Inhibin B in seminal plasma: testicular origin and relationship to spermatogenesis

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In men, inhibin B is the circulating isoform involved in the regulation of follicle stimulating hormone (FSH) secretion. Within the testis, inhibin B may have a role in Sertoli cell interactions, thus secretion into seminal plasma may reflect seminiferous tubule function. Using specific immunoassays, inhibin B was present in seminal plasma in fertile men (n = 105) and in unselected men attending an infertility clinic (n = 174) with a wide range in concentration from undetectable (<15 pg/ml) up to 54,100 pg/ml (geometric mean 280 pg/ml). There was a highly significant correlation between seminal plasma inhibin B concentration and sperm concentration (r = 0.46, P < 0.001), but no correlation with percentages of spermatozoa with progressive motility or normal morphology. Inhibin A and isoforms containing pro and αC immunoreactivity were not detectable. In post-vasectomy seminal plasma samples (18 of 20) inhibin B was undetectable, indicating that the testis is the predominant source. In unselected men attending an infertility clinic, inhibin B was undetectable in 17% (present in remainder; maximum concentration 26,200 pg/ml; mean 263 pg/ml), with a highly significant correlation between seminal plasma inhibin B and sperm concentration (r = 0.55, P < 0.0001). In men with oligo-azospermia (sperm concentration <20×10⁶/ml), seminal plasma inhibin B concentrations were lower in those with elevated plasma FSH concentrations (mean values 42 and 205 pg/ml, P < 0.05). Inhibin α and βB subunits were localized predominantly in Sertoli and Leydig cells, using immunohistochemistry. We conclude that inhibin B of testicular origin is present in normal human seminal plasma, but with a very wide range in concentration, and may reflect the functional state of the seminiferous epithelium.

Key words: inhibin/Sertoli cell/spermatogenesis/testis

Introduction
The inhibin/activin family of dimeric peptide hormones are produced in the testis and are postulated to have paracrine and autocrine roles in the regulation of steroidogenesis and spermatogenesis (Lin et al., 1989; Chen, 1993; Mathet et al., 1997) in addition to endocrine regulatory effects on follicle stimulating hormone (FSH) secretion (Burger and Igarashi, 1988). Inhibins are composed of a dimer of a common α subunit and either a βA (inhibin A) or βB (inhibin B) subunit, while the activins are β subunit dimers resulting in activin A (βAβA), activin B (βBβB) and activin AB (βAβB). The development of immunoassays specific for the dimeric forms has allowed the demonstration that inhibin B but not inhibin A is present in male serum in both fetus and adult (Anawalt et al., 1996; Illingworth et al., 1996; Wallace et al., 1997).

The various inhibin/activin subunits have been localized in testis as messenger RNA and proteins. In the adult rat, the α, βA and βB subunits were present in both Sertoli and Leydig cells (Roberts et al., 1989; Klaij et al. 1994) and in adult monkey, mRNA for all three subunits was localized predominantly in Sertoli cells (Zhang et al., 1997). The expression of mRNA in Sertoli cells varies with the stage of the seminiferous cycle (Bhasin et al., 1989), with differences between the various subunits possibly reflecting differential production of activin and inhibin (Klaij et al. 1994). In the human, α subunit immunostaining is present in both Leydig and Sertoli cells (Bergh and Cajander, 1990), with an increase in positive staining in Leydig cells after human chorionic gonadotrophin (HCG) treatment and increased staining in Sertoli cells in conditions of impaired spermatogenesis. The βA subunit has also been localized to both Leydig and Sertoli cells in normal adult testis, although the antibody used cross-reacted with the βB subunit (Vliegen et al. 1993). In the fetal human testis, α and βB but not βA subunits were immunolocalized in Leydig and Sertoli cells (Jafid et al. 1997), whereas βA mRNA was predominantly found in the interstitial cells, and βB mRNA was predominantly localized in the seminiferous tubules (Roberts, 1997). The distribution of the βB subunit in adult human testis has not been described.

