The aim of this study was to investigate inhibin B and follicle stimulating hormone (FSH) secretion in a large group of oligozoospermic subjects affected by different degrees of testicular damage, before and after FSH treatment. A total of 135 oligozoospermic subjects (sperm count <20 x 10^6/ml) were evaluated for seminal parameters and FSH, luteinizing hormone (LH), testosterone and inhibin B plasma concentrations. Testicular structure was analysed with bilateral fine needle aspiration cytology. Inhibin B showed an inverse correlation with FSH, no correlation with sperm concentration and a significant relationship with intratesticular spermatid number, demonstrating that testicular spermatids play an important role in the control of inhibin B production. Twenty-five subjects with sperm counts <10 x 10^6/ml were treated with FSH; 11 of these had basal FSH and inhibin B plasma concentrations in the normal range (group A), while in seven subjects FSH was elevated (>7 IU/l) with normal inhibin B (group B), and in seven patients FSH was high and inhibin B reduced (<80 pg/ml) (group C). During treatment, in group A patients inhibin B plasma concentrations increased significantly after 2, 3 and 4 weeks of FSH administration and declined thereafter to pre-treatment concentrations. Groups B and C did not show any modification during the treatment. In the same period, in group A FSH increased significantly after 2, 3 and 4 weeks and subsequently declined. In groups B and C, FSH increased significantly after 2 weeks and remained elevated during the following period. The results of the present study confirm the significant inverse correlation between inhibin B and FSH plasma concentrations in subjects with disturbed spermatogenesis, and demonstrate that inhibin B reflects Sertoli cell function and their interaction with spermatids. FSH and inhibin B concentrations are an expression of the spermatogenic status of seminiferous tubules. FSH treatment seems to modify inhibin B plasma concentrations only in subjects with normal basal FSH and inhibin B, independently from the effects of this therapy on sperm production.

**Key words:** cytology/fine needle aspiration/FSH therapy/inhibin B/oligozoospermia

**Introduction**

The development of a new generation of assays specific for each form of inhibins, the dimers inhibin A and B, and for the precursor inhibins containing the alpha subunit fragment pro-alpha-C, has allowed the physiological role of these hormones in the mechanisms regulating the reproductive system in males to be clarified. It has been demonstrated that: (i) inhibin B and not inhibin A is the circulating form both in human fetus and adult male (Illingworth et al., 1996; Wallace et al., 1997); (ii) inhibin B plasma concentrations are significantly lower in subjects affected by gonadotrophin-releasing hormone (GnRH) deficiency than in normal controls, and are normalized after pulsatile GnRH administration (Nachtigall et al., 1996); (iii) follicle stimulating hormone (FSH) is the principal stimulus for the secretion of testicular inhibin B (Anawalt et al., 1996); and (iv) the testis is the only source of inhibin B, as the latter is undetectable in orchiectomized men (Anawalt et al., 1996). Furthermore, a number of studies have provided substantial evidence that testicular inhibin B is the major regulator of the feed-back mechanisms of FSH secretion on the basis that inhibin B correlates with FSH plasma concentrations in normal and infertile men and in men with GnRH deficiency during pulsatile GnRH therapy (Anawalt et al., 1996; Nachtigall et al., 1996; Anderson et al., 1997). Taken together, these data are consistent with inhibin B being a testicular product that is stimulated by FSH and in turn regulates the production of this gonadotrophin. However, there are still some unresolved problems regarding the physiological mechanisms regulating the FSH–inhibin B feed-back axis. For example, immunoreactive inhibin B originates from Sertoli cells, but reflects interactions between these and neighbouring germ cells (Pineau et al., 1990). In addition, experimental studies in rats demonstrated that particularly the elongated spermatids are able to influence inhibin production (Allenby et al., 1991). Spermatids also seem to have a role in the regulation of inhibin B secretion in humans, since in subjects with severe oligozoospermia or azoospermia showing a testicular structure of partial or complete spermatidic arrest, normal FSH plasma concentrations were observed (Foresta and Varotto, 1992; Foresta et al., 1992, 1995). This hypothesis may justify previous studies demonstrating that, in oligozoospermic subjects, inhibin B is correlated with FSH plasma concentrations but not with sperm number (Illingworth et al., 1996; Anderson et al., 1997). In fact, oligozoospermia reflects the
end-point of different testicular alterations, including situations characterized by disturbances of spermiogenesis.

