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Original Contribution

Association of Sleep Disturbances With Reduced Semen Quality: A Cross-sectional Study Among 953 Healthy Young Danish Men

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Several studies have found an association between sleep duration and morbidity and mortality, but no previous studies have examined the association between sleep disturbances and semen quality. We conducted a cross-sectional study among 953 young Danish men from the general population who were recruited in Copenhagen at the time of determination of fitness for military service between January 2008 and June 2011. All of the men delivered a semen sample, had a blood sample drawn, underwent a physical examination, and answered a questionnaire including information about sleep disturbances. Sleep disturbances were assessed on the basis of a modified 4-item version of the Karolinska Sleep Questionnaire, which includes questions on sleep patterns during the past 4 weeks. Sleep disturbances showed an inverse U-shaped association with sperm concentration, total sperm count, percent motile and percent morphologically normal spermatozoa, and testis size. Men with a high level of sleep disturbance (score >50) had a 29% (95% confidence interval: 2, 48) lower adjusted sperm concentration and 1.6 (95% confidence interval: 0.3, 3.0) percentage points' fewer morphologically normal spermatozoa than men with a sleep score of 11–20. This appears to be the first study to find associations between sleep disturbances and semen quality. In future studies, investigators should attempt to elucidate mechanistic explanations and prospectively assess whether semen quality improves after interventions restoring a normal sleeping pattern.

fertility; male; reproduction; semen; sleep; stress

Abbreviations: BMI, body mass index; CI, confidence interval; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin.

Editor's note: *An invited commentary on this article appears on page 1038.*

Several studies have found a U-shaped relationship between sleep duration and morbidity, mortality, and obesity, with the lowest risk being observed among persons who sleep 7–8 hours per night (1–3). The mechanism may be mediated through changes in hormone regulation, metabolism, or an unhealthier lifestyle. Sleep disturbances also seem to affect mortality (4), but the health effects of sleep quality have been studied less extensively. The frequency of sleep disturbances has increased in the industrialized world during the past few decades, a period in which a decline in semen quality has also been reported (5). To our knowledge, no previous studies have directly examined the association between sleep disturbances and semen quality, but Leproult and Van Cauter (6) recently reported that 1 week of sleep restriction to 5 hours per night resulted in a 10%–15% decrease in serum testosterone levels. The suggested association with lower serum testosterone levels among the sleep-disturbed has been reported among older men, although some studies were unable to corroborate this finding (7–9).

We studied the association between self-reported quality of sleep during the past 4 weeks and semen quality and male reproductive hormone levels in a population of 953 young, healthy Danish men.

MATERIALS AND METHODS

Population

Because of the military draft in Denmark, all 18-year-old men, except those suffering from severe chronic disease, are required to undergo a compulsory physical examination to determine their fitness for military service. Some men postpone their examination until completion of their education. Since 1996, trained staff from the Department of Growth and Reproduction at Copenhagen University Hospital (Rigshospitalet, Copenhagen, Denmark) have approached the draftees when they have appeared for their compulsory physical examination and have invited them to participate in a study of semen quality. Men recruited from January 2008 to June 2011 were included in the present study, since the questionnaire they completed included detailed information about sleep disturbances. All participants completed a questionnaire, delivered a semen sample, had a blood sample drawn, and underwent a physical examination. They received compensation for their time (kr500, equal to approximately US$85). Participants did not differ from nonparticipants with regard to age, but they were generally better educated than nonparticipants (data not shown). Ethical approval was obtained from the local ethical committee. A detailed description of the study has previously been published (10).

Semen analysis

All men provided a semen sample by means of masturbation in a room close to the semen laboratory. The period of ejaculation abstinence (time since last ejaculation) was recorded, and the semen sample was analyzed for volume, sperm concentration, total sperm count, percent motile spermatozoa, and percent morphologically normal spermatozoa as described by Jørgensen et al. (10), which is in accordance with the most recent guideline from the World Health Organization (11). Since 1996, our laboratory has led a quality control program for assessment of sperm concentration; the laboratory has kept the interlaboratory difference unchanged (12), and the variation between technicians was less than 10%. The same 2 experienced technicians assessed sperm morphology according to strict criteria for the first 838 men (13).