Inhibin bioactivity is present in human seminal plasma (Scott and Burger, 1980, 1981; Robertson et al. 1989), and shows a positive relationship with sperm concentration and an inverse relationship with FSH concentration (Scott and Burger, 1981). However, bioactive inhibin concentrations in seminal plasma were normal following vasectomy. Cultured primate Sertoli cells secrete bioactive inhibin in a highly vectorial manner, the majority of secretion being into the adluminal compartment (Handelsman et al., 1990) and inhibin secretion...
in vitro is increased in response to FSH and in the presence of germ cells (Handelsman et al., 1990; Carreau, 1995). While inhibin B secretion into blood has been demonstrated to be of physiological relevance and to be under gonadotrophin, and particularly FSH, control (Anawalt et al. 1996; Illingworth et al. 1996; Nachtigall et al. 1996; Anderson et al. 1997), secretion into seminal plasma, which may more closely reflect the functional state of the seminiferous epithelium, has not been investigated. Here we report the presence of inhibin forms in seminal plasma and the relationship between inhibin B concentrations and spermatogenesis in men recruited from three groups: (i) men who had recently fathered pregnancies, (ii) men who had had a vasectomy and (iii) men attending an infertility clinic, with a wide range of reproductive function from normal to severely abnormal.

**Materials and methods**

**Seminal plasma samples**

Semen samples were obtained from three groups of men:

1. **Fertile men**
   - This group comprised 105 men (mean age 35 years, range 22–45) who were part of an ongoing programme to recruit semen donors for research purposes from antenatal parentcraft classes in a local maternity hospital and had fathered ongoing pregnancies 20–37 weeks previously. Clinical history and physical examination were normal in all cases and semen quality was assessed according to WHO criteria (World Health Organization, 1992). Peripheral blood samples were obtained simultaneously, the plasma separated and stored at –20°C until assay.

2. **Post-vasectomy men**
   - This group comprised 20 men (mean age 35 years, range 26–45) submitting routine semen samples 12 weeks after bilateral vasectomy.

3. **Infertility clinic men**
   - This group comprised 174 unselected men (mean age 35 years, range 23–50) attending an infertility clinic. An unselected population was studied to provide a wide range of semen quality, from normal to severely abnormal, and it is emphasized that not all these men would be expected to be infertile or subfertile. Indeed, 98 of them had normal sperm concentration. Conventional semen analysis was performed according to WHO criteria. FSH and luteinizing hormone (LH) determinations were available on 34 men from this group with oligo/azospermia (sperm concentration <20×10⁹/ml).

Semen samples were produced by masturbation after 3–7 days abstinence, except in the case of post-vasectomy samples where abstinence was not specified. Samples were centrifuged (3000 g; 5 min) and the seminal plasma stored at –20°C until assay. This study had the approval of the Reproductive Medicine Subcommittee of the Lothian Research Ethics Committee.

**Inhibin assays**

Dimeric inhibin B, inhibin A and inhibin forms containing pro- and αC immunoreactivity were measured using two-site enzyme-linked immunosorbent assays (ELISA) as described previously (Groome et al. 1994, 1995, 1996) with some additional minor modifications (Riley et al. 1996).

**Preparation of samples for inhibin assays**

All samples and standards were treated similarly. Dilutions were made for the dimeric inhibin assays using fetal calf serum as the diluent and assay buffer for the pro and αC ELISA. Sodium dodecyl sulphate (2% final volume) was added and the tubes mixed and heat-treated in a water bath for 3 min at 100°C, which both improves the signal and eliminates false positive results. Samples were allowed to cool before hydrogen peroxide was added (1% final volume), followed by mixing and incubation for 30 min at room temperature prior to addition to the appropriate ELISA plate. The peroxide step modifies the β-subunit epitopes to improve reactivity with the respective antibodies (Knight and Muttukrishna, 1994).

