Serum Inhibin B as a Marker of Spermatogenesis

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ABSTRACT

Inhibin B is produced by Sertoli cells, provides negative feedback on FSH secretion, and may prove to be an important marker for the functioning of seminiferous tubules. The purpose of the present study was to examine the relationship between the spermatogenic function of the testis of subfertile men and the plasma concentrations of inhibin B and FSH. These parameters were estimated in a group of 218 subfertile men.

Serum inhibin B levels were closely correlated with the serum FSH levels ($r = -0.78$, $P < 0.001$), confirming the role of inhibin B as feedback signal for FSH production.

The spermatogenic function of the testis was evaluated by deter-

mining testicular volume and total sperm count. Inhibin B levels were significantly correlated with the total sperm count and testicular volume ($r = 0.54$ and $r = 0.63$, respectively; $P < 0.001$).

Testicular biopsies were obtained in 22 of these men. Inhibin B was significantly correlated with the biopsy score ($r = 0.76$, $P < 0.001$). Receiver operating characteristic analysis revealed a diagnostic accuracy of 95% for differentiating competent from impaired spermatogenesis for inhibin B, whereas for FSH, a value of 80% was found.

We conclude that inhibin B is the best available endocrine marker of spermatogenesis in subfertile men. (*J Clin Endocrinol Metab* **83:** 3110–3114, 1998)

F SH is currently regarded as the most important endocrine parameter in the evaluation of male infertility (1). Its secretion can be suppressed by the testicular hormone inhibin, which is produced in Sertoli cells and may, therefore, be a serum marker for Sertoli cell function. Attempts to confirm this role of inhibin originally yielded contradictory results. Results of heterologous inhibin assays demonstrated that serum inhibin levels were stimulated with exogenous FSH and decreased after treatment with GnRH antagonists, radiotherapy-induced testicular damage, and testosterone (2–6). In contrast, a negative correlation between inhibin and FSH levels could not be shown, and inhibin levels in fertile controls and subfertile men with testicular disorders were not different (7).

This discrepancy can now be explained on the basis of the aspecificity of the inhibin assay that was used. Inhibin is a dimer of an α- and a β-subunit. Depending on the type of β-subunit, (β_A or β_B), inhibin A or inhibin B is formed. The antibodies used in the heterologous inhibin RIA were directed against the α-subunit, and they detected both dimeric inhibin and biologically inactive monomeric α-subunits (8). Since new specific sandwich assays for inhibin A, inhibin B, and uncombined α-subunits have been developed, studies have been undertaken to investigate the role of inhibins in male and female endocrinology.

One major finding is that inhibin B is the physiologically important form of inhibin in the male, serum inhibin A levels being undetectable (9). The finding that castration results in

undetectable inhibin B levels indicates that circulating inhibin B is produced by the testes (10). Furthermore, recent papers have reported a strong negative correlation between FSH and inhibin B in fertile and subfertile men (9, 11–14).

Little information is available on the correlation of inhibin B with the severity of spermatogenic defects in subfertile men. So far, lower inhibin B levels were reported in a limited number of subfertile men, compared with fertile controls (10). More recently, inhibin B was found to be correlated with the sperm concentration in a study of 349 normal men (12) and in a mixed group of 65 men with normal and impaired spermatogenesis (13).

The aim of this study was to further investigate the clinical value of inhibin B estimations in subfertile men and to correlate inhibin B levels with clinical history, testicular volume, testicular biopsy score, and sperm characteristics. Subsequently, we analyzed the additional value of inhibin B, compared with that of FSH, with special emphasis on the differentiation between normal and impaired spermatogenesis.

Subjects and Methods

Patients

The study comprised 218 consecutive patients that were referred to our andrology outpatient clinic with fertility problems (age, 21–57 yr). In the period September 1996 until October 1997, 235 new patients were enrolled, of which 17 were excluded from further study on the basis of medication (n = 7; androgens or anti-estrogens), unilateral castration (n = 4), hypogonadotropic hypogonadism (n = 3), systemic disease (n = 1; renal failure), or chromosome translocation (n = 2).

