r) and p values are given only in case of significance ($p < 0.05$). ——-, regression line for group I; - - -, regression line for group II.

promising candidate. A limited number of studies so far have investigated the relationship between inhibin B and ovarian follicular development (4,12) or even pregnancy outcome (13,14) in patients treated with exogenous FSH following pituitary downregulation. Inhibin B levels measured early during FSH stimulation seem to reflect the number of recruited follicles and may be useful as an early indicator of ovarian response. However, the usefulness of basal inhibin B as an additional predictive parameter to basal FSH and patient age is not undoubted (15,16). A number of recent studies lead to the assumption that hormonal manipulation of the ovarian milieu by preceding luteal phase estrogens, GnRH agonists or exogenous

FSH may strengthen the correlation between early follicular phase inhibin B and follicular development to various degrees (15,17,18). In the current study, inhibin B serum levels on days 1 and 6 are positively correlated with the number of large follicles and retrieved oocytes only in patients treated with GnRH agonists.

To date, there are no published studies on inhibin B serum measurements in controlled clinical trials using GnRH antagonists for pituitary suppression. In our study, the correlations of inhibin B levels with the number of large follicles or retrieved oocytes described above were not found in the GnRH antagonist group. On day 1, the mean serum levels of inhibin B are significantly lower in women treated with GnRH agonist. Notably, the total number of antral follicles as determined by sonography on that day as well as the relevant patient characteristics such as age and early follicular phase FSH or inhibin B in a previous untreated cycle are not different between study groups. Probably inhibin B production is significantly suppressed by GnRH agonist application without an effect on antral follicle number, but the degree of inhibin B suppression might serve as an indicator for follicular response to exogenous FSH.

The delay of follicular growth in patients treated with GnRH agonists compared to GnRH antagonist protocols might be caused by the missing early FSH surge in the late luteal phase of the preceding cycle (6,19). Indeed, FSH serum levels on day 1 are significantly lower in the agonist as compared to the antagonist group in our study. Various studies have indicated that the early surge in FSH is accompanied by a parallel rise of inhibin B (2). Only in the GnRH antagonist group were inhibin B serum levels on days 1 and 6 significantly and negatively correlated with the total and daily FSH dose. Only in these patients were inhibin B serum levels on day 1 significantly correlated with the number of FSH stimulation days. Thus, differences in terms of required FSH stimulation length and FSH dose seem to be associated only with unsuppressed inhibin B serum levels in the early follicular phase.

Whether the effects on follicular development are based on GnRH analogue action at the pituitary level or directly at the ovary is unknown. *In vitro* experiments performed in the murine (20) and human (21,22) model have demonstrated that the ovary is a putative site of GnRH receptor expression. Until now, the data from the human model are conflicting, and the functional relevance of GnRH receptor expression in the ovary has not yet been elucidated. Aside from progesterone accumulation in response to *in vitro* stimulation, differences in granulosa cell steroidogenesis have not been found when comparing GnRH antagonist versus agonist administration for IVF treatment (23). Nevertheless, different ovarian functions may be affected by GnRH analogues; for example, GnRH was found to inhibit FSH-induced inhibin B production in the rat granulosa cell model (24). However, the limitations of these studies will not allow final conclusions about the causal relationships between GnRH analogues and inhibin B production at the cellular level. Future studies will have to clarify whether inhibin B merely reflects differences in the follicular growth pattern or if it actively contributes to the differences as a paracrine modulator of ovarian folliculogenesis.

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