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Serum inhibin-b in fertile men is strongly correlated with low but not high sperm counts: A coordinated study of 1,797 European and US men

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Abstract

OBJECTIVE—To describe associations between serum inhibin-b and sperm counts adjusted for effect of time of blood sampling in larger cohorts than previously reported.

DESIGN—Cross-sectional studies of spermatogenesis markers.

SETTING—Four European and four US centres.

STUDY POPULATION—1,797 fertile men included and examined October 1996 to February 2005.

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MAIN OUTCOME MEASURES—Associations between inhibin-b and semen variables controlled for time of blood sampling, and other co-variates.

RESULTS—Inhibin-b decreased about 2.00% per hour from 8 AM to 12 PM and then about 3.25% per hour from 12 PM to 4 PM. There was a strong positive association between inhibin-b below 150 pg/ml and both sperm concentration and total sperm count (slopes of the regression lines were β =0.011 and β =0.013 for natural-logarithm-transformed sperm concentration and total sperm count, respectively). For inhibin-b 150–300 pg/ml the associations were not as steep (β =0.002 for both), but still significant. For inhibin-b above 300 pg/ml there was there was little association to the sperm counts. Neither sperm motility nor morphology was significantly related to inhibin-b in any group.

CONCLUSIONS—Serum inhibin-b levels decrease non-linearly during day-time, and is positively correlated with sperm counts, but the predictive power is best when inhibin-b is low.

Keywords

inhibin-b; follicle-stimulating hormone; sperm concentration; total sperm count; fertile men

Introduction

The relationship between inhibin-b and spermatogenesis was not established until specific assays for the inhibin-a and inhibin-b dimers were developed in the early 1990's (1). Following the development of these dimer-specific assays, publications have described the associations between serum levels of the hormone and semen parameters, however, utilizing either men from infertile couples or relatively small study populations. In a study of 349 Danish young couples planning their first pregnancy a positive correlation (r=0.38) between cubic root transformed serum inhibin-b and cubic root transformed sperm concentration was detected (2). Significant positive correlations between inhibin-b and sperm concentration and total sperm count were also detected among 303 Danish men with proven fertility as well as 289 infertile men (3). A Dutch study of 218 subfertile men also confirmed the positive correlation between inhibin-b and total sperm count, and suggested serum inhibin-b concentration of 139 pg/ml as a cut-off to identify men with impaired spermatogenesis (4). A more recent US study of 388 US men from infertile couples showed that men having inhibin-b in the medium or high tertiles were more likely to have a sperm concentration above 20 million/ml than men in the lowest tertile, and men in the lowest tertile had a lower percentage of morphologically normal spermatozoa (5).

Of the mentioned studies only that of Meeker et al (5) adjusted their calculations of the effects of inhibin-b for hour of blood sampling. A study of diurnal changes in serum inhibin-b levels in 13 healthy volunteers detected declining values of inhibin-b by approximately 3% per hour from 09:00 to 17:00 (6). In that study, no diurnal variation was detected for FSH and no evidence for a role of FSH in the diurnal variation of inhibin-b was found.

Here, we present data from two coordinated, large multi-center studies of fertile men to further elucidate the associations between inhibin-b and semen quality, after controlling for confounders including the important diurnal variation of the hormone.

Materials and Methods

Study populations

In total, 1,797 men from four centers in Europe (N=1,073) and four centers in the Unites States (N=724) were included in this study (Table 1). These men, all partners of pregnant women, were participants in studies designed to examine geographic variation in semen quality. Detailed descriptions of methods have been reported previously (7,8), and are summarized briefly here.

