0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 12
*Printed in U.S.A.*

# Inhibin B as a Serum Marker of Spermatogenesis: Correlation to Differences in Sperm Concentration and Follicle-Stimulating Hormone Levels. A Study of 349 Danish Men*

TINA KOLD JENSEN, ANNA-MARIA ANDERSSON, NIELS HENRIK I. HJOLLUND, THOMAS SCHEIKE, HENRIK KOLSTAD, ALEKSANDER GIWERCMAN, TINE BRINK HENRIKSEN, ERIK ERNST, JENS PETER BONDE, JØRN OLSEN, ALLAN MCNEILLY, NIGEL P. GROOME, AND NIELS E. SKAKKEBÆK

*Department of Growth and Reproduction (T.K.J., A.-M.A., A.G., N.E.S.), Rigshospitalet, DK-2100 Copenhagen, Denmark; Department of Occupational Medicine (N.H.I.H., H.K., J.P.B.), University of Aarhus; Department of Biostatistics (T.S.), University of Copenhagen; Department of Obstetrics and Gynaecology (T.B.H., E.E.), Aarhus University Hospital; The Danish Epidemiology Sciences Centre (J.O.), Aarhus University; Centre for Reproductive Biology (A.M.), Medical Research Council, Edinburgh; and School of Biological and Molecular Sciences (N.P.G.), Oxford Brookes University, Oxford*

**ABSTRACT**

Recent studies have focused on reproductive health of men in the general population. However, semen samples are difficult to obtain within sampling frames that allow comparisons. Blood samples are easier to obtain than ejaculates. Therefore, serum biomarkers of spermatogenesis are of major interest for population studies. FSH has previously been used as a marker of spermatogenesis, although it is also influenced by the hypothalamus. Serum inhibin B was recently suggested as a possible, more direct serum marker of spermatogenesis in men with testicular disorders. In a Danish nationwide collaborative study, we found an unexpected difference in semen concentration between two groups of men recruited from two different centres. We, therefore, analyzed reproductive hormones in blood, including inhibin B, to test whether the observed difference in semen concentration was reflected in the reproductive hormones.

From 1992 to 1995, a total of 430 men, 20–35 yr old, who lived with a partner and who had not previously attempted to achieve a pregnancy, were recruited. The couples were enrolled into the study in one of two centres (centre A, n = 231; and centre B, n = 199) when they discontinued birth control. At enrollment, they provided a semen sample (n = 419), and a blood sample was drawn (n = 349). The semen analysis was performed in accordance with the WHO 1992 guidelines, and interlaboratory differences were tested. Inhibin B was measured in an enzyme immunometric assay, which has previously been described. All blood samples were analyzed in the same laboratory.

Median sperm concentration and the percentage of morphologically normal spermatozoa were significantly higher among men from centre A (56.0 mill/mL and 42.5%), compared with men from centre B (44.8 mill/mL and 39%). Men from centre B had a significantly higher median FSH (3.42 IU/L) and a lower inhibin B (186 pg/mL) than men from centre A (3.21 IU/L and 209 pg/mL). The differences persisted after control for potentially confounding variables. A significant correlation was found between the cubic root-transformed serum FSH and inhibin B levels ($r = -0.61$, $P < 0.001$), between the cubic root-transformed serum FSH and sperm concentration ($r = -0.40$, $P < 0.001$), and between the cubic root-transformed inhibin B and sperm concentration ($r = 0.38$, $P < 0.001$). The predictive power of detecting sperm counts below 20 mill/mL among men who's inhibin B and FSH both were below 80 pg/mL and above 10 IU/L, respectively, was 100%.