In this study we evaluated the reciprocal behaviour of inhibin B and FSH secretion in a large group of oligozoospermic subjects with well-defined testicular alterations in order to investigate which maturational stage of spermatogenesis was able to influence the production of inhibin B by Sertoli cells. Furthermore, to clarify the clinical meaning of inhibin B concentrations in oligozoospermic subjects, the behaviour of this hormone was evaluated after FSH treatment in patients affected by different degrees of testicular damage.

Materials and methods

Subjects

The subjects were 135 adult oligozoospermic men (mean age 32 ± 3.4 years; range 27–38 years) who had complained of infertility for at least two years. Each man was studied on two different occasions, separated by a 3-week interval, with 3 days of sexual abstinence. Semen and blood samples for the measurement of concentrations of luteinizing hormone (LH), FSH, inhibin B and testosterone were obtained on each occasion. Semen analysis always showed oligozoospermia (sperm count <20×10⁶/ml). Forty-one subjects were affected by left varicocele, six by bilateral varicocele, and 20 had undergone orchidopexy when they were aged between 2 and 11 years (cryptorchids). Eight had a history of post-mumps orchitis, six of spermatic cord torsion. The pathogenesis of oligozoospermia was unknown in the remaining 48 subjects (idiopathic). Twenty age-matched fertile subjects were considered as controls for seminal and hormonal parameters. Semen samples were obtained by masturbation; after liquefaction at room temperature, semen volume, pH, sperm concentration, motility and morphology were determined following WHO guidelines for semen analysis (WHO, 1992). The presence of antisperm antibodies was evaluated utilizing the immunobead and the Sperm-Mar test (Ortho Diagnostic (WHO, 1992). The presence of antisperm antibodies was evaluated utilizing the immunobead and the Sperm-Mar test (Ortho Diagnostic System, Milan, Italy). All patients underwent ultrasound scanning of the testis to evaluate testicular size and morphology.

The study was approved by the Hospital Ethical Committee and informed consent was obtained from each subject.

Hormone assays

FSH and LH plasma concentrations were measured in each subject by radioimmunoassay using 125I-labelled FSH and LH (Ares-Serono, Milan, Italy). Inter-assay and intra-assay coefficients of variation were 2.8 and 3.7% respectively for LH and 2.6 and 3.6% respectively for FSH.

Testosterone plasma concentrations in all subjects using the double antibody radioimmunoassay utilizing commercial kits (Radim, Rome, Italy). All specimens were measured in duplicate in the same assay. Intra- and inter-assay coefficients of variation were 7.8 and 7.0% respectively.

Inhibin plasma concentrations were measured by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) specific for the dimeric inhibin-B form (Serotec, Oxford, UK) (Groome et al., 1996; Illingworth et al., 1996). The first antibody is directed to the βB-subunit, and the second antibody to the α-subunit and conjugated to alkaline phosphatase. The assay has a cross-reactivity of <0.1% with activins and ~1% with inhibin A. Assay sensitivity was 15 pg/ml and the inter- and intra-plate coefficients of variation were 6.4 and 6.8% respectively.

Fine needle aspiration cytology

The testicular structure was analysed in all patients by means of bilateral fine needle aspiration cytology (FNAC), in order to evaluate the tubular status related to oligozoospermia. The methods of aspiration and cytopathological analysis have been described previously in detail (Foresta and Varotto, 1992; Foresta et al., 1992, 1995).