Infertility of the couple was defined as a duration of infertility of more than 1 yr. Patients were subjected to a thorough clinical evaluation according to the WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple (15). Patients were diagnosed with normospermia (n = 49; ≥20 million sperm/mL), idiopathic moderate oligozoospermia (n = 69; 5–20 million sperm/mL), idiopathic severe oligozoospermia (n = 58; >0 and <5 million sperm/mL), idiopathic

azoospermia (n = 15), obstructive azoospermia (n = 6, normal FSH, normal testicular size, and Johnsen score > 8; in 3 of these men, congenital absence of the vas deferens was diagnosed with ultrasonography), history of cryptorchidism (n = 17, accompanied by oligozoospermia), or Klinefelter's syndrome (n = 4).

Hormone analyses

Serum samples were stored for a period of 1 day to maximal 5 weeks at −20 C before analysis. Inhibin B was measured using kits purchased from Serotec Limited, Oxford, UK (16). The within-assay coefficient of variance (CV) was less than 9%, and the between-assay CV was less than 15%. The lowest detectable inhibin B concentration was 5 pg/mL (based on the mean value of the blanks + 2 sD). A value of 2.5 pg/mL (the mean of the undetectable range) was assigned to test results below 5 pg/mL. Serum FSH and LH were determined with the Amerlite FSH and LH assays (Orange-Clinical Diagnostics, Amersham, UK). Within-assay and between-assay CVs are less than 3, less than 8% and less than 5, less than 15% for FSH and LH, respectively. Total serum testosterone was determined by RIA, as described earlier (17) (within- and between-assay CV: less than 6 and less than 9%). Using the above assays, mean (sD) FSH, LH, and testosterone levels in a group of normal men were 2.5 (1.3) IU/L, 3.6 (1.9) IU/L, and 17.6 (6.8) nmol/L (18). Per patient, all hormone concentrations were analyzed in the same blood sample.

Semen analysis

Semen analyses were carried out according to the WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction (19). Per patient, the results of the two semen analyses that were performed closest in time to the hormone analyses were selected. Per patient, the average sperm count was calculated. The median time difference (semen analysis date − blood sampling date) was 22 days (10th and 90th percentiles: -23, 51.4 days). Semen samples were obtained and assessed in 205 of the 218 patients.

Testicular evaluation

Testicular volume was estimated with the Prader orchidometer. Bilateral biopsy specimens were available on 22 of the 218 patients. Testicular biopsies were performed to discriminate impaired spermatogenesis from excurrent duct obstruction as a cause for azoospermia or severe oligozoospermia. Criteria for testicular biopsy were: azoospermia accompanied by a normal FSH level, or less than 5 million sperm/mL ejaculate. Biopsy specimens were scored using the method described by Johnsen (20), as modified by Aafjes et al. (21). Seminiferous tubule cross-sections were rated with a score from 1 to 10, based on the most advanced stage of spermatogenesis observed. The mean score of at least 50 tubules was calculated per biopsy, both for the left and right testis. Tubules scored 10 for complete and abundant spermatogenesis with at least 5 condensed spermatids; 8 when all stages of spermatogenesis were present, but less than 5 condensed spermatids were seen; 7 when no condensed spermatids, but at least 5 round spermatids were present; 6 when no condensed spermatids, and less than 5 round spermatids present; 5 when no spermatids, but 5 or more spermatocytes present; 4 when no spermatids and less than 5 spermatocytes were present; 3 only spermatogonia present; 2 for Sertoli cells only; and 1 for no cells in the tubular section. It was previously shown that spontaneous pregnancy is possible when a biopsy score of ≥8 is present, but highly unlikely below a Johnsen score of 8 (21).

Statistical analysis

The FSH, LH, testosterone, and sperm count variables were transformed logarithmically to achieve a normal distribution. Correlations were calculated with Pearson's method. Differences between patient groups were tested with one-way ANOVA, followed by Fisher's least-significant difference method for pairwise comparisons.

The performance of inhibin B or FSH estimations in discriminating between normal and impaired spermatogenesis (Johnsen score ≥ 8 or <8) was described by receiver operating characteristic (ROC) statistics. ROC curves were drawn by plotting the sensitivity against the false positive rate (1-specificity) for varying cutoff levels of inhibin B and FSH.