Serum samples

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone, and sex hormone-binding globulin (SHBG) were determined using a time-resolved immunofluorometric assay (Delfia; Wallac Oy, Turku, Finland). Testosterone and estradiol levels were determined using time-resolved fluoroimmunoassays (Delfia; Wallac Oy). Inhibin B level was determined by means of a specific 2-sided enzyme immunometric assay (Inhibin B Gen II; Beckman Coulter Ltd., High Wycombe, United Kingdom). Intra- and interassay coefficients of variation for measurements of FSH and luteinizing hormone were 3% and 4.5%, respectively. Coefficients of variation for testosterone and SHBG were <8% and <5% respectively. The intraassay coefficients of variation for estradiol and inhibin B were <4% and <7%, respectively, and the interassay coefficients of variation were <4% and <6%, respectively. The hormones were all measured within same time period and in the same assay batches. Therefore, even though some variation in analyses over time may have been present, this would not have affected the results. At the time of submission of this article, reproductive hormone levels had been measured in the first 672 men. Free testosterone was calculated on the basis of the measured serum concentrations of total testosterone and SHBG using the method of Vermeulen et al. (14) and a fixed albumin concentration of 0.62 mmol/L. Because changes in the concentration of albumin have only minor effects on the ratio of total testosterone to free testosterone, it is justifiable to use a fixed mean albumin concentration when individual albumin measurements are not available, provided that there is no reason to suspect significantly abnormal albumin levels (14). In addition, the ratios between inhibin B and FSH, testosterone and estradiol, and calculated free testosterone and luteinizing hormone were calculated.

Physical examination

Physicians assessed Tanner stage of pubic hair and genital development, testicular volumes (determined using a Prader wooden orchidometer (Pharmacia & Upjohn, London, United Kingdom)), the possible presence of a varicocele (grades 1–3) or hydrocele, and the location of the testes in the scrotum, and the consistency of the testis and epididymis were recorded. Weight and height was measured, and body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. Conditions detected at the physical examination that may affect semen quality (varicocele grade 2 or 3 or abnormal position of the testes) were summarized in a single variable designated "conditions found at the physical examination."

Questionnaire

Prior to the examination, all participants completed a questionnaire that collected information on previous and/or current diseases and genital diseases such as inguinal hernia, varicocele, epididymitis, gonorrhea, chlamydia, and surgery for testicular torsion. The participants were asked whether they had been born with both testicles in the scrotum. In addition, they reported whether they had had fever greater than 38°C (100.4°F) within the previous 3 months and whether they had ever been diagnosed by a physician with depression. Self-reported diseases of the reproductive organs affecting semen quality (torsion of testes, epididymitis, or inguinal hernia) were transformed into 2 variables: "self-reported genital conditions" and "sexually transmitted diseases" (gonorrhea or chlamydia).

Sleep disturbances were assessed on the basis of a modified 4-item version of the Karolinska Sleep Questionnaire

(15, 16), which includes questions on sleep patterns during the past 4 weeks: How often have you 1) "slept badly or restlessly"; 2) "found it difficult to fall asleep"; 3) "woken up too early in the morning and not been able to go back to sleep"; and 4) "woken up several times during the night and found it difficult to go back to sleep"? The response categories were "all the time" (scoring 100%), "a large part of the time" (scoring 67%), "rarely" (scoring 33%), and "never" (scoring 0%) and were scored according to the Copenhagen Psychosocial Questionnaire (15). Because responses to the 4 questions were correlated ($\rho = 0.25–0.57$), a sleep score was created as the mean of the percentages answered in the 4 questions. Thirteen men did not respond to all of the sleep questions, and a score could not be computed for these men.