The unexpected significant difference in semen concentration between two groups of normal Danish men was probably caused by differences in sampling procedures in the two centres where the men were recruited, rather than geographical differences. However, similar differences in serum levels of inhibin B and FSH between centres were found. These findings suggest that a real difference in spermatogenic potential between the two groups of men existed. We suggest that serum inhibin B, in future population studies on male reproductive health, may serve as a new marker of spermatogenesis, in addition to sperm concentration and serum FSH. (*J Clin Endocrinol Metab* **82:** 4059–4063, 1997)

RECENT studies have focused on semen quality of men in the general population (1–3). However, the interpretation of semen data, over time, has been hampered by significant intra- and interindividual differences in sperm counts, high levels of nonresponse, inappropriate sampling procedures, and intralaboratory differences in methods. Some of these problems are difficult to overcome because semen analysis has to be performed soon after ejaculation, and the analysis must be performed in a laboratory at the site of collection, in contrast to blood analysis. Furthermore, in most semen quality studies, the participation rate is low, which could introduce selection bias. Blood samples are easier to obtain than ejaculates. Therefore, a valid serum biomarker of spermatogenesis is of particular interest for population studies. Circulating FSH has long been considered a valuable marker for Sertoli cell function and spermatogen-

Received June 13, 1997. Revised August 18, 1997. Accepted September 9, 1997.

Address all correspondence and requests for reprints to: Tina Kold Jensen, Department of Growth and Reproduction, The National University Hospital, Rigshospitalet, sect. GR 5064, 9-Blegdamsvej, DK-2100 Copenhagen, Denmark.

* This study was supported by a grant from Aarhus University Research Foundation (J.nr. 1994–7430-1). Additional support was given by the Danish Medical Research Council (J.nr. 12–2042-1) and the Danish Medical Health Insurance Foundation (J.nr. 11/236–93 and J.nr. 11/243–91).

esis, but FSH is influenced by hypothalamic function, as well as by testicular factors and steroid hormones. Serum inhibin B was recently suggested as a promising, more direct serum marker of Sertoli cell function and spermatogenesis than FSH (4, 5).

In a Danish, nationwide, collaborative study, we had the opportunity to investigate semen samples and reproductive hormones, including FSH and inhibin B, among 349 normal Danish men. The men were enrolled differently in two regions. Although the primary objective of the study was not to identify regional differences, we found a significant difference in sperm counts between the two populations. Given that serum inhibin B concentrations correlate with semen quality, it was of interest to see whether a parallel difference in inhibin B was present.

## Subject and Methods

### *Study population and enrollment*

The aim of the original study was to describe the association between time to pregnancy and semen quality in a population with unknown fertility. Four nationwide unions (*i.e.* metal workers, trade and office workers, nurses, and day care workers) provided personal identification numbers of all members throughout the country. Members between 20–35 yr of age, cohabiting with a person of the opposite sex and in the same age range, with no children at the same address, were identified (n = 52,255). From August 1992 through September 1995, they were mailed a personal letter and invited to join the study. Inclusion criteria were: no previous pregnancies; no prior knowledge of fertility; living with a person of the opposite sex; current use of contraception but planning to discontinue within the study period to conceive. A total of 430 couples from all over Denmark participated. It was estimated that 10–20% of eligible couples participated (6). Because the couples lived in different parts of the country, they were enrolled in the study in two centres (centre A, n = 231; and centre B, n = 199). The protocol was approved by The National Ethical Committee.

At enrollment, both partners filled in a questionnaire on demographic, medical, reproductive, occupational, and lifestyle factors. The males provided a semen sample (n = 419) and a blood sample (n = 349). At centre A, the couples who lived far from the centre hospital were interviewed at their home address, because of the long distances, whereas all participants at centre B were interviewed at the centre hospital. Blood samples were collected from 162 men (82.7%) from centre B and 187 men (83.9%) from centre A. At centre B, a semen sample was collected at the home address among couples who achieved a pregnancy before the interview (n = 16). Therefore, no blood samples were drawn from these couples. All questions regarding diseases of the reproductive organs were analyzed, in relation to fecundability, and those found to affect fecundability (adult parotitis, testicular cancer, and cryptorchidism) were transformed into one variable: present or not present.