European men were enrolled in the study “Partners of Pregnant Women” in Copenhagen (Den-mark), Paris (France), Edinburgh (United Kingdom) and Turku (Finland) from October 1996 to June 1998. US men were enrolled in the “Study for Future Families” (SFF) in Los Angeles (California), Minneapolis (Minnesota), Columbia (Missouri) and Iowa City (Iowa) from September 1999 to Febru-ary 2005. Pregnant women of couples fulfilling the eligibility were approached during routine visits to antenatal care units, and, if the women agreed, their partners were invited to participate in the study. The eligibility criteria for each man were: partner pregnant at time of enrollment, 20 to 45 years of age at the time of invitation, residing in the local referral area of the hospital to which he was recruited, and, in the European study, being born in that country. The study pregnancy had to be achieved by normal sexual relations, and not as a result of any treatment for subfertility or infertility. Men included in the present analysis were those who delivered both a semen sample and a blood sample at the same study visit. The participation rates were 43% for men from Copenhagen, 15% Paris, 19% Turku,, Los Angeles 14%, Minnesota 19%, Missouri 16% and Iowa 14%. The inclusion process in Edinburgh did not allow for calculation of a participation rate.

Correlations between sperm concentration and inhibin-b for 303 of the 345 Danish men included here have been previously reported (3), although the statistical methods differed from those used here.

Human subject committees or Institutional Review Boards for all participating institutions approved these studies and all participants signed consent forms.

Questionnaires

At the time of sample collection, participants completed a questionnaire providing information on age, previous or current diseases and some life-style factors. Standardized questionnaires were developed in English and translated into Danish, Finnish, French and for the US, Spanish. These translated questionnaires were back-translated to minimize translation errors.

Semen samples

Semen samples were obtained by masturbation and ejaculated into a clean collection tube. In all US centers, Paris and Edinburgh all samples were collected in the privacy of a room at the study center. Due to limited facilities, approximately 20% of samples from Turku and approximately 80% of samples from Copenhagen were collected at the men’s home and

delivered to the study center. If collected at home, the samples were protected from extremes of temperature (<20 °C and >37 °C) during transport to the laboratory (7). The ejaculation abstinence was calculated as the time between the current and previous ejaculation as reported by the men. Furthermore, the time from ejaculation to assessment of motility was recorded.

The analysis of semen samples was performed following the then current WHO guidelines (9), except for assessment of semen volume and morphology. Ejaculate volume was estimated by weighing the ejaculate (10). Phase-contrast microscopy was used for examination of the fresh semen.

For assessment of sperm motility, 10 μl of well-mixed semen was placed on a clean glass slide (which had been kept at 37 °C), covered with a 22 × 22 mm coverslip and immediately examined at a total magnification of x400. The spermatozoa were classified as either motile (WHO class A+B+C) or immotile (WHO class D) (9) in order to report the proportion of motile spermatozoa.

For the assessment of sperm concentration, each semen sample was thoroughly mixed, and an aliquot of the sample was put into the diluent using a positive displacement pipette and further mixed. The sperm concentration was assessed using haemocytometers (Bürker-Türk chamber in Copenhagen and Turku, Thoma chamber in Paris and Neubauer chamber in Edinburgh and all four US centres). Only spermatozoa with tails were counted.

Smears for morphology were made, air-dried, fixed and Papanicolaou-stained. The slides from the European men were assessed in Turku and the US slides at the University of California Davis according to strict criteria (11).

Physical examination

A physical examination of each participant was performed at the day of semen and blood sampling. Body weight and height of the participants were assessed. The Tanner stage of pubic hair and the presence of varicocele noted, and testicular size measured by use of an orchidometer.

Blood samples

A blood sample was drawn from the cubital vein, centrifuged, and the serum separated and kept frozen at minus 20 °C. The time of sampling was recorded. The frozen serum samples were sent from all centers (including the US) to Denmark for a centralized analysis at the University Department of Growth and Reproduction, Rigshospitalet, Denmark. The serum level of inhibin-b was determined by a specific two-sided enzyme linked immunoassay (Serotec, UK) and levels of follicle stimulating hormone (FSH) were determined using a time-resolved immunofluorometric assay (Delfia, Wallac, Turku, Finland). The intra- and inter-assay coefficients of variation (CV) for measurement of inhibin-b were 15% and 18% respectively. CV’s for FSH were 3 and 4.5%, respectively.