Briefly, bilateral FNAC was performed aspirating by a 23-G (0.6 mm) butterfly needle attached to a 20 ml syringe. The retrieved material was placed on two or more microscope slides for each testis, allowed to air-dry for 24 h, stained with May–Grunwald and Giemsa stains, and examined under a light Orthoplan microscope (Wild-Leitz, Wetzlar, Germany) at magnifications of ×125, ×400 and ×1250, counting at least 200 cells per smear. Tubular cells were recognized because of their staining and morphological aspects, such as cell diameter, chromatin pattern and cytoplasm size, as described previously (Foresta et al., 1992). Cytological analysis allowed identification of the following spermatogenetic cell types: spermatogonia, primary and secondary spermatocytes, early and late spermatids (corresponding to Sa–Sb and Sc–Sd steps of spermatogenesis respectively) and spermatozoa. The relative number of each cell type was expressed as a percentage. Sertoli cells were expressed as the Sertoli index (SEI; the number of Sertoli cells/100 spermatogenic cells), which has been found to be a reliable index of the tubular germ cell potential, and constitutes an attempt to simplify the interpretation of cytopathological results. Since the number of Sertoli cells is constant per unit of tubular length, an increase in the SEI indicates tubular germ cell depopulation (hypospermatogenesis).

The proportion of spermatozoa was expressed as the spermatic index (SI; the number of spermatozoa/100 spermatogenic cells) that reflects the final maturational step and the spermiogenic process.

Forty normozoospermic infertile subjects, whose characteristics have been reported in previous studies (16 autoimmune, 24 idiopathic), were considered as controls for testicular cytology analysis (Foresta et al., 1992). Studies on the reproducibility of the cytopathological analysis have been performed previously, and have reported very low coefficient of variation between differential counts (Foresta et al., 1992).

On the basis of the testicular cytological findings, all considered subjects were divided into four different groups as follows: (i) severe hypospermatogenesis, defined as a strong quantitative reduction of germ cell line with respect to Sertoli cells, as indicated by a higher SEI (>300, where normal value is <50); (ii) spermatogonia and/or spermatocytic arrest, characterized by a significant increase of spermatogonia and/or spermatocytes (>40%, where normal value is <20%), with very low percentages of spermatids and spermatozoa; (iii) spermatid arrest, characterized by a significant increase of both early and late spermatids (>65%, where normal value is <45%), with a strong reduction of spermatozoa; and (iv) mild hypospermatogenesis, defined as a moderate reduction of germ cell line with respect to Sertoli cells, with a slight increase of SEI (100–300, where normal value is <50).

Treatment with FSH

Twenty-five of the 135 patients were treated with human highly purified FSH (Metrodin HP; Serono, Milan, Italy) at the dose of 75 IU i.m. on alternate days for 3 months. All these patients were affected by idiopathic oligozoospermia and showed a sperm count <10×10⁶/ml. Seminal parameters, inhibin B, FSH, LH and testosterone plasma concentrations were measured weekly for the first month, and then monthly.

Statistical analysis

The results are given as mean ± SD. Statistical analyses were performed using the Statview statistical package (ABACUS, Berkeley,
Results

Cytological analysis

Comparing cytological quantification of fine needle aspiration in oligozoospermic patients with those obtained from normozoospermic controls, four different groups were identified independently from hormone concentrations; group 1 (n = 38) had an elevated SEI compared with controls (486.3 ± 168.6 versus 30.4 ± 11.6%, P < 0.001) and a reduction of all germ cell types including spermatids and spermatozoa. In these patients, FSH plasma concentrations were elevated (15.7 ± 2.6 versus 2.6 ± 1.6 IU/l, P < 0.001 controls); the mean testicular volume was reduced (11.3 ± 2.8 versus 16.6 ± 4.6 ml, P < 0.001); inhibin B plasma concentrations were lower than those of controls (44.7 ± 10.3 versus 178.5 ± 37.3 pg/ml, P < 0.001), while LH and testosterone plasma concentrations were in the normal range. In group 2 (n = 26), there was a significant increase in spermatozoa and spermatoctyes with very low percentages of spermatids and spermatozoa, indicating partial arrest at the first maturational steps. In these subjects, the SEI was lower than controls (94.3 ± 36.8 versus 30.4 ± 11.6%); FSH plasma concentrations were higher than in controls (11.7 ± 5.4 versus 2.6 ± 1.6 IU/l, P < 0.01), whereas the mean testicular volume was normal (13.7 ± 2.8 versus 16.6 ± 4.6 ml). Inhibin B plasma concentrations were lower than in controls (54.8 ± 21.5 versus 178.5 ± 46.8 pg/ml, P < 0.001), while LH and testosterone plasma concentrations did not differ from those of controls. In group 3 (n = 34), there was a significant increase in both early and late spermatids with strong reduction of spermatozoa, indicating a partial arrest at the late maturational steps. In these subjects, FSH plasma concentrations were higher than in controls (11.7 ± 5.4 versus 2.6 ± 1.6 IU/l, P < 0.01), whereas the mean testicular volume was normal (13.7 ± 2.8 versus 16.6 ± 4.6 ml). Inhibin B plasma concentrations were lower than in controls (54.8 ± 21.5 versus 178.5 ± 46.8 pg/ml, P < 0.001), while LH and testosterone plasma concentrations did not differ from those of controls. In group 4 (n = 37), the percentages of the different spermatogenic cells were similar to those observed in controls, with a slight