### *Semen and blood collection and analysis*

Semen samples were obtained by masturbation only and were collected at the home residence. We encouraged sampling after 3 days of abstinence but emphasized that samples obtained outside this time window would still be useful. The ejaculates were collected directly into a 50-mL polyethylene container, and 93.8% were examined within the first 2 h (no difference between centres). Men from centre B poured the semen sample from a large to a smaller portable container at home, whereas this was done by a technician at centre A. The semen analysis was performed in accordance with the WHO 1992 guidelines (7). After complete liquefaction, the sample was kept in a heated chamber at 37 C until analysis. The semen volume was measured in a graded tube with 0.1 mL accuracy. Sperm concentration was measured in a Makler chamber at centre A and in a Bürger-Türk chamber at centre B. An appropriate dilution was determined after a preliminary examination of the undiluted sample. Counting was undertaken using a phase-contrast microscope at a magnification of 200. The sample was counted twice, and if there was more than 10% difference between the two counts, the sample dilution was remixed and the counting procedure was repeated. Morphological evaluation was performed only by one technician. The interlaboratory variation in sperm count was tested by comparing sperm counts on the same 28 samples made by a technician from each of the two centres, and no systematic difference was found (interlaboratory coefficient 0.96, paired $t$ test = 0.23, $P = 0.82$).

Serum was separated after clotting and stored at −20 C until hormone measurements. No differences between storage times existed between centres. With regard to the different hormone assays, all serum samples were analyzed in the same laboratory. Inhibin B was measured in an enzyme immunometric assay, which has previously been described (4, 8). The inhibin B assay, which is specific for the bioactive inhibin B dimer ($\alpha$-$\beta_B$), uses an immobilized monoclonal capture antibody raised against a sequence from the human $\beta_B$-subunit and a secondary enzyme-conjugated monoclonal antibody raised against a sequence from the human $\alpha$-subunit. The sensitivity of the inhibin B assay was 20 pg/mL, and the intra- and interassay coefficients of variation were <12% and <17%, respectively. Serum concentrations of FSH and LH were measured using time-resolved immunofluorometric assays from Wallac, Turku, Finland. The sensitivity of the FSH and LH assays was 0.06 IU/L and 0.05 IU/L, respectively. In both assays the intra- and interassay coefficients of variation were <10%. Serum testosterone was measured using an RIA (Coat-a-Count) from Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of the Diagnostic Products Corporation testosterone assay was 0.23 nmol/L, and the intra- and interassay coefficients of variation were both <10%.

### *Statistical analysis*

Frequency distributions of the various semen and blood parameters were highly skewed, and nonparametric tests were used to test differences in parameters between centres. Cubic root transformation showed good approximation to the normal distribution in the residual plots. Multiple linear regression analysis was done on the cubic root-transformed semen and blood parameters as dependent variables, and potentially confounding variables were included in the model. The potential confounding variables were excluded stepwise if they had no impact on the dependent variable.

## Results

### *Semen*

Table 1 shows the values for semen parameters in the two groups of men. Sperm concentration, volume, total sperm count, and the percentage of morphologically normal spermatozoa were significantly higher among men from centre A, compared with men from centre B. Men from centre B had a smaller seminal volume, which was probably because of the fact that they poured the sample from one container to another at home, which the men from centre A did not. This may also explain the observed difference in total sperm count (sperm concentration multiplied by seminal volume).

No significant difference was noted with respect to age among men in the two centres, but men from centre A had a higher weekly alcohol intake and a higher percentage smoked and had had diseases of the reproductive organs (Table 1). More men from centre B delivered their semen sample in the winter, from September to March. The median time of the day for blood sampling among men at centre B was 1030 h, whereas it was 1430 h among men at centre A. The men from whom no blood sample was drawn did not differ from the rest, with respect to semen quality, age, weekly alcohol intake, percentage of smokers, or percentage with diseases of the reproductive organs.