All hormone assessments of the European men were done June 1998 at the end of the European study. For the US men from California, Minnesota and Missouri approximately

22%, 35% and 63% of the samples were assessed in November 2001 for inhibin-b, and the remaining in July-October 2002. FSH was assessed in September 2002. Samples from 44% of Iowa men were assessed for hormones in November 2003 and the remaining in October 2005. For the European samples the median duration from blood sampling to hormonal assessment was 11 months (range 0–20 months) and for the US samples 17 months (0–37 months).

Statistical analysis

We examined the relationships between time of blood sampling and hormone level using General Linear Models. Using these models, we identified a significant diurnal variation for inhibin-b which was the same for men from the eight centers, and we therefore used the data as originating from one group only in the following calculations for the diurnal variation. We did not detect any diurnal variation for FSH. Regression analyses included both linear and quadratic terms for time of blood sampling. We also used the model to estimate the inhibin-B level expected had the man's blood been drawn at 8:00 am (using a transformed time of blood sampling, ie 8:00=0:00, 9:00=1:00 etc), denoted here as adjusted inhibin-b. The effect of other co-variates (e.g. BMI) was tested but not included in the final model because they did not change the estimates significantly.

After determining that the relationships between inhibin-B and sperm concentration (and total count) were nonlinear, we used piece-wise regression to model the relationship between sperm concentration (as well as total sperm count), allowing for varying slopes as inhibin-B varied. This modeling indicated that the associations between inhibin-b and sperm counts were non-linear but fell into three groups: low (adjusted inhibin-b <150 pg/ml), medium (adjusted inhibin-b 150–300 pg/ml) and high (adjusted inhibin-b >300 pg/ml) groups. Our final models included age, age-squared, abstinence time and abstinence time-squared as covariates. BMI, season, investigation center and other potential covariates were also tested but found to be non-significant and therefore not included in the final model.

Hormone levels, hour of blood sampling, semen variables, duration of abstinence and anthropometric data between low, medium, and high adjusted inhibin-b level groups were compared using linear regression analyses. Sperm concentration, total sperm count and semen volume were normalized by taking natural logarithms. Sperm motility and morphology, which were approximately normally distributed, were not transformed. Duration of ejaculation abstinence period was included in regression models for the comparisons of sperm concentration, total sperm counts, and semen volume. Time from ejaculation to assessment of motility was included in regression models comparing percentages of motile spermatozoa.

Statistical analyses were performed using the statistical packages SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA) and SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Descriptive statistics of the participating men by (adjusted) inhibin-b category (low, medium or high) are presented in Table 1. This table includes p-values comparing the low and high

inhibin-b groups, respectively, to the middle group. Men in the low inhibin-b group had lower values of all semen parameters, except percentage of morphologically normal spermatozoa, than the groups with higher adjusted inhibin-b levels. More men in the low inhibin-b group had cryptorchidism in their medical history (12.2% vs. 7.3% and 5.7% for the intermediate and high inhibin-b groups, respectively). The average testis size differed between the three groups with smallest volume in the low inhibin group, which also had the highest body weight and BMI. Cryptorchidism in the medical history, smaller testis size and increasing BMI, analyzed individually or in combination, were all negatively associated with adjusted inhibin-b as well as sperm concentration and total sperm count (all $p<0.0001$). However, neither cryptorchidism, testis size nor BMI affected the associations between inhibin-b and sperm concentration or total sperm counts. Thus, the curves presented in the figures did not change when excluding men with these conditions from the calculations (not shown).

Table 2 shows the time of day of blood sampling, and the unadjusted and time adjusted inhibin-b values for men from the eight participating centers. The time of blood sampling differed between the centers ($P<0.0005$), however the effect of time of blood sampling on inhibin-b levels did not differ between centers ($P=0.6$). The results from the centers were therefore merged in subsequent analyses. Figure 1 illustrates the decline of inhibin-b over the course of the day. From 8:00 to 12:00 inhibin-b fell to 92%, corresponding to an hourly decrease of 2.00%. The following four hours the hormone level decreased further to 79%, corresponding to an hourly decrease of 3.25% (R^2 of the model 0.061, $p<0.0001$). The adjusted inhibin-b levels in Table 2 are corrected for this diurnal variation and calculated to the levels expected had all blood samples been drawn at 08:00. FSH levels were not affected by the time of blood sampling ($p=0.8$). Season was not significantly associated to inhibin-b or FSH levels ($p=0.7$ and 0.6, respectively).