CA, USA). Statistical analysis was performed using an analysis of variance (ANOVA). Correlations were performed after logarithmic transformation of the data. Probability (P) values < 0.05 were regarded as statistically significant.

Table I. Cytological analysis of oligozoospermic subjects compared with controls (normozoospermic subjects)

<table>
<thead>
<tr>
<th>Cytological picture</th>
<th>n</th>
<th>Spermatogonia/ spermatocytes (%)</th>
<th>Spermatid (%)</th>
<th>Spermatozoa SI (%)</th>
<th>Sertoli index SEI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe hypospermatogenesis</td>
<td>38</td>
<td>52.6 ± 21.4a</td>
<td>17.2 ± 8.7a</td>
<td>30.2 ± 6.9b</td>
<td>486.3 ± 168.6b</td>
</tr>
<tr>
<td>Spermatogonia/spermatocytic arrest</td>
<td>26</td>
<td>60.3 ± 22.7a</td>
<td>22.4 ± 15.2a</td>
<td>17.3 ± 8.8a</td>
<td>94.3 ± 36.8</td>
</tr>
<tr>
<td>Spermatid arrest</td>
<td>34</td>
<td>10.6 ± 5.6</td>
<td>71.5 ± 31.3a</td>
<td>17.9 ± 11.3a</td>
<td>54.8 ± 21.5</td>
</tr>
<tr>
<td>Mild hypospermatogenesis</td>
<td>37</td>
<td>34.9 ± 19.3b</td>
<td>35.5 ± 18.1b</td>
<td>29.6 ± 9.8b</td>
<td>127.2 ± 37.4b</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>9.3 ± 3.1</td>
<td>41.9 ± 12.9</td>
<td>48.8 ± 13.2</td>
<td>30.4 ± 11.6</td>
</tr>
</tbody>
</table>

SI = spermatic index.

*P < 0.001 versus controls.

P < 0.01 versus controls.

Table II. Clinical and hormonal features of oligozoospermic patients, compared with controls (normozoospermic subjects)