**TABLE 1.** Mean (and SD) and median (and 5 and 95 percentiles) semen parameters, age, and potential confounders among men from centre A and B

| Variables | Centre A | | Centre B | | $P^a$ |
|---|---|---|---|---|---|
| | Men giving blood and semen samples | Men giving only semen samples | Men giving blood and semen samples | Men giving only semen samples | |
| Number | 187 | 223 | 162 | 196 | |
| Sperm density (million/mL) | | | | | 0.007/ 0.03 |
| Mean | 79.2 (71.6) | 76.0 (70.5) | 57.0 (48.2) | 57.1 (46.5) | |
| Median | 60.0 (4.8–233.6) | 56.0 (4.4–230.8) | 41.5 (1.2–144.7) | 44.8 (2.6–143.3) | |
| % <20 mill/ml | 16.0 | 17.0 | 22.8 | 20.4 | |
| % with azospermia | 1.6 | 1.8 | 2.5 | 2.0 | |
| Sperm volume (mL) | | | | | <0.001/ |
| Mean | 3.4 (1.6) | 3.3 (1.6) | 2.7 (1.3) | 2.7 (1.3) | <0.001 |
| Median | 3.3 (0.8–6.0) | 3.1 (0.8–6.0) | 2.5 (0.9–5.3) | 2.5 (0.9–5.2) | |
| Total sperm count (million) | | | | | <0.001/ <0.001 |
| Mean | 250.2 (229.7) | 232.1 (219.5) | 156.2 (145.6) | 152.3 (137.1) | |
| Median | 171.6 (9.3–726.8) | 153.0 (8.1–716.4) | 119.4 (15.–457.7) | 118.1 (3.6–436.4) | |
| Morphologically normal spermatozoa (%) | | | | | 0.02/ 0.03 |
| Mean | 39.9 (11.4) | 39.8 (11.9) | 37.2 (12.8) | 37.6 (12.7) | |
| Median | 42.0 (17.2–57.2) | 42.5 (17.0–56.5) | 38.8 (2.6–54.5) | 39.0 (14.9–54.6) | |
| Abstinence (days) | | | | | 0.48/ |
| Mean | 4.5 (4.1) | 4.1 (3.9) | 4.0 (2.9) | 4.1 (3.5) | 0.55 |
| Median | 3.0 (1.0–14.0) | 3.0 (1.0–12.1) | 3.0 (1.0–9.0) | 3.0 (1.0–10.0) | |
| Age (yr) | | | | | |
| Mean | 28.5 (3.0) | 28.4 (3.0) | 28.2 (2.9) | 27.9 (3.0) | |
| Median | 28.2 (23.8–33.7) | 28.1 (24.0–33.4) | 27.6 (23.3–33.4) | 27.5 (23.2–33.3) | |
| Diseases in reproductive organs[b] (%) | 10.7 | 12.1 | 9.3 | 9.7 | |
| Smokers (%) | 37.4 | 36.3 | 26.5 | 27.0 | |
| Alcohol intake (drinks/ week) | | | | | |
| Mean | 10.2 (9.7) | 10.0 (9.3) | 8.2 (7.4) | 8.2 (7.2) | |
| Median | 7.0 (0–30.6) | 7.0 (0–29.6) | 7.0 (0–25.6) | 7.0 (0–23.0) | |
| Semen samples delivered from September–February (%) | 82.0 | 77.6 | 95.1 | 92.8 | |
| Time for blood sample collection | | | | | |
| Mean | 2.05 P.M. (3.28) | | 11.40 A.M. (3.11) | | |
| Median | 2.30 P.M. (8.15 A.M.–9.10 P.M.) | | 10.30 A.M. (8.00 A.M.–5.00 P.M.) | | |

[a] Mann-Whitney U-test. P value for differences between men delivering blood and semen samples/*P* value for differences between men delivering only semen sample.
[b] Fertility related diseases include: adult parotitis, testicular cancer and cryptorchidism

*Reproductive hormones*

Significant correlations were found between serum FSH and inhibin B levels [r = −0.44, $P < 0.001$ (nontransformed parameters); or r = −0.61, $P < 0.001$ (cubic root-transformed parameters), Fig. 1]. The correlations did not differ according to centres.