A nondiscriminating test would follow the diagonal line of the figure, whereas a 100% accurate so-called gold standard test would coincide with the upper left corner of the box. By comparing the areas under the curve (AUCs) for inhibin B and FSH, the diagnostic values of both hormones were compared (22). AUCs were estimated with the Wilcoxon statistic (23).

Independent variables predictive of the biopsy score were identified with linear multiple regression analysis. Two-sided P values less than 0.05 were considered significant. Statistical analyses were carried out with the SPSS 7.5 for Windows statistical software package.

Results

Inhibin B was detectable in all but 6 men, with a mean concentration of 144.2 ± 4.9 (sEM) pg/mL. Subdivided by diagnosis, the mean (sEM) serum inhibin B concentrations were 244.0 (31.6) for obstructive azoospermia, 181.9 (9.1) for normospermia, 166.1 (7.3) for moderate oligozoospermia, 128.4 (8.8) for severe oligozoospermia, 52.0 (14.4) for idiopathic azoospermia, 7.3 (2.5) for Klinefelter's syndrome, and 118.1 (18.3) pg/mL for patients with a history of cryptorchidism (Fig. 1). Compared with the group with normospermia, the mean serum inhibin B levels were significantly lower in the groups with severe oligozoospermia, idiopathic azoospermia, Klinefelter's syndrome, and a history of cryptorchidism. The mean inhibin B level in patients with obstructive azoospermia, in which spermatogenesis may be normal, was significantly higher, when compared with the other groups.

Table 1 shows the correlations of inhibin B with parameters related to spermatogenesis. The inhibin B levels were correlated with the total sperm count (r = 0.54, P < 0.001; Fig. 2); patients with obstructive azoospermia were excluded from this correlation and other correlations with the sperm count. The inhibin B levels were also significantly correlated with the total bilateral testicular volume (Fig. 3), the Johnsen score (Fig. 4), and negatively with serum FSH (Fig. 5) and LH levels. The correlation of inhibin B with LH was not signif-
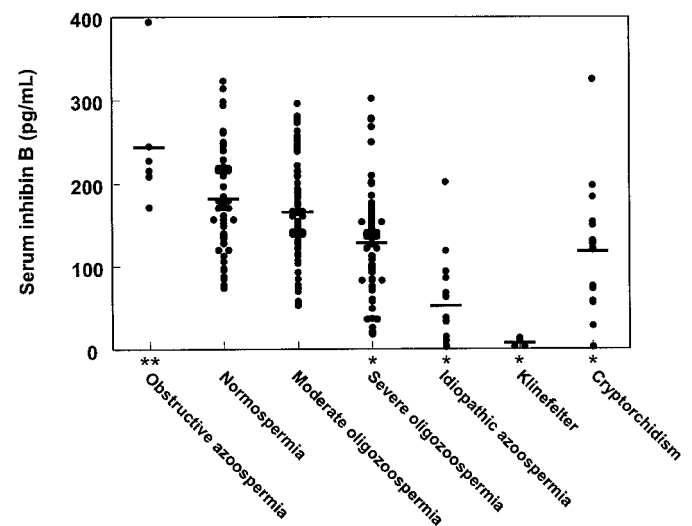

FIG. 1. Serum inhibin B levels in subgroups of subfertile men. The *horizontal line* per group indicates the mean level (*, the mean inhibin B level was significantly lower, compared with normospermia, P < 0.05; **, the mean inhibin B level in patients with obstructive azoospermia was significantly higher, compared with other groups, P ≤ 0.01).

TABLE 1. Correlations between Inhibin B, FSH, LH, testosterone (T), testicular volume (TV, sum of left and right testis), mean bilateral Johnsen score, and sperm count are *in italic*, and number of patients (*P* value) are *boldface*.