<table>
<thead>
<tr>
<th>Cytological picture</th>
<th>n</th>
<th>Sperm concentration (×10^9/ml)</th>
<th>Testicular volume (ml)</th>
<th>FSH (IU/l)</th>
<th>LH (IU/l)</th>
<th>Testosterone (ng/ml)</th>
<th>Inhibin B (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe hypospermatogenesis</td>
<td>38</td>
<td>2.1 ± 1.1</td>
<td>11.3 ± 2.8a</td>
<td>15.7 ± 2.6a</td>
<td>4.4 ± 1.9</td>
<td>4.0 ± 3.1</td>
<td>44.7 ± 10.3a</td>
</tr>
<tr>
<td>Spermatogonia/spermatocytic arrest</td>
<td>44</td>
<td>4.2 ± 2.0</td>
<td>13.7 ± 2.8</td>
<td>11.7 ± 5.4a</td>
<td>3.1 ± 1.4</td>
<td>4.4 ± 2.3</td>
<td>54.8 ± 21.5a</td>
</tr>
<tr>
<td>Spermatid arrest</td>
<td>25</td>
<td>8.7 ± 3.2</td>
<td>14.3 ± 2.6</td>
<td>3.8 ± 2.5</td>
<td>3.9 ± 1.7</td>
<td>4.1 ± 2.0</td>
<td>164.1 ± 37.3</td>
</tr>
<tr>
<td>Mild hypospermatogenesis</td>
<td>28</td>
<td>12.0 ± 3.9</td>
<td>14.5 ± 3.2</td>
<td>4.2 ± 2.1</td>
<td>3.6 ± 1.6</td>
<td>4.5 ± 2.4</td>
<td>176.4 ± 31.6</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>5.1 ± 0.9</td>
<td>13.2 ± 3.0</td>
<td>9.0 ± 8.9</td>
<td>3.5 ± 2.2</td>
<td>4.2 ± 2.9</td>
<td>124.0 ± 66.7</td>
</tr>
<tr>
<td>Fertile subjects</td>
<td>20</td>
<td>61.2 ± 38.8</td>
<td>16.6 ± 4.6</td>
<td>2.6 ± 1.6</td>
<td>2.8 ± 1.5</td>
<td>4.4 ± 2.2</td>
<td>178.5 ± 46.8</td>
</tr>
</tbody>
</table>

*P < 0.01 versus fertile controls.

P < 0.01 versus fertile controls.

FSH = follicle stimulating hormone; LH = luteinizing hormone.

Table III. Oligozoospermic subjects subdivided on the basis of inhibin B and follicle stimulating hormone (FSH) plasma concentrations

<table>
<thead>
<tr>
<th></th>
<th>FSH (pg/ml)</th>
<th>Inhibin B (×10^9/ml)</th>
<th>Sperm concentration (ml)</th>
<th>Testicular volume (ml)</th>
<th>Sertoli index SEI (%)</th>
<th>Spermatids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>3.5 ± 1.8</td>
<td>158.0 ± 81.9</td>
<td>10.4 ± 3.7</td>
<td>14.5 ± 2.5</td>
<td>279.0 ± 220.1</td>
<td>43.2 ± 20.1</td>
</tr>
<tr>
<td>Group B</td>
<td>12.5 ± 5.8a</td>
<td>107.4 ± 36.6</td>
<td>3.5 ± 3.9a</td>
<td>13.2 ± 2.0</td>
<td>507.6 ± 284.5</td>
<td>28.5 ± 11.6</td>
</tr>
<tr>
<td>Group C</td>
<td>18.9 ± 10.7a</td>
<td>50.2 ± 19.3a</td>
<td>1.9 ± 2.5a</td>
<td>10.8 ± 2.0a</td>
<td>727.5 ± 225.6a</td>
<td>17.7 ± 9.4a</td>
</tr>
</tbody>
</table>

*P < 0.01 versus group A.
concentrations and low inhibin B concentrations (n = 25, 18.5%); and group C, those with high FSH plasma concentrations and high FSH concentrations (subjects with normal FSH and inhibin B plasma concentrations in inhibin B and FSH concentrations: group A, characterized by parameters in the evaluation of testicular tubular function. A significant inverse correlation was observed between inhibin B and testicular volume (r = -0.412, P < 0.0001; Figure 1). To explore the presence of a relationship between inhibin B plasma concentrations and testicular tubular structure, correlation coefficients between this hormone, testicular volume and cytological indexes were analysed. A significant inverse correlation was observed between inhibin B and testicular volume (r = -0.412, P < 0.0001), SEI (r = -0.282, P < 0.001) and spermatids percentage (r = -0.329, P < 0.001) (not shown).

If FSH plasma concentrations at least two SD higher than mean values (7.0 IU/l) and inhibin B plasma concentrations at least two SD lower than mean values of normal controls were considered pathological, it was clear that when inhibin B was pathologically low (<80.0 pg/ml; n = 33), FSH plasma concentrations were high in each case. In contrast, when subjects with pathologically high FSH plasma concentrations were considered pathological, it was clear that when inhibin B and inhibin B plasma concentrations were increased significantly in study weeks 2, 3 and 4 with respect to baseline concentrations (P < 0.01). In groups B and C, FSH plasma concentrations remained significantly elevated in study weeks 8 and 12, while in group A FSH plasma concentrations declined to baseline concentrations (Figure 2). Inhibin B plasma concentrations were unmodified during the first week of FSH administration in all patients. In patients of group A, inhibin B plasma concentrations were increased significantly in study weeks 2, 3 and 4 with respect to baseline concentrations (P < 0.01), and declined thereafter. In groups B and C, inhibin B plasma concentrations remained unchanged throughout the study period (Figure 2).