Serum FSH and sperm concentration were correlated among all men [r = −0.25, $P < 0.001$ (nontransformed parameters); or r = −0.40, $P < 0.001$ (cubic root-transformed parameters), Fig. 2] and among men from the two centres. Similar associations were found between serum inhibin B and sperm concentration [r = 0.26, $P < 0.001$ (nontransformed parameters); or r = 0.38, $P < 0.001$ (cubic root-transformed parameters), Fig. 3]. We performed all correlations after exclusion of men with azospermia or by exclusion of men with a sperm count below 20 mill/mL. This had an adverse effect on the correlations, although they were still significant.

Among men with oligospermia (sperm count below 20 mill/mL) 17.9% had an inhibin B concentration below 80 pg/mL and 17.9% had an FSH concentration above 10 IU/L (sensitivity). Among the men with sperm counts above 20 mill/mL, 95.7% had an inhibin B concentration above 80 pg/mL and 99.3% an FSH concentration below 10 IU/L (specificity). The predictive power of detecting oligospermia among men with an inhibin concentration below 80 pg/mL or an FSH concentration above 10 IU/L was 80.0% and 85.7%, respectively. The predictive power of detecting oligospermia among men whose inhibin B and FSH both were below 80 pg/mL and above 10 IU/L, respectively, was 100%.

Blood parameters of the participating men from the two centres are shown in Table 2. The differences in serum FSH and inhibin B levels between men from the two centres were statistically significant, whereas there was no significant difference in LH and testosterone levels (Table 2).

Multiple linear regression analysis, using the cubic root-

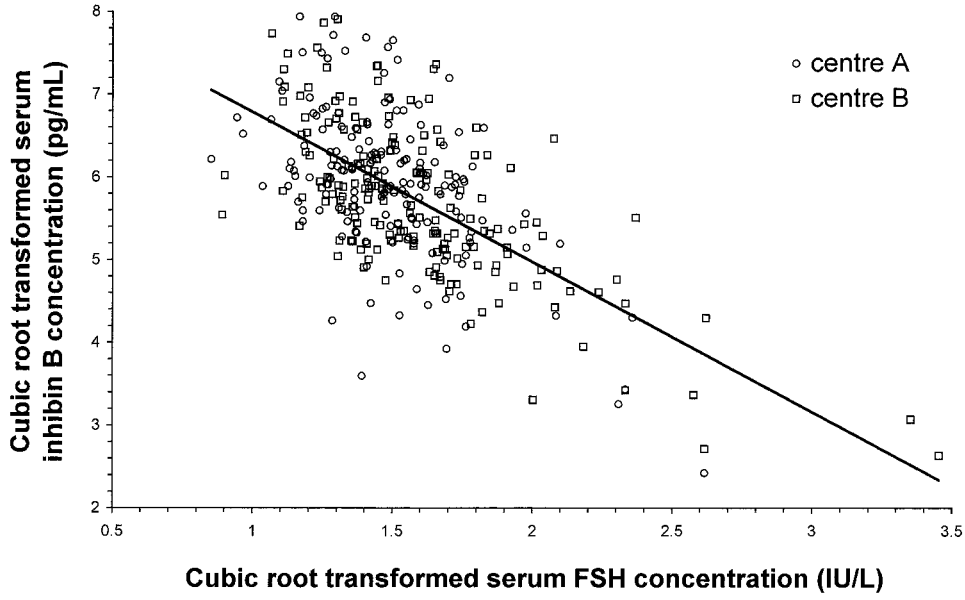


FIG. 1. Correlation between the cubic root transformed serum inhibin B and FSH levels in men from both centres ($r = -0.61$, $P < 0.001$).