The young men responded to a standard questionnaire about maternal education, which was coded as below 9 years of schooling, 9–10 years of schooling, and more than 10 years of schooling. Participants were asked: "How much of the following beverages did you consume during the last week?" Possible responses were: bottles of cola (0.5 L), other sodas (0.5 L), diet cola (0.5 L), other diet sodas (0.5 L), caffeine-containing energy drinks (0.25 L), and number of chocolate bars (50 g). In addition, participants were asked how many cups of coffee, tea, and chocolate-containing beverages they had consumed daily during the last week. Each man's daily caffeine intake was estimated assuming that a cup contained 150 mL and that caffeine content was 117 mg in 1 cup of coffee, 70 mg in 1 cup of tea, 5 mg in 1 cup of chocolate beverages, 70 mg in a 0.5-L cola or diet soft drink, 117 mg in 1 energy drink, and 7 mg in a 50-g chocolate bar (17). The men were informed that 1 beer, 1 glass of wine, or 40 mL of spirits contained 1 unit of alcohol, whereas 1 strong beer or 1 alcopop contained 1.5 units of alcohol and 1 bottle of wine contained 6 units of alcohol. They were asked about their daily unit intakes of red and white wine, beer, strong alcoholic drinks, and alcopops during the last week. Alcohol intake was calculated as the sum of daily reported unit intakes within the last week. Information on physical activity was coded into watts per week according to the method of Craig et al. (18).

Statistics

Exposure variables were the 4 individual sleep disturbances and a sleep score. Sleep score was calculated as the mean of the participant's replies to the 4 sleep questions: "all the time" (scoring 100%), "a large part of the time" (scoring 67%), "rarely" (scoring 33%), or "never" (scoring 0%) and was categorized as 0, 1–10, 11–20 (reference), 21–30, 31–40, 41–50, or >50. The category 11–20 was used as the reference category because this group of men had the highest sperm count. First, semen quality, testis size, and reproductive hormone levels were compared for men in relation to sleep disturbances and sleep score categorized as 0, 1–20, 20–40, or >40. Then we compared the distributions of the variables from the questionnaires and physical examinations among men with different sleep disturbance scores by χ^2 test in order to identify potential confounders. Finally, data were analyzed using multiple linear regression analyses. Normally distributed outcome variables were entered directly into the model as continuous variables.

Because of the nonnormal (skewed) distributions of sperm concentration, total sperm count, calculated free testosterone, and FSH and increasing variances, they were analyzed on a natural logarithmic scale and back-transformed to obtain the percentage of change in these parameters. Covariates initially included factors possibly associated with semen parameters, reproductive hormone levels, or sleep score and were then excluded stepwise if they did not change the estimate by more than 10%. The same set of confounders was used for all semen parameters: period of abstinence (transformed by natural logarithm), alcohol intake, smoking, and age and, for sperm motility, also duration between the time of ejaculation and analysis of the sample, categorized as shown in Table 1. The analyses of reproductive hormones were adjusted for time of blood sampling, smoking, and BMI. Tests for trend were performed by inserting the categorical sleep variable into the model, assuming the association to be linear.

Because depression may cause sleep disturbances, we repeated the analyses after excluding the 21 men who had ever had depression diagnosed by a physician. Because the association between sleep disturbances and semen quality and reproductive hormone levels may be mediated through alcohol consumption and smoking habits, we repeated the analyses without adjustment for these factors. In addition, because being overweight may lead to sleep disturbances, we evaluated whether BMI modified the effect by stratifying results according to BMI. The results are presented as regression coefficients with 95% confidence intervals. We evaluated the fit of the regression models by testing the residuals for normality and by inspecting the residual plots.

RESULTS

A total of 966 men participated, of whom 953 responded to the questions about sleep and had a sleep score calculated. A total of 13% reported having slept badly and restlessly, 15% found it difficult to fall asleep, 6% had woken up too early and not been able to go back to sleep, and 4% had woken up several times and found it difficult to go back to sleep. The median sleep score was 16.7 (interquartile range, 8.3–33.3), and 49 men (5.7%) scored more than 50.