Discussion

There is growing experimental evidence that the secretion of inhibins by Sertoli cells is subject to paracrine control by germ cells (Pineau et al., 1990). In rats, mRNA concentrations for inhibin subunits and the concentration of immunoactive inhibins vary according to the stage of seminiferous cycle (Bhasin et al., 1989); moreover, experiments in which specific germ cell types have been depleted (local heating, gamma-irradiation, methoxy acetic acid treatment) have shown that concentrations of FSH in the blood are increased when elongated spermatids are absent from seminiferous epithelium (Jegou et al., 1984; Bartlett et al., 1988; Pinon-Lataillade et al., 1988). Furthermore, it has been shown that the addition of spermatids or spermatid-conditioned medium to immature rat Sertoli cells increased inhibin secretion (Pineau et al., 1990). One aim of the present study was to ascertain whether a similar germ cell control of inhibin production was also present in humans. The findings provide evidence suggesting that also in man the secretion of inhibin B by Sertoli cells may be regulated by spermatids. Independent of the concentration of inhibin B, and follicle stimulating hormone (FSH) in 135 oligozoospermic subjects (r = 0.55, P < 0.0001).

Inhibin B in oligozoospermic subjects

Figure 1. Correlation between plasma concentrations of inhibin B and follicle stimulating hormone (FSH) in 135 oligozoospermic subjects (r = 0.55, P < 0.0001).
An inverse relationship between the plasma concentrations of inhibin B and the functional state of the seminiferous epithelium (Anderson et al., 1998) could be a marker of testicular function than FSH (Jensen et al., 1997). It has been made recently as to whether plasma concentrations of inhibin may be considered a better indicator of exocrine than endocrine function, the investigation of Sertoli cell function and spermatogenesis, but represents an intricate combination of tubular dysfunction, but a careful analysis of results shows that FSH and inhibin B plasma concentrations do not always behave similarly. In fact, when inhibin B serum concentrations are low (<80 pg/ml), plasma concentrations of FSH are inevitably high, while high plasma FSH concentrations (>7 IU/l) may be associated with low or normal inhibin B concentrations.

The presence of high FSH plasma concentrations despite normal inhibin B concentrations remains to be clarified, but shows that elevated FSH is not always a marker of decreased inhibin B production; it also suggests that in these cases a residual function of tubular cells exists. One hypothesis could explain the elevated plasma FSH concentrations as being secondary to a partial alteration of testicular tubular function determining a decrease of inhibin B negative feed-back, and thus inducing a compensatory Sertoli cell response, resetting the pituitary–testicular axis and maintaining normal inhibin B production. In these patients, cytological analysis of tubular status showed that the number of spermatids was higher than that observed in patients with elevated FSH and normal inhibin B plasma concentrations. Furthermore, it is important to underline that in these patients the testicular volume was not different from that in controls.

On the basis of these findings, inhibin B appears to represent a more specific marker of tubular function than FSH, and permits the identification among oligozoospermic subjects with high FSH plasma concentrations of two sub-populations, each characterized by different degrees of testicular damage and spermatogenic cell content.