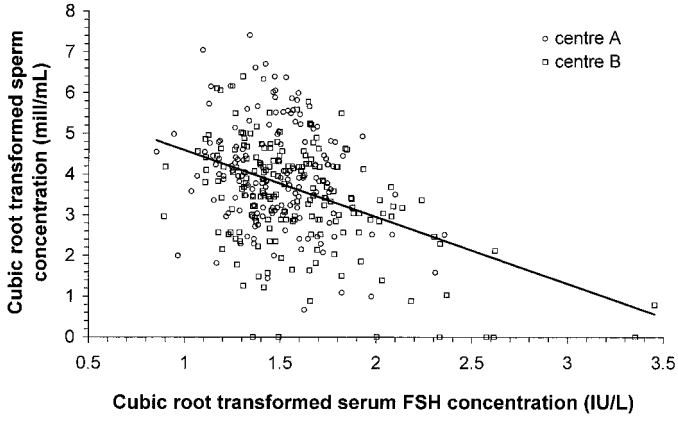


FIG. 2. Correlation between the cubic root-transformed sperm concentration and serum FSH levels in men from both centres ($r = -0.40$, $P < 0.001$).

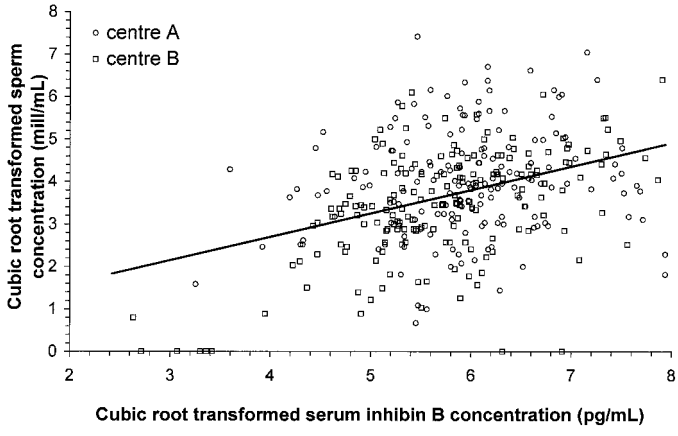


FIG. 3. Correlation between the cubic root-transformed sperm concentration and serum inhibin B levels in normal men from both centres ($r = 0.38$, $P < 0.001$).

transformed sperm concentration as a dependent variable and the following explanatory variables: smoking, age, period of abstinence (cubic root-transformed), season at delivery, diseases of the reproductive organs, smoking, and alcohol intake, was performed. Sperm concentration was higher among men from centre A than among men from centre B ($P < 0.001$) after control for length of abstinence, season at delivery, and diseases of the reproductive organs. Men from centre A had a higher inhibin B concentration ($P < 0.001$) and a lower FSH concentration ($P < 0.001$) after control for hour of blood sampling and diseases of the reproductive organs.

## Discussion

We found an unexpected difference in sperm concentration and sperm morphology between men recruited in two different centres in Denmark. The difference in sperm concentration could apparently not be explained by methodological differences in semen analysis between the two laboratories, nor by differences in length of abstinence, season at delivery, or diseases of the reproductive organs. Sampling procedures differed between the two centres, because men living far from the centre hospital in centre B had more inconvenience in participating than did men living far from the hospital in centre A (who were interviewed at their home address). Furthermore, the men from centre B decanted the seminal fluid at home, whereas this was performed by a technician in centre A. Therefore, differences in sampling and recruitment procedures may explain our findings. Furthermore, care should be taken in assessing spermatogenic potential by use of only one semen sample, because intraindividual variation in sperm concentration exists (9–11).