Figure 2 illustrates the associations between adjusted inhibin-b and the untransformed sperm concentration and total sperm counts, respectively. The strength of the association between sperm count and inhibin-b decreases with increasing inhibin-b level. The increase is steepest for men in the low inhibin-b group. In this group, the slope of the regression lines between inhibin-B and semen variables were $\beta = 0.011$ and $\beta = 0.013$ for natural logarithmic transformed sperm concentration and total sperm count, respectively. For men in the medium group, the increases were not as steep ($\beta = 0.002$ for both), but still significant. However, for men in the high group there was little relationship between either sperm concentration ($\beta = 0.0001$) or total count ($\beta = 0.0001$) and inhibin-b. These trends did not differ by center ($P=0.2$).

Discussion

Previous publications have described associations between inhibin-b and sperm concentration and/or total sperm count, and some have also stated that inhibin-b and sperm counts are better correlated at lower inhibin-b levels (2–5). Here we strengthen those findings in an analysis on a much larger population (N=1,797) than examined in the past and with control for time of blood sampling and other important covariates. Our primary finding is that the relationships between inhibin-b and sperm concentration and number are non-

linear. The greatest increase in sperm count is seen as inhibin-b increased from 37 pg/ml (which was the lowest adjusted inhibin-b in our study population) to approximately 150 pg/ml. Between 150 pg/ml and approximately 300 pg/ml, we saw a more moderate increase in sperm counts, while sperm counts are nearly independent of inhibin-b above 300 pg/ml. Secondly, we showed a non-linear decrease in inhibin-b level with the time of blood sampling. Previously, this question had only been addressed in a longitudinal study of 13 men who, however, had blood samples drawn throughout a 24 hour period (6). That study detected an average decline in inhibin-b of 3% per hour during the day. The non-linear association between inhibin-b and sperm counts as well the nonlinear, and pronounced, inhibin-b decrease during daytime has implications both for clinicians interpreting a patient's inhibin-b level and for researchers planning to use inhibin-b as a marker of spermatogenesis. Ideally, blood samples should be drawn at a predetermined time of day. If this is not possible, the inhibin-b results should be adjusted according to time of blood sampling to aid in interpreting results. This will, however, not account for inter-individual differences in diurnal variation, and therefore a correction may be most useful in epidemiologic studies.

We present data on fertile men, but it is likely that our observations are applicable to the general population. Firstly, we detected similar diurnal variations and associations with sperm count and concentration across our eight different populations. Among these the Finnish men have previously been shown to have a better semen quality than Danish men and men from Minnesota better quality than men from Missouri (7,8). Secondly, the study subjects were partners of pregnant women, inevitably implying that subfertile men were under-represented, however, some of the investigated men had suboptimal semen quality (12–14). Thirdly, the estimated associations between inhibin-b and sperm counts were not altered depending on whether the men had cryptorchidism in their medical history, whether their testis size was reduced or whether they were obese.

Our results cannot provide an explanation for the non-linear association between inhibin-b and sperm concentration and total sperm counts or the lack of associations for the high levels of inhibin-b. We speculate, that there may be more than one underlying mechanism for the associations, which then in combination result in the associations we have detected, but we don't have any direct information to elucidate this further.