**Dimeric inhibin B assay**

This ELISA uses a monoclonal capture antibody (C5) raised against the human inhibin Bβ subunit, immobilized passively onto 96-well plates (Nunc Maxisorp, Life Technologies Ltd, Paisley, UK) utilizing dry coating reagent (diluted 1:1 with H₂O; Bionostics Ltd, Wyboston, UK) and stored dry. The standard used was inhibin, partially immunopurified from human follicular fluid and calibrated against recombinant 32 kDa human inhibin B (Genentech, San Francisco, CA, USA). Plates were incubated for 16 h at 23°C; washed, then incubated with the detection antibody, a mouse monoclonal antibody (R1) raised against the inhibin αC-subunit conjugated to alkaline phosphatase. The alkaline phosphatase activity was detected using an amplification kit (Life Technologies) and absorbency was measured with a microplate reader (Thermomax, Molecular Devices Corp., Menlo Park, CA, USA) with dedicated software (Softmax, Molecular Devices). The detection limit of the assay was 15 pg/ml. The cross-reactivities of this assay for activin A, activin B, follistatin and purified human pro-αC were <0.1%, with cross-reactivity with recombinant inhibin A of 0.2% (Groome et al. 1996). The intra- and inter-plate coefficients of variation were 7 and 10% respectively. Recovery of inhibin B standard from seminal plasma was quantitative (92 ± 2%, n = 13) and serial dilution of seminal plasma gave a dose-response parallel to that of the standard (Figure 1).

**Dimeric inhibin A assay**

This assay is similar to the inhibin B assay, except that it uses a mouse monoclonal antibody (E4) raised against the βA subunit as the capture antibody. The same partially immunopurified follicular fluid preparation was used as standard but calibrated against 32 kDa recombinant human inhibin A (Genentech) with the same detection antibody (R1). The amount of alkaline phosphatase activity bound to the plate was determined using p-nitrophenylphosphate substrate (pNPP, Kirkegaard and Perry Laboratories, Gaithersburg, MA, USA). Cross-reactivities of this assay with activin A, activin B, follistatin, purified human pro-αC and inhibin B were all <0.1% (Groome et al. 1996).
Assay for inhibin forms containing pro and αC immunoreactivity

Inhibin isoforms containing pro and αC were detected using an ELISA similar to the dimeric inhibin assays (Groome et al. 1995). As standard this assay uses a highly purified preparation of pro-αC with a monoclonal capture antibody (INPROM) against a sequence of the pro-portion of the α-subunit and the same detection antibody (R1) was used as in the dimeric inhibin assays. Alkaline phosphatase activity was measured using pNPP substrate as for inhibin A. Recombinant forms of inhibin A, inhibin B and follistatin all cross-reacted <0.02%, although this antibody may cross-react with the larger dimeric inhibin isoforms containing the α-subunit pro sequences as demonstrated by immunoblotting studies (Groome et al. 1995). The detection limit was 3 pg/ml. Recovery of immunoreactive pro-αC standard from seminal plasma was quantitative (95 ± 2%, n = 10).

FSH assays

Plasma concentrations of FSH and LH were measured by immunoassay (Maiaclone, Biodata Diagnostics, Rome, Italy).

Tissue samples

Testicular biopsies were obtained from four young adult men (aged 26–34 years) undergoing investigation for infertility. All had normal testicular morphology and azoospermia due to epididymal obstruction. FSH concentrations were in the normal range. Tissues were fixed in Bouin’s fluid for 24 h then washed and mounted in paraffin.