The study gave us, however, an opportunity to compare reproductive hormones in two populations of normal men with different semen quality. Interestingly, men from centre A who had significantly higher sperm concentration also had significantly higher inhibin B and lower FSH levels than did men from centre B, after controlling for hour of sampling and diseases of the reproductive organs. The fact that serum inhibin B and FSH point in the same direction strengthen our findings and indicate that both serum markers may be useful

**TABLE 2.** Mean (and SD) and median (and 5 and 95 percentiles) hormone parameters among men from centre A and B

| Variable | Centre A | Centre B | $P^a$ |
|---|---|---|---|
| Number | 187 | 162 | |
| FSH (IU/L) | | | 0.03 |
| Mean | 3.66 (2.24) | 4.70 (4.79) | |
| Median | 3.21 (1.44–7.73) | 3.42 (1.59–11.08) | |
| Inhibin B (pg/mL) | | | 0.01 |
| Mean | 219.8 (90.8) | 201.4 (93.1) | |
| Median | 209.0 (80.8–421.5) | 186.0 (83.2–392.0) | |
| Testosterone (nmol/L) | | | 0.26 |
| Mean | 16.51 (5.80) | 15.88 (4.97) | |
| Median | 15.91 (7.21–24.86) | 15.90 (8.22–24.10) | |
| LH (IU/L) | | | 0.62 |
| Mean | 3.57 (1.51) | 3.66 (1.67) | |
| Median | 3.22 (1.52–6.41) | 3.34 (1.62–6.64) | |

[a] Mann-Whitney U-test.

proxy measures of spermatogenic potential in population studies. In fact, the predictive power in detecting oligospermia (semen concentration below 20 mill/mL), among men with a serum inhibin B below 80 pg/L and a serum FSH above 10 IU/L, was 100%.

We do not believe that the observed differences in serum levels of FSH and inhibin B can be explained by differences in the hour of sampling. Blood samples were generally drawn in the late morning at centre B and in the early afternoon at centre A. Several studies have found decreasing testosterone concentrations during the day (12–14), but no clear pattern regarding FSH secretion has been found (12, 15–18). No report on the secretion pattern of inhibin B has been published.

Inhibin B is a marker of Sertoli cell function. Because the Sertoli cells can only support, and thereby help, the maturing of a given number of spermatozoas, serum inhibin B is probably a more direct marker of spermatogenesis than is FSH, which has been used traditionally as a marker of spermatogenesis in the clinical evaluation of male infertility. Serum FSH levels are not only determined by the testis but are also influenced by hypothalamic function. However, measurement of inhibin has previously been problematical. Inhibin is a glycoprotein dimer, consisting of two disulphides linked subunits ($\alpha$ and either $\beta_A$ or $\beta_B$) (19). In addition to the bioactive inhibin A ($\alpha$-$\beta_A$) and inhibin B ($\alpha$-$\beta_B$) dimers, biologically inactive inhibin forms, such as free inhibin subunits and unprocessed or partially processed inhibin forms, are also present in the circulation (20–22). The first inhibin assays developed could not distinguish between the bioactive and inactive forms of inhibin. Studies that used these assays failed to observe an inverse relationship between circulating inhibin and FSH levels in different testicular disorders, presumably because the results obtained with these assays did not reflect the level of bioactive inhibin present in the circulation (23, 24).

The inhibin B assay used in this study was recently developed. It specifically measures the bioactive inhibin B dimer (8). Inhibin B is the main circulating inhibin form in adult men, and an inverse relationship exists between circulating inhibin B and FSH, both in men with normal and abnormal spermatogenesis (4, 5). We found a significant correlation between the serum FSH and inhibin B levels.

In conclusion, we have used serum levels of inhibin B and FSH as biomarkers of spermatogenesis to confirm a difference in semen quality between two groups of normal Danish men. Our findings suggest that serum inhibin B, in addition to FSH,, may serve as a new marker of spermatogenesis in future population studies on male reproductive health of normal men.

## Acknowledgments

We are indebted to support from several trade union officials. Technicians from the laboratories are greatly acknowledged for performing the semen and blood analyses.