The 4 individual items of the sleep score were each individually associated with semen quality, but few men reported that they had woken up too early and not been able to go back to sleep or had woken up several times and found it difficult to go back to sleep (data not shown). Sleep disturbance score was associated with sperm concentration, total sperm count, percent morphologically normal spermatozoa, and testis size in an inverse U-shaped manner (Table 2), so that men with a sleep score below or above 11–20 had poorer semen quality and smaller testicles. Unadjusted serum testosterone and calculated free testosterone levels were higher, whereas the inhibin B:FSH ratio was lower, among men with a high sleep disturbance score (Table 2).

Men with sleep disturbances generally had an unhealthier lifestyle; they had a higher BMI, had higher alcohol and

Table 1. Information (%) Obtained From Questionnaires and Physical Examination Among 953 Young Danish Men With Different Stress and Sleep Scores, January 2008–June 2011

	No. of Participants	%	Sleep Score			
			0 (*n* = 143)	1–20 (*n* = 284)	21–40 (*n* = 165)	>40 (*n* = 101)
Information obtained at the physical examination						
Season of examination October–March	516	54	54	55	58	49
Conditions found at the physical examination[a]	136	15	14	16	12	15
Fever >38°C within the past 3 months	64	7	10	6	7	7
Body mass index[b]						
<20	152	17	16	15	20	17
20–24.99	578	64	65	65	64	59
≥25	177	20	18	20	17	24
Information obtained from the questionnaire						
Age >20 years at time of examination*	219	23	21	20	28	27
Physical activity >400 W/week	429	45	44	45	46	49
Alcohol intake >21 units/week[c]*	238	26	17	28	25	31
Total caffeine intake >300 mg/day	221	23	23	23	21	28
Maternal education, years						
<9	34	4	2	3	6	6
9–10	176	21	20	20	24	21
>10	627	75	78	76	70	73
Current smoking*	360	38	28	37	38	54
Exposure to mother's smoking in utero	224	27	22	26	28	34
Self-reported genital conditions[d]	62	7	7	5	8	9
Sexually transmitted diseases[e]*	93	10	5	11	10	14
Born with cryptorchidism[f]	59	6	8	5	8	5
Physician-diagnosed depression*	21	2	0	1	3	8

* $P < 0.05$ (by χ^2 test).
[a] Varicocele or abnormal testes found at physical examination.
[b] Weight (kg)/height $(m)^2$.
[c] 1 unit = 12 g of alcohol.
[d] Self-reported information about torsion of the testes, epididymitis, or inguinal hernia.
[e] Gonorrhea or chlamydia.
[f] If information was missing, the man was categorized as not having cryptorchidism.

caffeine intakes, more often smoked, and more often had been exposed to smoking in utero. They were older and had more often had sexually transmitted diseases (gonorrhea or chlamydia) or depression, and their mothers had a lower educational level (Table 1).

After control for confounders, sperm concentration, total sperm count, percent motile spermatozoa, percent morphologically normal spermatozoa, and testis size were significantly lower among both men with higher sleep scores and men with lower sleep scores in comparison with the reference group (sleep score 11–20). A sleep score above 50 (poor sleep) was associated with a 29% (95% confidence interval (CI): 2, 48) reduction in sperm concentration, a 25% (95% CI: −4, 46) reduction in total sperm count, and 0.9 (95% CI: −3.1, 4.9) and 1.6 (95% CI: 0.3, 3.0) percentage points' fewer motile and morphologically normal spermatozoa, respectively, in comparison with the reference group (Table 3), and statistically significant decreasing trends in semen parameters with increasing sleep disturbance score were found (Figures 1–3). Interestingly, a low sleep score (zero, no sleeping problems) was associated with poorer semen quality as well, although the finding was not statistically significant (Table 3, Figures 1–3). No association with semen volume was observed.

In order to define a group of men with severe sleep disturbances, we identified 17 men who reported that they had slept badly and restlessly *and* had difficulties falling asleep all the time during the last 4 weeks. They had a median sperm concentration, total sperm count, percent motile spermatozoa, and percent morphologically normal spermatozoa of 28 million/mL, 90 million, 68.7%, and 6.3%, respectively, as compared with 48 million/mL, 153 million, 69.7%, and 7%, respectively, among the other men.