In a previous analysis of data obtained from the European part of the study it was shown that increasing sperm concentration up to approximately 55 million/ml and increasing total count up to approximately 145 million were inversely correlated with waiting time to pregnancy (TTP) (14). In a study of young couples planning their first pregnancy it has also been indicated that increasing sperm concentration up to 40 million/ml was inversely correlated with TTP (12). Similarly, Guzick *et al* detected inverse correlations between sperm concentration up to 48 million/ml and TTP (13). These studies seem to indicate a "threshold" value for declining fecundity at sperm concentration around 40–55 million/ml. It is therefore striking that the cutoff for the strongest association between adjusted inhi-bin-b (ie 150 pg/ml) and sperm concentration corresponded to a sperm concentration of 52 million/ml as illustrated in Figure 2A. Thus, in epidemiologic studies adjusted inhibin-b values below 150 pg/ml may indicate sub-fertility and impaired semen quality. Previous

studies have not indicated any clear-cut “cut-offs”. Jensen et al suggested a cut-off at inhibin-b at 80 pg/ml mainly because 95.7% of men having a sperm concentration above 20 million/ml had inhibin-b above 80 pg/ml (2). In contrast Uhler et al found that sperm concentration increased steadily with increasing inhibin-b (15). Our finding of approximately 150 pg/ml is in better agreement with the Dutch study of sub-fertile men finding 139 pg/ml as a cut-off (4). The size of our study population, the fact that we detected the same tendencies in eight different study centers and the combined statistical analyses make us believe that the suggested 150 pg/ml is a more relevant “cut-off” than previously published suggestions. However, our study design did not allow for repeated measures from the individual men to test the predictive value of the model any further.

All men were investigated according to coordinated, and standardized protocols, and all analyses were controlled for known confounders. All hormone analyses were conducted in one laboratory, thus eliminating inter-laboratory variation. All routine quality control (both internal and external) programs were followed and inter-assay variations were low. The fact that associations were similar across different centers suggests that assay variation is unlikely to explain these findings. Also, we don’t believe that our results are biased by long or different storage time of serum samples before hormone assessments. Previously, Andersson et al have indicated that there was no time trend in inhi-bin-b level according to storage time at minus 20 °C for up to 15 years (16). The samples we used had not previously been thawed before assessment of inhibin-b and FSH. Thus, repeated freezing and thawing procedures cannot have influenced the hormonal levels we detected. Furthermore, our laboratory has previously investigated for changes in hormone levels following repeated freezing and thawing cycles, without being able to show any significant or consistent changes in inhibin-b or FSH levels (not shown).

Also, inter-laboratory differences in assessment of sperm concentrations were controlled by external quality control programs within the US and European laboratories, respectively, as previously reported (7,8). We did not have any quality control that directly could evaluate the inter-laboratory variation in assessment of sperm concentration between the US and European laboratories. However, the Danish, the Finnish and the Californian laboratories participated in the same quality control program during 2001 and 2002 when the Californian study was ongoing, and the variation between these three laboratories did not differ significantly (data not shown). Furthermore, imprecisely obtained data or significant inter- or intra-laboratory variations would tend to blur the associations we have detected. Collecting the semen sample at home rather than at the study site did not bias the results. We could not detect any difference in the association between sperm counts and inhibin-b for men from Copenhagen (where 80% collected at home), men from Finland (20% collected at home) and the remaining centers where all men collected at the study site. Thus, we do not believe that technical errors can explain our findings.

The absence of any diurnal variation in FSH, together with an increase in FSH paralleling the decrease in inhibin-b, suggests that other factors in addition to inhibin-b contribute to the endocrine feed-back loop from the testicles. The study by Carlsen et al detected a negative feed-back effect of FSH on the mean levels of inhibin-b (6), which was also the case in our study. Neither in that study nor this one, changes in FSH levels could explain the diurnal

variation of inhibin-b. Some studies have detected a seasonal variation in sperm counts (7, and references therein) but whether season also might affects FSH is not clear (17). We did not detect any effect of season on inhibin-b or FSH. Thus, our study does not indicate that seasonality needs to be accounted for when interpreting inhibin-b results. However, we had no opportunity to evaluate the potential confounding effects of photoperiods, humidity or temperatures directly, as suggested by Adamopoulos (17).