To clarify further the relationship between FSH and inhibin B and the meaning of inhibin B evaluation, three groups of patients characterized by: (i) normal FSH and normal inhibin B plasma concentrations (group A); (ii) high FSH and normal inhibin B plasma concentrations (group B); and (iii) high FSH and low inhibin B plasma concentrations (group C) were treated with FSH. In this study, doses of FSH were used that had been shown previously to influence human Sertoli cell functions and human spermatogenesis in vivo (Foresta and Varotto, 1994; Foresta et al., 1998). The findings of this study provide considerable insight into the dynamics of testicular inhibin B secretion in response to FSH administration. In fact, when FSH plasma concentrations were high, independently from those of inhibin B, FSH treatment did not induce any modification of inhibin B production. In contrast, in subjects with normal FSH and normal inhibin B plasma concentrations, FSH treatment stimulated inhibin B production. The lack of any increase of inhibin B after FSH treatment in subjects with high basal FSH and low inhibin B plasma concentrations is not surprising, and possibly reflects an incompetence of Sertoli cells in the production of this hormone, even if it is impossible to distinguish an intrinsic alteration of these cells from their dysfunctional status related to the lack or reduction of germ cells. In subjects with high plasma FSH and normal inhibin B concentrations, the absence of any increase in plasma inhibin

![Diagram](image)

**Figure 2.** Mean serum concentrations of inhibin B and follicle stimulating hormone (FSH) before and during treatment with FSH (75 IU i.m. on alternate days for 3 months) in three groups of oligozoospermic subjects (●, group A, n = 11; ■, group B, n = 7; ▲, group C, n = 7). *, P < 0.001 versus pre-treatment; †, P < 0.01 versus pre-treatment.

ejaculated spermatozoa, inhibin B plasma concentrations were reduced when intratesticular spermatids were grossly depleted and were significantly related to the Sertoli cell/spermatid ratio.

These results may justify the lack of any correlation between concentrations of spermatozoa and plasma inhibin B observed in this study, since in a large number of patients oligozoospermia was related to a maturational disturbance at the spermatidic level.

Circulating FSH has long been considered a reliable marker of Sertoli cell function and spermatogenesis, but represents an indirect parameter of tubular function, since FSH is produced by the pituitary and is influenced by hypothalamic regulation as well as by testicular factors and steroids. Since inhibin B is produced directly and solely from testis, the investigation has been made recently as to whether plasma concentrations of inhibin may be considered a better indicator of exocrine testicular function than FSH (Jensen et al., 1997; Klingmüller and Haidl, 1997). At present, this aspect has not been completely clarified, although some authors have suggested that seminal plasma inhibin B concentrations could be a marker of the functional state of the seminiferous epithelium (Anderson et al., 1998). The results of this study demonstrate a significant inverse relationship between the plasma concentrations of inhibin B and FSH in subjects with disturbed spermatogenesis, confirming the supposed role of inhibin B in the mechanisms regulating FSH secretion. This relationship, at first sight, may suggest that both hormones express the same evaluation of tubular dysfunction, but a careful analysis of results shows that FSH and inhibin B plasma concentrations do not always behave similarly. In fact, when inhibin B serum concentrations are low (<80 pg/ml), plasma concentrations of FSH are inevitably high, while high plasma FSH concentrations (>7 IU/l) may be associated with low or normal inhibin B concentrations.

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To clarify further the relationship between FSH and inhibin B and the meaning of inhibin B evaluation, three groups of patients characterized by: (i) normal FSH and normal inhibin B plasma concentrations (group A); (ii) high FSH and normal inhibin B plasma concentrations (group B); and (iii) high FSH and low inhibin B plasma concentrations (group C) were treated with FSH. In this study, doses of FSH were used that had been shown previously to influence human Sertoli cell functions and human spermatogenesis in vivo (Foresta and Varotto, 1994; Foresta et al., 1998). The findings of this study provide considerable insight into the dynamics of testicular inhibin B secretion in response to FSH administration. In fact, when FSH plasma concentrations were high, independently from those of inhibin B, FSH treatment did not induce any modification of inhibin B production. In contrast, in subjects with normal FSH and normal inhibin B plasma concentrations, FSH treatment stimulated inhibin B production. The lack of any increase of inhibin B after FSH treatment in subjects with high basal FSH and low inhibin B plasma concentrations is not surprising, and possibly reflects an incompetence of Sertoli cells in the production of this hormone, even if it is impossible to distinguish an intrinsic alteration of these cells from their dysfunctional status related to the lack or reduction of germ cells. In subjects with high plasma FSH and normal inhibin B concentrations, the absence of any increase in plasma inhibin
B after FSH treatment is unclear. In these patients the high endogenous plasma FSH concentrations may have induced a maximal functional activation of Sertoli cells which cannot be further stimulated. Another explanation for the lack of inhibin B production in these subjects following FSH administration could lie in the germ cell depletion seen in subjects not responding to FSH in terms of inhibin B production, thus strengthening the hypothesis that the presence of differentiated germ cells is necessary for Sertoli cells to produce inhibin B. This hypothesis seems to be confirmed by the observation that the higher the Sertoli cell index, the poorer the response to FSH in terms of inhibin B production.