## References

1. **Carlsen E, Giwercman A, Keiding N, Skakkebæk NE.** 1992 Evidence for decreasing quality of semen during past 50 years. Br Med J. 305:609–613.
2. **Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J.** 1996 Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. Br Med J. 312:467–471.
3. **Auger J, Kunstmann JM, Czyglik F, Jouannet P.** 1995 Decline in semen quality among fertile men in Paris during the past 20 years. N Engl J Med. 332:281–285.
4. **Illingworth PJ, Groome NP, Bryd W, et al.** 1996 Inhibin-B: a likely candidate for the physiologically important form of inhibin in man. J Clin Endocrinol Metab. 81:1321–1325.
5. **Anawalt BD, Bebb RA, Matsumoto AM, et al.** 1996 Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab. 81:3341–3345.
6. **Krause W, Pflüger KH.** 1989 Treatment with the gonadotropin-releasing hormone agonist burselin to protect spermatogenesis against cytotoxic treatment in young men. Andrologia. 21:265–270.
7. **World Health Organization.** 1992 WHO laboratory manual for the examination of human semen, and sperm-cervical mucus interaction. 3rd ed. Cambridge: Cambridge University Press.
8. **Groome NP, Illingworth PJ, O'Brien M, et al.** 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab. 81:1401–1405.
9. **Heuchel V, Schwartz D, Price W.** 1981 Within-subject variability and the importance of abstinence period for sperm count, semen volume and pre-freeze and post-thaw motility. Andrologia. 13:479–485.
10. **Poland ML, Moghissi KS, Giblin PT, Ager JW, Olson JM.** 1985 Variation of semen measures within normal men. Fertil Steril. 44:396–400.
11. **Mallidis C, Howard EJ, Baker HWG.** 1991 Variation of semen quality in normal men. Int J Androl. 14:99–107.
12. **Faiman C, Winter JSD.** 1971 Diurnal cycles in plasma FSH, testosterone and cortisol in men. J Clin Endocrinol Metab. 33:186–192.
13. **Bremner WJ, Vitiello MV, Prinz PN.** 1983 Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J Clin Endocrinol Metab. 56:1278–1281.
14. **Winters SJ.** 1991 Diurnal rhythm of testosterone and luteinizing hormone in hypogonadal men. J Androl. 12:185–190.
15. **Krieger DT, Ossowski R, Fogel M, Allen W.** 1972 Lack of circadian periodicity of human serum FSH and LH levels. J Clin Endocrinol Metab. 35:619–623.
16. **Spratt DI, O'Dea LSL, Schoenfeld D, Butler J, Narasimha R, Crowley WF.** 1988 Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone. Am J Physiol. 254:E658–E666.
17. **Veldhuis JD, Iranmanesh A, Johnson ML, Lizarralde G.** 1990 Twenty-four-hour rhythms in plasma concentrations of adenohypophyseal hormones are generated by distinct amplitude and/or frequency modulation of underlying pituitary secretory bursts. J Clin Endocrinol Metab. 71:1616–1623.
18. **Brennemann W, Sommer L, Stoffel-Wagner B, Bidlingmaier F, Klingmüller D.** 1994 Secretion pattern of immunoreactive inhibin in men. Eur J Endocrinol. 131:273–279.
19. **De Kretser DM, McFarlane JR.** 1996 Inhibin in the male. J Androl. 17:179–182.
20. **Schneyer AL, Mason AJ, Burton LE, Ziegner JR, Crowley Jr WF.** 1990 Immunoreactive inhibin alpha-subunit in human serum: implications for radioimmunoassay. J Clin Endocrinol Metab. 70:1208–1212.
21. **Robertson D, Burger HG, Sullivan J, et al.** 1996 Biological and immunological characterization of inhibin forms in human plasma. J Clin Endocrinol Metab. 81:669–676. 6
22. **Robertson DM, Sullivan J, Watson M, Cahir N.** 1995 Inhibin forms in human plasma. J Endocrinol. 144:261–269.
23. **De Kretser DM, McLachlan R, Robertson D, Burger HG.** 1989 Serum inhibin levels in normal men and men with testicular disorders. J Endocrinol. 120:517–523.
24. **Plymate SR, Paulsen CA, McLachlan RI.** 1992 Relationship of serum inhibin levels to serum follicle stimulating hormone and sperm production in normal men and men with varicoceles. J Clin Endocrinol Metab. 74:859–864.