In conclusion, we have shown that the associations between inhibin-b and sperm concentration and total sperm count are non-linear, with pronounced increases in sperm counts as inhibin-b increases up to approximately 150 pg/ml and virtually no associations when inhibin-b levels are above 300 pg/ml. We have also confirmed that inhibin-b levels decrease significantly during the day and have provided tables and figures that may be of practical value for those working clinically or epidemiolog-ically with inhibin-b.

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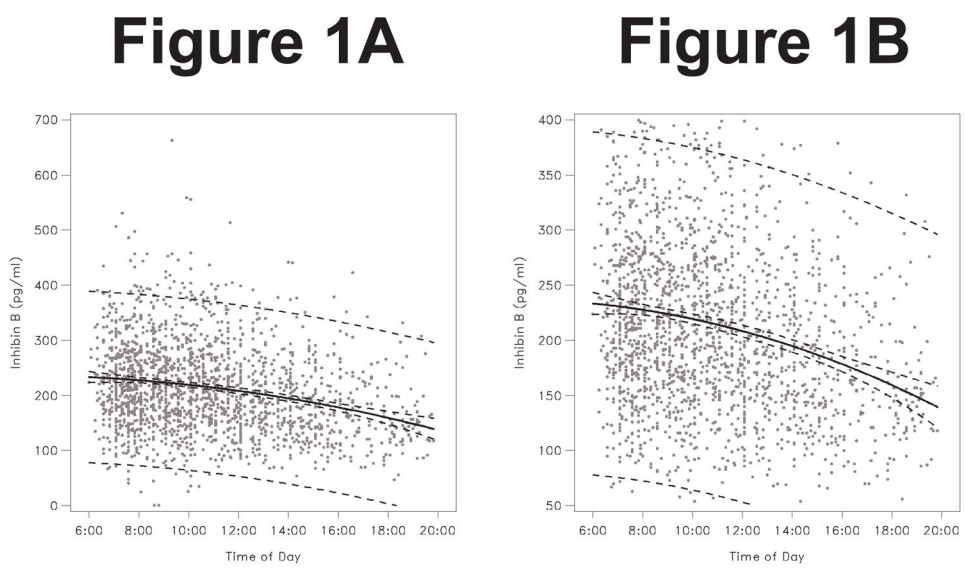

Figure 1.

The black full line and the two black broken lines closest to this represent the calculated average inhibin-b level according to hour of blood sampling and the 95% confidence intervals of this, respectively. The broken lines at highest and lowest represents 95% of the data set. **A** shows the entire data set and **B** a higher magnification that more clearly shows the decreasing inhibin-b level over the day.