	Inhibin B	FSH	LH	T	TV	Johnsen score	Sperm count
Inhibin B		*−0.78[a]*	*−0.41[a]*	*0.20[a]*	*0.63[a]*	*0.76[a]*	*0.54[a]*
FSH	**218 (<0.001)**		*0.55[a]*	*−0.17[a]*	*−0.56[a]*	*−0.64[a]*	*−0.55[a]*
LH	**218 (<0.001)**	**218 (<0.001)**		*0.01*	*−0.34[a]*	*−0.35*	*−0.32[a]*
T	**208 (<0.004)**	**208 (0.02)**	**208 (0.97)**		*0.18[a]*	*0.38*	*0.13*
TV	**166 (<0.001)**	**166 (<0.001)**	**166 (<0.001)**	**164 (0.02)**		*0.34*	*0.38[a]*
Johnsen score	**22 (<0.001)**	**22 (0.001)**	**22 (0.11)**	**21 (0.11)**	**20 (0.14)**		*0.53[a]*
Sperm count	**205 (<0.001)**	**205 (<0.001)**	**205 (<0.001)**	**202 (0.07)**	**156 (<0.001)**	**17 (0.03)**	

[a] Correlation coefficients are statistically significant.
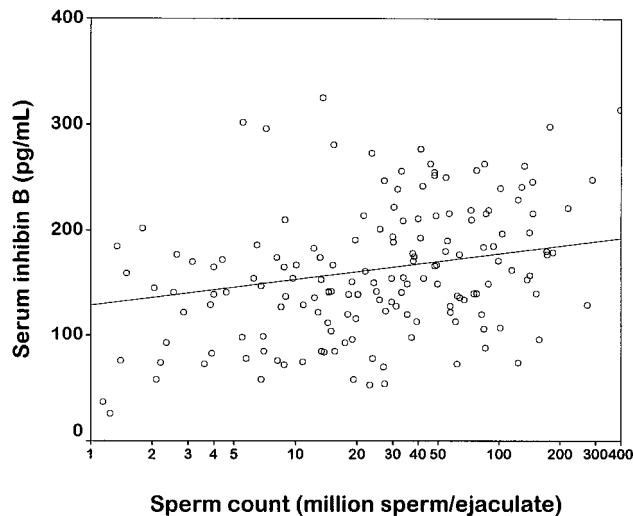
FIG. 2. Serum inhibin B plotted against the total sperm count in 205 subfertile males (r = 0.54, *P* < 0.001).
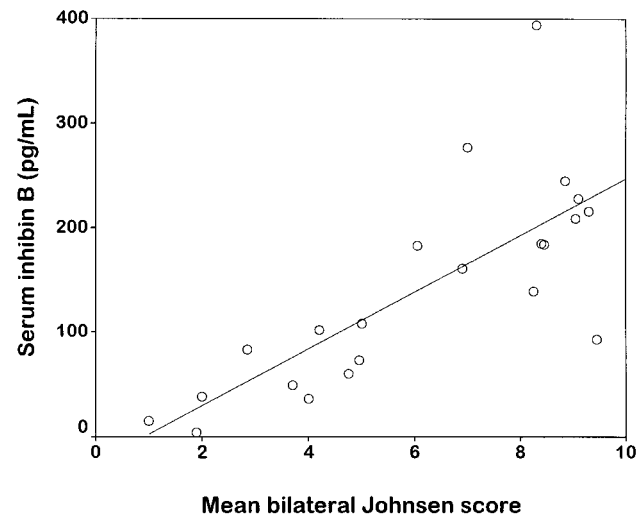
FIG. 4. Serum inhibin B plotted against the mean bilateral Johnsen scores of 22 subfertile males (r = 0.76, *P* < 0.001).
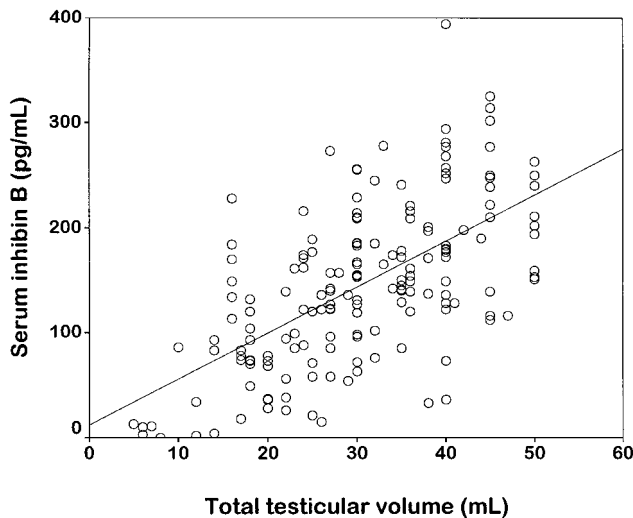
FIG. 3. Serum inhibin B levels plotted against the testicular volume of 166 subfertile males (r = 0.63, *P* < 0.001).
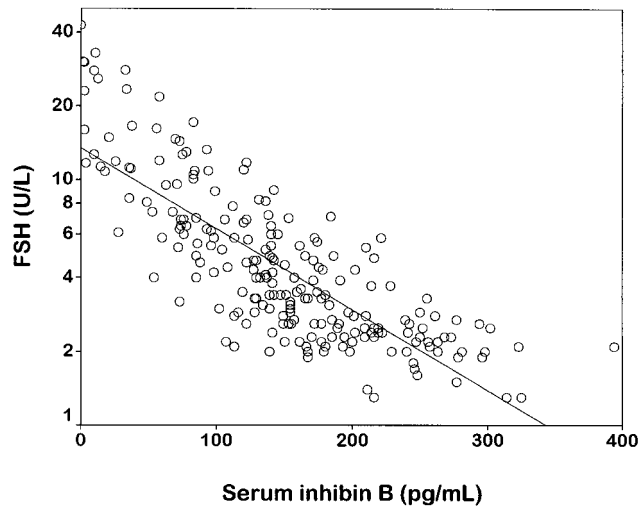
FIG. 5. Serum FSH concentration plotted against serum inhibin B in 218 subfertile males (r = −0.78, *P* < 0.001).