In subjects with normal plasma FSH concentrations, the increase in inhibin B was evident in the second week of treatment, although circulating FSH was raised significantly after the first week of treatment, and declined—as did plasma FSH concentrations—after 4 weeks of therapy. The decline in plasma concentrations of inhibin B and FSH after 8 and 12 weeks of treatment may be due to the negative influence of increased inhibin B plasma concentrations on endogenous FSH production, leading to a decline in FSH secretion and, in turn, of inhibin B production. This hypothesis seems to be confirmed by the persistently increased plasma FSH concentrations seen throughout the treatment period in patients with high FSH concentrations but who showed no increase in inhibin B production.

The reason for the delayed response of inhibin B production to exogenous FSH administration is unknown, but confirms previous observations in monkeys during chronic treatment with FSH (Arslan et al., 1992) and in humans after acute administration of recombinant FSH (Kamischke et al., 1998). One possible explanation could lie in the requirement of germ cell replication for inhibin B production to occur after FSH administration. In respect of this, Anderson et al. (1997) observed that the recovery of inhibin B secretion following gonadotrophin suppression was related to the delay in reinitiation of spermatogenesis. Recently, it has been reported that inhibin B concentrations remained unchanged after treatment with recombinant FSH in a large group of oligozoospermic subjects (Kamischke et al., 1998). However, in that study the evaluation of inhibin B was performed at 6 and 12 weeks after the initiation of treatment, and therefore the initial increase in inhibin B production may have not been observed.

The ability of Sertoli cells to increase inhibin B production after FSH treatment may allow the identification of those oligozoospermic patients responsive to FSH treatment. To verify this hypothesis, treated patients were distinguished as those who doubled sperm concentrations after FSH treatment (responder), and those who did not (non-responder), as reported recently (Foresta et al., 1998). The similar behaviour of inhibin B production in responder and non-responder subjects excludes the possibility that inhibin B production after FSH administration could be utilized as a marker for the response to this treatment in terms of an increase in sperm production. On the other hand, in both cases the spermatid population evaluated by cytopathological analysis of the testis was well represented, thus justifying these findings.

In conclusion, the results of this study demonstrate that: (i) in humans, the production of inhibin B by Sertoli cells reflects interactions between these cells and spermatids; (ii) in oligozoospermic subjects, low plasma concentrations of inhibin B as well as high plasma FSH concentration identify pathological conditions characterized by a severe depopulation of seminiferous tubules; (iii) elevated plasma FSH concentrations are not always indicative of a decrease of inhibin B production, and in this case a normal inhibin B concentration demonstrates the presence of a residual function of tubular cells related to the presence of spermatids; (iv) FSH treatment causes an increase in inhibin B production only when plasma FSH concentrations are in the normal range; and (v) an increase of inhibin B production after FSH treatment may occur both in responders and non-responders, thus not allowing distinction between patients responsive to this treatment in terms of an increase in the production of spermatozoa.

References
Jegou, B., Laws, A.O. and de Kretser, D.M. (1984) Changes in testicular endocrinology and in this case a normal inhibin B concentration demonstrates the presence of a residual function of tubular cells related to the presence of spermatids; (iv) FSH treatment causes an increase in inhibin B production only when plasma FSH concentrations are in the normal range; and (v) an increase of inhibin B production after FSH treatment may occur both in responders and non-responders, thus not allowing distinction between patients responsive to this treatment in terms of an increase in the production of spermatozoa.

Inhibin B in oligozoospermic subjects

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