Figure 2A

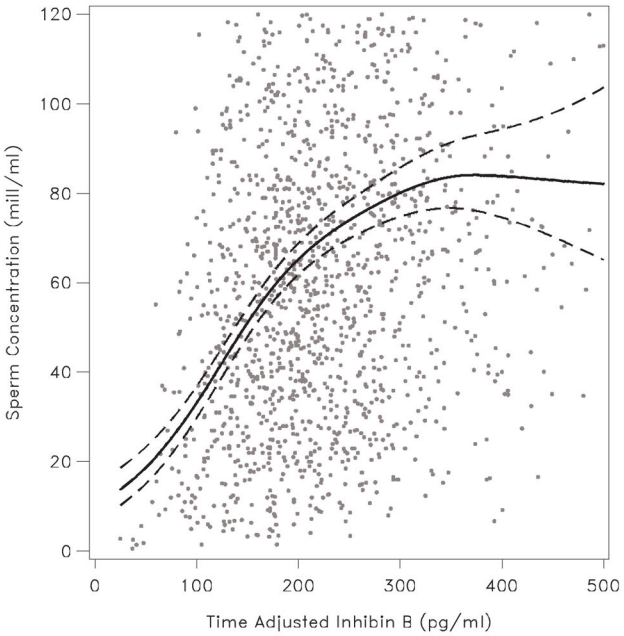

Figure 2B

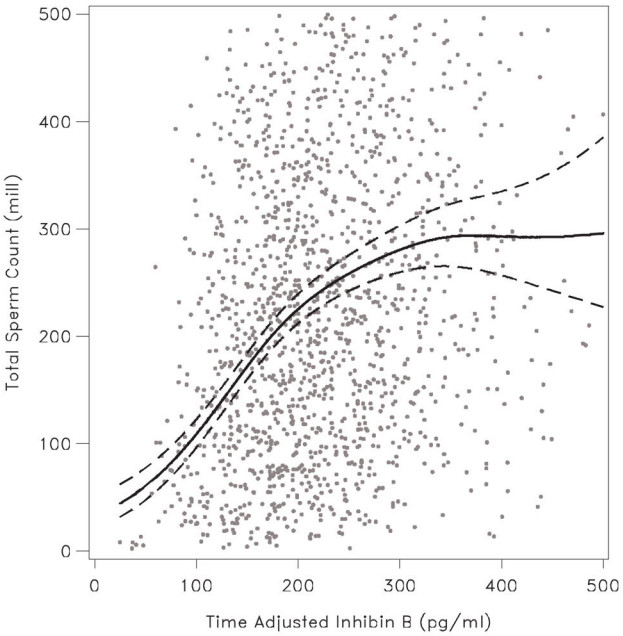

Figure 2.

The associations between time adjusted inhibin-b values and non-transformed sperm concentrations (**A**) and total sperm counts (**B**) are shown as function of inhibin-b adjusted for the time of day of the blood drawing. Sperm counts increased steeply with increasing time adjusted inhibin-b values up to approximately 150 pg/ml, followed by a less steep increase up to approximately 300 pg/ml where after further increase in time adjusted inhibin-b was not reflected by a further increase in sperm counts.

Table 1

Descriptive statistics of the men categorized by serum inhibin-b levels (adjusted for time of blood sampling).

	Low (<150 pg/ml) (N=279)				Adjusted inhibin-b Medium (150–300 pg/ml) (N=1,202)			High (>300 pg/ml) (N=316)			
	N	**Mean (SD)**	**Median (5–95)**	**p-value**	**N**	**Mean (SD)**	**Median (5–95)**	**N**	**Mean (SD)**	**Median (5–95)**	**p-value**
Adjusted inhibin-b (pg/ml)	279	119 (25)	126 (69–147)	**<0.0005**	1,202	220 (41)	217 (159–289)	316	366 (64)	347 (305–481)	**<0.0005**
FSH (U/l)[a]	279	5.3 (2.8)	4.6 (2.2–10.6)	**<0.0005**	1,201	3.5 (1.7)	3.2 (1.5–6.8)	316	2.3 (1.1)	2.1 (1.0–4.7)	**<0.0005**
Adjusted inhibin-b/FSH[a]	279	29 (16)	27 (8–57)	**<0.0005**	1,201	78 (45)	68 (27–165)	316	190 (105)	164 (71–374)	**<0.0005**
Hour of blood drawing	279	11:10	10:30 (7.30–17:35)	0.51	1,202	11:04	10:25 (07:20–18:00)	316	11:17	10:38 (07:15–17:45)	0.28
Abstinence (hours) [b]	274	89 (69)	72 (32–203)	0.18	1,183	108 (202)	75 (35–240)	310	147 (488)	73 (36–214)	0.72
Age (years)	279	31.6 (5.6)	31.4 (22.3–41.9)	0.65	1,194	31.4 (5.1)	30.9 (23.1–39.9)	312	31.1 (5.1)	30.8 (22.8–40.5)	0.34
Weight (kg)	271	90 (18)	86 (66–128)	**<0.0005**	1,168	83 (15)	81 (63–111)	210	81 (13)	79 (64–103)	**0.01**
Height (cms)	271	179 (7)	179 (168–192)	0.61	1,168	179 (7)	180 (168–191)	310	179 (8)	179 (166–191)	0.2
BMI[c]	271	28 (5)	27 (21–39)	**<0.0005**	1,168	26 (4)	25 (20–34)	310	25 (4)	25 (20–32)	**0.05**
Testis size, mean (ml)[d]	265	20.0 (4.6)	20.0 (13.2–27.5)	**<0.0005**	1,164	22.4 (4.7)	22.5 (15.0–30.0)	308	24.4 (5.2)	25.0 (15.2–35.0)	**<0.0005**
Semen volume (ml)	279	3.6 (1.7)	3.4 (1.3–6.5)	**0.02**	1,200	3.9 (1.7)	3.7 (1.5–7.0)	315	3.9 (1.7)	3.5 (1.6–7.1)	0.98
Sperm conc. (mill/ml)[e]	279	61 (53)	47 (6–171)	**<0.0005**	1,202	88 (64)	72 (16–218)	316	104 (72)	83 (27–263)	**<0.0005**
Total sperm count (mill)	279	202 (176)	152 (20–517)	**<0.0005**	1,200	329 (274)	252 (44–903)	315	386 (301)	308 (81–999)	**<0.0005**
Motile spermatozoa (%)	278	59 (13)	59 (35–79)	**0.029**	1,201	60 (12)	61 (40–78)	316	61 (12)	61 (43–79)	0.26
Morphologically normal (%)[f]	266	10.4 (5.7)	9.6 (2.2–20.8)	0.88	1,125	10.4 (5.3)	10.0 (2.6–19.9)	299	10.6 (5.0)	10.0 (3.0–19.4)	0.46