Because depression may cause sleep disturbances, we repeated the analyses after excluding the 21 men with physician-diagnosed depression, which strengthened the

Table 2. Semen Quality, Testis Size, and Reproductive Hormone Levels Among 953 Young Danish Men According to Sleep Score, January 2008–June 2011

	Sleep Score							
	0 ($n = 194$)		1–20 ($n = 398$)		21–40 ($n = 227$)		>40 ($n = 134$)	
	Median	5th–95th Percentile Range	Median	5th–95th Percentile Range	Median	5th–95th Percentile Range	Median	5th–95th Percentile Range
Semen quality								
Testis size, mL[a]	21	15–28	22	14–30	22	15–30	20	14–28
Semen volume, mL	3.2	1.3–5.9	3.2	1.3–6.1	3.2	1.2–6.6	3.1	1.1–7.0
Sperm concentration, millions/mL	47	3–170	52	5–177	47	5–167	42	3–150
Total sperm count, millions	138	10–535	165	17–582	160	14–628	123	9–486
Motile sperm, %	68	36–87	71	44–88	69	35–85	69	50–86
Morphologically normal sperm, %[b]	6.8	1.5–16.0	7.5	0.5–16.3	7.0	0.5–16.0	6.5	0.9–14.1
Period of abstinence since last ejaculation, hours	66	36–138	64	37–134	62	37–157	63	37–137
Time between ejaculation and sample analysis, % >1 hour	16		13		18		12	
Reproductive hormones[c]								
FSH, IU/L	2.2	1.5–3.0	2.2	1.5–3.3	2.4	1.7–3.4	2.4	1.6–3.1
LH, IU/L	3.0	2.3–4.2	2.9	2.2–3.9	3.1	2.3–4.1	3.0	2.2–4.1
Testosterone, nmol/L	19.0	16.3–22.3	18.8	15.2–23.5	18.7	15.2–23.3	19.4	15.8–24.8
SHBG, nmol/L	27	20–34	26	21–33	24	19–31	24	18–31
Free testosterone, pmol/L	441	263–780	449	278–728	467	262–808	462	291–967
Inhibin B, pg/mL	186	153–227	167	141–205	168	136–202	157	125–207
Estradiol, nmol/L	0.08	0.05–0.11	0.08	0.05–0.12	0.08	0.04–0.12	0.08	0.04–0.14
Testosterone:LH ratio	5.9	3.1–13.2	6.5	3.1–15.0	6.3	3.0–11.9	6.6	2.8–13.4
Inhibin B:FSH ratio	86.0	24.4–343.8	79.5	25.1–279.1	67.3	20.0–239.4	66.7	13.6–347.1
Testosterone:estradiol ratio	244	158–371	244	162–371	249	152–417	249	152–372
Free testosterone:LH ratio	153	75–355	148	65–293	150	70–306	156	72–403
Time of blood sampling, AM	9:50	9:20–10:10	9:50	9:27–10:20	9:52	9:33–10:20	10:00	9:35–10:30

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin.
[a] Mean of measurements for the 2 testicles.
[b] Measured for 838 men.
[c] Measured for 672 men.

findings. Among men without depression, a sleep score above 50 (poor sleep) was associated with a 33% (95% CI: 5, 53) reduction in sperm concentration, a 30% (95% CI: 0.1, 51) reduction in total sperm count, and 1.3 (95% CI: −3.0, 5.7) and 1.9 (95% CI: 0.4, 3.4) percentage points' fewer motile and morphologically normal spermatozoa, respectively, compared with the reference group. In addition, we repeated the analyses without adjustment for alcohol consumption and smoking, since these may be intermediate factors between sleep disturbances and reproductive health. We also stratified the analyses according to BMI; neither change altered the direction of the findings.

No clear associations between sleeping disturbances and serum reproductive hormone levels were detected (Table 3) (data for FSH, luteinizing hormone, and inhibin B not shown).