Immunohistochemistry

Immunoreactive inhibin α- and β-subunits were localized in testis using methodologies described in detail previously with slight modifications (Majdic et al. 1997). Briefly, tissue sections (5 µm) on aminoalkylsilane coated slides (Sigma Chemical Co, St Louis, MO, USA) were dewaxed and rehydrated. Sections were boiled in citrate buffer (0.01 M; pH 6) by microwave then allowed to cool, to retrieve antigenic sites. Endogenous peroxidase activity was inhibited by incubation in H2O2 (3% solution for 20 min). Blocking steps with avidin (0.01 M; 20 min) and biotin (0.001 M; 20 min), followed by normal horse serum (20 min), were performed to eliminate non-specific binding. The primary antibodies were then applied at 4°C for 16 h. The following panel of mouse monoclonal antibodies were used, the specificities of which have been reported elsewhere (Majdic et al. 1997): α-subunit, 173/9 K (2 µg/ml optimal final concentration); βB-subunit, two antibodies, 12/13 (0.12 µg/ml) and C5 (0.24 µg/ml). Sections were then washed and the primary antibody was detected using a biotinylated mouse anti-horse antibody, an avidin–peroxidase complex (according to the manufacturer’s instructions; Vector Laboratories, Burlingame, CA, USA) and visualized using 3,3’-diaminobenzidine chromagen (Vector). Sections were counterstained, dehydrated, mounted and visualized by light microscopy.

Statistical analysis

Inhibin B concentrations in seminal plasma showed a skewed distribution, which was normalized after logarithmic transformation. Data are presented as geometric mean and interquartile range. Correlations were performed and groups were compared (Student’s t-test) after logarithmic transformation.

Results

Inhibin in seminal plasma

Inhibin B was present in seminal plasma samples from men in the normal and infertility clinic groups, with a wide range in concentration from undetectable to 54 100 pg/ml, mean 280 pg/ml, interquartile range 976 pg/ml (n = 20). There was a highly significant correlation between seminal plasma inhibin B concentration and sperm concentration (r = 0.46, P < 0.0001), but no correlation between seminal plasma inhibin B and percentages of spermatozoa showing progressive motility or normal morphology (P < 0.05).

Group 1. Men of proven fertility

The range of concentrations of inhibin B in seminal plasma from normal men from the proven fertility group was very wide, from undetectable (13/105 men, 12%) to 54 100 pg/ml, mean 310 pg/ml, interquartile range 715 pg/ml. Inhibin B was detectable in all peripheral plasma samples collected simultaneously but with a much narrower range, from 66.5 to 945 pg/ml, mean 230 pg/ml, interquartile range 135 pg/ml. The correlation between inhibin B concentrations in seminal plasma and sperm concentration was lower in this group than when groups 1 and 3 were combined, but remained significant (r = 0.26, P < 0.01, Figure 2a). There were also significant correlations between inhibin B in seminal plasma and peripheral plasma (r = 0.27, P < 0.01) and between inhibin B concentration in peripheral plasma and sperm concentration (r = 0.36, P < 0.002). As these men were recruited on the
basis of recent conception rather than semen analysis, it was expected that some would have semen quality outside WHO limits for normality. Indeed, in 13 men (12.4%) sperm concentration was \(<20 \times 10^6/ml\). Thirteen men had undetectable inhibin B in seminal plasma, of whom four had oligozoospermia (sperm concentration \(<20 \times 10^6/ml\)). A further four men also had oligozoospermia but had detectable seminal plasma inhibin B concentrations (range 104–869 pg/ml). There was no correlation between inhibin B concentration and percentage or number of spermatozoa with progressive motility, or with subject’s age.

Seminal plasma samples from 10 normal men were also assayed for inhibin A and forms containing the pro and αC immunoreactivity. Inhibin A was undetectable in all samples, and inhibin isoforms containing immunoreactive pro and αC isoforms were only detectable in one sample (50.1 pg/ml).

**Group 2. Post-vasectomy men**

To investigate the origin of inhibin B, seminal plasma samples were obtained from 20 men following bilateral vasectomy. All samples were azoospermic. Inhibin B was undetectable in samples from 18 men, and low concentrations were found in the other two samples (46 and 158 pg/ml).