N: Number of men for which data were available.

SD: Standard deviation.

5–95: 5–95th percentile.

[a]: FSH, follicle stimulating hormone.

[b]: Abstinence, ejaculation abstinence period.

[c]: BMI, body mass index.

[d]: Mean of left and right testis size.

[e]: Sperm conc., sperm concentration.

[f]: Percentage of morphologically normal spermatozoa assessed according to strict criteria.

p-value: Comparisons of low vs. medium and high vs. medium groups based on linear regression. For semen volume, sperm conc and total sperm count the comparison was controlled for effect of duration of ejaculation abstinence time and for motile spermatozoa for duration of ejaculation to assessment time. See text for further explanation.

Table 2

Time of day of blood sampling, and the unadjusted and time adjusted inhibin-b values for men from four European countries and four US states.

		Blood sampling hour			Unadjusted inhibin-b (pg/ml)			Adjusted inhibin-b (pg/ml)[a]		
	No. of men	Mean	Median	Min-Max	Mean (SD)	Median (5–95)	Min-Max	Mean (SD)	Median (5–95)	Min-Max
Europe										
Copenhagen, Denmark	345	08:07	07:50	06:25–12:45	225 (87)	215 (102–378)	41–718	226 (87)	218 (102–376)	41–719
Edinburgh, Scotland	250	14:57	14:58	06:40–20:15	189 (81)	171 (87–329)	47–769	232 (90)	216 (109–408)	61–815
Paris, France	204	12:08	11:27	07:35–19:50	202 (70)	193 (99–342)	35–404	222 (74)	215 (115–360)	37–420
Turku, Finland	274	10:55	10:40	06:45–16:15	219 (80)	208 (106–371	62–514	234 (85)	218 (119–396)	65–560
United States										
Los Angeles, California	179	11:20	10:50	06:40–17:15	217 (88)	203 (88–378)	66–663	234(93)	219 (100–406)	82–683
Iowa City, Iowa	143	11:03	11:30	07:00–16:30	201 (67)	196 (96–321)	45–356	214 (70)	211 (108–336)	46–409
Minneapolis, Minnesota	206	09:39	09:15	07:15–12:55	229 (77)	224 (109–373)	58–457	235 (79)	230 (114–388)	60–462
Columbia, Missouri	196	12:10	12:00	06:30–17:15	217 (83)	208 (108–378)	25–556	240 (89)	237 (123–403)	25–582

No. of men: Number of men included from each of the participating centers.

Min-Max: Minimum-Maximum.

SD: Standard deviation.

5–95: 5–95th percentile.

1: Inhibin-b values corrected for diurnal variation by replacing measured value with value expected had the sample been drawn at 08:00.