DISCUSSION

In this cross-sectional study of 953 young Danish men from the general population, we detected an inverse

Table 3. Results From Linear Regression Analyses (Adjusted β Coefficient or Percent Change) of Semen Quality and Reproductive Hormone Levels Among 953 Young Danish Men According to Sleep Score, January 2008–June 2011

Sleep Score	No. of Participants	Semen Volume, mL[a]		Sperm Concentration, millions/mL[a,b]		Total Sperm Count, millions[a,b]		% Motile Spermatozoa[a,c]		% Morphologically Normal Spermatozoa[a,d]		Testis Size, mL[a]		Testosterone Level, nmol/L[b,e]		Free Testosterone Level, pmol/L[b,e]	
		β	95% CI	% Change	95% CI	% Change	95% CI	β	95% CI	β	95% CI	β	95% CI	% Change	95% CI	% Change	95% CI
0	194	−0.1	−0.3, 0.3	−18	−36, 4	−19	−37, 3	−4.8	−7.7, −1.8	−0.6	−1.6, 0.5	−0.4	−1.3, 0.5	−0.2	−1.7, 1.4	−3.4	−11.2, 1.0
1–10	192	0.1	−0.2, 0.4	−15	−33, 8	−10	−29, 14	−2.5	−5.5, 0.4	−0.3	−1.4, 0.7	−0.3	−1.2, 0.6	−1.0	−2.6, 0.5	−7.0	−14.5, 1.0
P-trend[f]		0.87		0.10		0.07		<0.01		0.27		0.36		0.86		0.42	
11–20	206	Reference		Reference		Reference		Reference		Reference		Reference		Reference		Reference	
21–30	122	0.3	−0.1, 0.6	−6	−28, 24	−2	−25, 30	−3.2	−6.5, 0.2	0.1	−1.1, 1.3	0.3	−0.7, 1.3	−0.3	−2.1, 1.5	−2.4	−11.1, 7.3
31–40	105	0.1	−0.3, 0.5	−7	−30, 25	−7	−31, 24	−5.4	−8.9, −1.9	−1.1	−2.4, 0.1	−0.3	−1.4, 0.8	0.0	−1.9, 1.9	4.1	−6.0, 15.1
41–50	58	−0.1	−0.6, 0.3	−22	−46, 11	−29	−50, 2	−1.2	−5.6, 3.1	−0.5	−2.1, 1.0	−1.1	−2.5, 0.2	0.1	−2.2, 2.4	−0.4	−11.8, 12.5
>50	76	0.2	−0.2, 0.6	−29	−48, −2	−25	−46, 4	−0.9	−4.9, 3.1	−1.6	−3.0, −0.3	−1.3	−2.5, −0.1	1.1	−1.0, 3.2	6.5	−4.7, 19.1
P-trend[f]		0.75		0.03		0.03		0.34		<0.01		0.02		0.34		0.23	

[a] Adjusted for period of abstinence since last ejaculation (transformed using the natural logarithm), alcohol consumption, smoking, and age, categorized according to Table 2.
[b] Transformed using the natural logarithm and back-transformed, giving percent change.
[c] Also adjusted for duration between time of ejaculation and analysis of the sample, categorized according to Table 2.
[d] Measured for 838 men.
[e] Measured for 672 men and adjusted for a blood sampling time of 8:00 AM, body mass index, and smoking.
[f] Test for trend was performed by inserting the categorical sleep variable into the model, assuming the association to be linear.