**Group 3. Infertility clinic men**

Seminal plasma samples were obtained from the male partners of 174 couples attending an infertility clinic. A wide range in inhibin B concentration was found, from undetectable levels in 17% of samples to a maximum of 26 200 pg/ml, mean 263 pg/ml, interquartile range 1193 pg/ml. The range and mean were therefore similar to that found in the normal population (group 1) and similarly there was a significant correlation between inhibin B in seminal plasma and sperm concentration \((r = 0.55, P < 0.0001, \text{Figure } 2b)\). The mean inhibin B concentration in samples with normozoospermia \((>20 \times 10^6/ml, n = 98)\) was 561 pg/ml, range 15–17 000 pg/ml, interquartile range 2344 compared with 99 pg/ml (range 15–26 250 pg/ml, interquartile range 379) in men with oligo/azoospermia \((n = 76)\) \((P < 0.001)\). Inhibin B was undetectable in 2% of samples with a normal sperm concentration and in 22% with oligo/azoospermia. In 13 individuals, a second semen sample was available for inhibin B determination 4–12 weeks later. Seminal plasma inhibin B concentrations varied from undetectable to 17 000 pg/ml in this group (Table I).

**Table I.** Seminal plasma inhibin B concentrations (pg/ml) of subjects from whom two samples were analysed

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<th>Subject no.</th>
<th>First sample</th>
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There was a highly significant correlation between samples from individuals \((r = 0.98, P < 0.0001)\). In particular, for five of these men in whom inhibin B was undetectable in one sample, it was also undetectable in the other sample in three men and \(<40\) pg/ml in two.

There was a significant correlation between seminal plasma inhibin B concentration and percentage of spermatozoa with progressive motility \((r = 0.19, P < 0.05)\) but not with percentage of spermatozoa with normal morphology. After multiple regression analysis (including the variables sperm concentration, motility and morphology), only the correlation between seminal plasma inhibin B and sperm concentration remained statistically significant \((P < 0.0001)\).

Concentrations of FSH and LH were obtained in 34 men with oligo/azoospermia. Sperm concentration and seminal plasma inhibin B concentrations in these men are shown in Figure 3, grouped according to whether FSH was elevated \((>7\text{ IU/l})\). While there was a greater number of azoospermic men in the high FSH group, sperm concentration was not
Figure 4. Localization by immunohistochemistry of inhibin α subunit (a and b) and inhibin βB (c and d) subunits in testis from men assessed to have normal testicular morphology [(c) and (d) with the two βB antibodies (C5 and 12/13 respectively)]. Both α and βB subunits are localized predominantly in Sertoli cells (arrowheads) and Leydig cells (L; arrows); (e) negative control section (primary antibody substituted with mouse IgG) with no specific positive immunostaining [original magnifications: (a), (b) and (d) ×750, (c) ×300, (e) ×350].

Discussion
These results demonstrate that human seminal plasma contains dimeric inhibin B but not inhibin A or inhibin isoforms containing pro- and αC immunoreactivity. The principal origin of this inhibin B in seminal plasma is the testis, as demonstrated by the absence of inhibin B in post-vasectomy samples. While the Sertoli cell is known to secrete inhibin (Grootenhuis et al. 1990; Handelsman et al., 1990; Carreau, 1995) and is likely to be the predominant source, α and βB subunits are also present in Leydig cells, suggesting another site of secretion. The concentration of inhibin B in seminal plasma is positively related to spermatogenesis, and there was an inverse relationship to FSH in men with abnormal spermatogenesis and men.
Inhibin B in seminal plasma

with oligo/azoospermia with elevated FSH concentrations had lower seminal plasma inhibin B concentrations than those with normal FSH concentrations.

The range of inhibin B in seminal plasma was very wide, being undetectable in 12% of the normal, fertile group, the significance of which is unclear. Subjects were instructed to abstain from sexual intercourse for 3–5 days prior to sample submission, but it is possible that some did not. This might increase the variability of the results, but it is difficult to account for the wide range by this factor alone. There was however considerable intra-individual consistency in seminal plasma inhibin B concentration in the small number of subjects from whom two samples were analysed. The testicular/epididymal contribution to the volume of the ejaculate has been estimated to be ~5% (Purvis et al. 1975). The mean concentration of inhibin B in seminal plasma in fertile men was ~300 pg/ml; thus the concentration of inhibin B in the seminiferous tubules may be in the order of 6000 pg/ml. This is ~20-fold higher than found in normal male serum, where a concentration of inhibin B in seminal plasma in fertile men was undetectable in

with oligo/azoospermia, although sperm concentration was not lower, seminal plasma inhibin B concentration in men with lower seminal plasma inhibin B concentrations in men with oligo/azoospermia was undetectable in 12% of the normal, fertile group, the reasons for these discrepancies are unclear, but it appears that the male accessory glands secrete substances other than inhibin which have FSH-suppressing activity (Robertson et al. 1989).