icant if it was adjusted for the FSH level (r = 0.03, *P* = 0.63), with which LH is closely correlated.

The accuracy for differentiating adequate (Johnsen score ≥ 8) from impaired spermatogenesis (Johnsen score <8), on the basis of inhibin B and FSH levels, was estimated from the area under the ROC curve (Fig. 6). The AUC was 0.95 (SE:

0.07) for inhibin B and 0.80 (SE: 0.12) for FSH. The areas under the ROC curves for inhibin B and FSH were statistically different (*P* = 0.04). Depending on the desired sensitivity or specificity of inhibin B or FSH, a cutoff level can be deduced from the ROC curves. When the point on the curve closest to the upper left corner of the box corresponding to 100% sensitivity and 0% false positives (100% specificity) was selected,
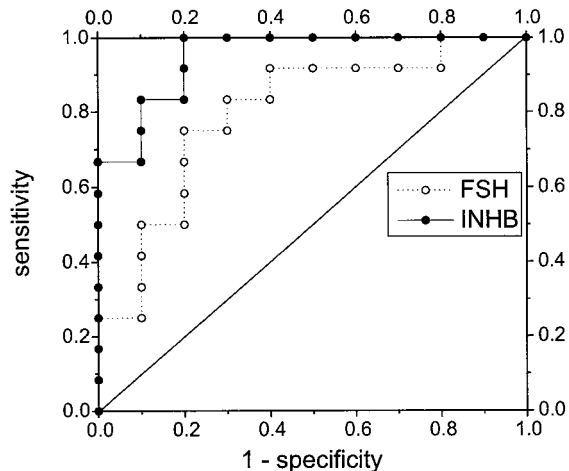
FIG. 6. ROCs of inhibin B and FSH for discriminating normal and disturbed spermatogenesis. INHB, Inhibin B.

this resulted in cutoff levels of inhibin B less than 139 pg/mL and FSH more than 4.9 IU/L to identify patients with impaired spermatogenesis. The sensitivity and specificity corresponding to these cutoffs were 83 and 90% for inhibin B, and 75 and 80% for and FSH, respectively.

In addition, linear multiple regression was performed to study whether hormonal variables could account for the variation in the mean bilateral Johnsen score. The Johnsen score was predicted from FSH and inhibin B, the variables correlated with it in Table 1. Sperm count was not entered as an independent variable, because an unrevealed (partial) obstruction may bias the biopsy score *vs.* sperm count correlation. The regression procedure indicated inhibin B as the best predictor of the Johnsen score, explaining 58% of the variance. In the resulting regression equation [Johnsen score $= 4.78 + (0.017 \times \text{inhibin B}) + (0.05 \times \text{FSH})$], the coefficient for inhibin B was statistically significant ($P = 0.007$), whereas FSH did not improve the regression model ($P = 0.24$).

Discussion

In this study, we demonstrate a significant correlation between sperm concentration, sperm count, and testicular volume on the one hand, and serum inhibin B levels on the other. These results provide strong evidence that inhibin B is an important marker of the competence of Sertoli cells and spermatogenesis in the human, which is in accordance with the few reports on inhibin B and quality of spermatogenesis up to now (9, 10, 12, 13). In the first two studies, a lower inhibin B concentration was noted in small groups of men with azoospermia, testicular disorders, and infertility, as compared with fertile controls. More recently, results of two larger study populations became available, showing a significant positive correlation of inhibin B with sperm concentration in 349 normal men (12), and with sperm concentration and testicular volume in 65 men with normal and impaired spermatogenesis (13).

We now provide further evidence for the value of inhibin B as a marker of spermatogenesis by the novel finding of a statistically significant positive correlation with the most ac-

curate assessment of spermatogenesis in our setting, the testicular biopsy score. We compared the accuracy of FSH and inhibin B levels to distinguish between patients with competent and impaired spermatogenesis, based on the Johnsen score. The area under the ROC curve, corresponding to the accuracy of the diagnostic method, was significantly larger for inhibin B. Multiple linear regression analysis also revealed that FSH had no significant additional predictive value for the Johnsen score above inhibin B.

The choice for a cutoff level for inhibin B or FSH to discriminate competent from impaired spermatogenesis depends on the priority of a high sensitivity or specificity. We arbitrarily chose the cutoff level closest to the upper left corner of the box. The resulting cutoff levels for inhibin B (<139 pg/mL) and FSH (>4.9 IU/L) were surprisingly close to the cutoff levels for these hormones based on control populations. A lower normal limit for inhibin B has not been defined, but it was 140.6 pg/mL (95% confidence interval, 140.6–225.7) for a group of 18 semen donors (9). We use 5.1 IU/L as the upper normal limit for FSH based on the mean plus 2 SD [2.5 + (2 × 1.3)] in a population of normal men (17).

The present data show significant differences in mean inhibin B levels between diagnostic subgroups. The inhibin B levels were significantly lower in patients with a spermatogenic defect, as compared with the group with normospermia. Patients with obstruction as the sole identified cause for azoospermia had normal inhibin B levels, which were significantly higher, compared with other subgroups. With the aspecific heterologous assay for inhibin, no differences in inhibin levels between comparable subgroups were found (7).

A further advantage of inhibin B measurement is that it reflects the function of the total testicular tissue, whereas a biopsy may not be representative for the entire testis. Multiple biopsies, which are nowadays performed for testicular sperm extraction, often show a large variation in the completeness of spermatogenesis (24). This heterogeneity of spermatogenesis is even more conspicuous in patients with impaired spermatogenesis, where sections with complete spermatogenesis may be found among others with germinal cell aplasia, referred to as focal spermatogenesis (25). It has to be established whether inhibin B levels can demonstrate the presence of focal spermatogenesis and, in this way, could reduce the need for invasive testicular biopsies. It is not unlikely that, in many cases, the area of spermatogenesis is too small to substantially increase serum inhibin B levels.

FSH was regarded the most important endocrine marker for testicular function until now (26). The diagnostic value of inhibin B for spermatogenetic disorders seems to be better. This may be explained by the fact that inhibin is a direct product of the seminiferous tubules, and that its secretion is stimulated by the presence of advanced stages of spermatogenesis (27). In contrast, FSH levels are also affected by GnRH, estradiol, and testosterone.

In conclusion, we have confirmed the role of inhibin B in FSH regulation, and we have found a strong correlation of inhibin B levels with spermatogenesis. Our results provide further and novel evidence that inhibin B is the best known endocrine marker for spermatogenesis. Inhibin B estimation

may prove an alternative for testicular biopsy in the differentiation between normal and impaired spermatogenesis.

Acknowledgments

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