Inhibin bioactivity in seminal plasma has been previously described (Scott and Burger, 1980, 1981; Robertson et al. 1989). As inhibin B is the only dimeric bioactive isoform we have detected, the bioactivity in the assay used by Scott and Burger should reflect inhibin B concentrations. There are, however, both similarities and discrepancies with the present results. Thus bioactive inhibin was low in men with azoosperma and elevated FSH levels, but was detectable in all men following vasectomy and in 16/17 men with obstructive azoospermia (Scott and Burger, 1981). The reasons for these discrepancies are unclear, but it appears that the accessory glands secrete substances other than inhibin which have FSH-suppressing activity (Robertson et al. 1989).

Inhibin B in seminal plasma was significantly related to sperm concentration in both the fertile population (group 1) and an unselected population of men attending an infertility clinic (group 3). These two groups were selected to include men with a wide range of spermatogenic quality rather than being artificially defined according to WHO norms. Thus the first and fertile group had recently fathered ongoing pregnancies (although paternity was not positively proven). The second group would be expected to include men with the full range of potential fertility from fertile to sterile. This was supported by the range of semen quality subsequently found. Undetectable inhibin B concentrations were found more often in men with oligo/azoospermia than in men with normal sperm concentration. The suggestion that the inhibin B concentration in seminal plasma may be a marker of the functional state of the seminiferous epithelium is further supported by the observation that high FSH concentrations were associated with lower seminal plasma inhibin B concentrations in men with oligo/azoospermia, although sperm concentration was not significantly different in the two groups. Inhibin B was undetectable in >50% of such men compared to 17% overall in the infertility clinic population and 12% in the fertile population. There was no direct correlation between seminal plasma inhibin B concentration and plasma FSH concentration, whereas such a relationship has been demonstrated between the plasma concentrations of the two hormones in both normal and pathological states (Anawalt et al. 1996; Illingworth et al. 1996; Anderson et al. 1997). A direct correlation would not be expected if seminal plasma inhibin B reflects Sertoli cell function and/or germ cell interaction, but is not under direct trophic control by FSH. While seminal plasma inhibin B measurement may be of value in the assessment of the state of the seminiferous epithelium, the wide range of values seen limits its applicability without a greater understanding of its functional significance.

Inhibin B in peripheral plasma showed a much narrower range, and was detectable in all samples. There are thus fertile men with normal sperm concentration and detectable plasma inhibin B but undetectable seminal plasma inhibin B. There were significant correlations between inhibin B concentrations in seminal plasma and peripheral plasma, and between plasma inhibin B and sperm concentration. We have previously found no correlation between plasma inhibin B and sperm concentration (Anderson et al., 1997) in a group of men volunteering for a study of hormonal male contraception. Men were required to have normal sperm concentration for that study, whereas for the present study men were recruited on the basis of fertility, thus the range of sperm concentration was wider and included some men with oligo/azoospermia.

In conclusion, inhibin B of testicular origin is present in normal human seminal plasma but with a very wide range in concentration in both fertile and infertile men, and is related
to sperm concentration. Sertoli cells may be the predominant site of production. Other inhibin forms were not detected. While no direct relationship between seminal plasma inhibin B and FSH concentrations was detected, men with abnormal spermatogenesis and elevated FSH concentrations had lower inhibin B concentrations than those with normal FSH concentrations despite similar sperm concentrations. Inhibin B in seminal plasma may therefore reflect the functional state of the seminiferous epithelium.

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References


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