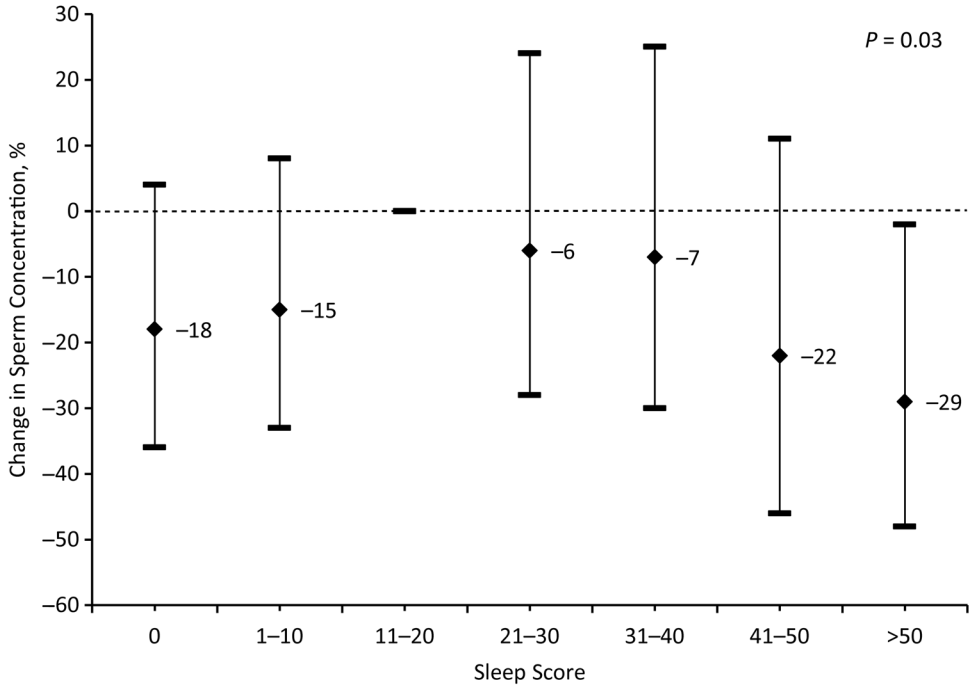

Figure 1. Adjusted (for period of abstinence, alcohol intake, smoking, and age) change in sperm concentration (%) according to sleep score (reference score: 11–20) among young Danish men ($n = 838$), January 2008–June 2011. The *P* value refers to the linear trend from the reference sleep score to the highest score (>50).

U-shaped association between self-reported sleep disturbances and sperm concentration, total sperm count, morphologically normal spermatozoa, and testis size. Men in the highest category of sleep disturbances had an approximately 25% lower total sperm count and 1.6 percentage points' fewer morphologically normal spermatozoa than the men with less disturbed sleep. Interestingly, men who reported no sleep disturbances also had a trend towards lower semen quality. No differences in the associations of serum reproductive hormone levels with sleep score were found.

In several studies, sleep duration has been found to be associated with BMI, morbidity, and mortality in a U-shaped manner (1–3). To our knowledge, this is the first study to relate sleep disturbances to semen quality. In previous large, community-based studies, investigators have reported an association between sleep disturbances and calculated free testosterone level. A longitudinal study carried out among 1,312 US men above 65 years of age found no association between testosterone and sleep duration (8) but found lower sleep efficiency among men with low testosterone levels. However, this association was absent after adjustment for BMI. In a cross-sectional study among 531 Chinese men, longer sleep duration was related to increased testosterone level and free androgen index (9)—findings which could not be confirmed in a cross-sectional study among 375 Austrian men (7). However, the cross-sectional nature of the studies makes it difficult to rule out reverse causation, and the effect of BMI was not accounted for. We did not find any clear associations between serum reproductive hormone levels and sleeping disturbances, but the cross-sectional nature of our study makes it difficult to draw firm conclusions about this association. In addition, we cannot exclude the possibility that sleeping disturbances may change the circadian rhythm of reproductive hormone levels (19).

It remains to be investigated whether improved sleeping patterns restore semen quality. In a recent study, Leproult and Van Cauter (6) restricted sleep among 10 young men for 1 week and reported a 10%–15% decrease in serum testosterone levels during the daytime—findings which are in line with other studies of young military personal (20–22). Penev (23) reported an association between longer sleep duration, as measured in a laboratory, and higher testosterone levels in 12 elderly men. However, it is questionable whether we can compare results from population-based studies with results of studies carried out in laboratory settings. In laboratory settings, artificially induced sleep disturbances might be viewed more as an interesting challenge than as a real-life stressor by the participants, and the measured cortisol level may mirror an arousal more than a response to poor sleep. In addition, in laboratory studies, the participants are informed about the test setting and may therefore perceive a degree of control. Poor sleep during everyday life may be caused by work-related circumstances or life events that the individual cannot control, and that may influence the individual emotionally.

We can only speculate about the possible mechanism by which sleep disturbances may affect semen quality, as we

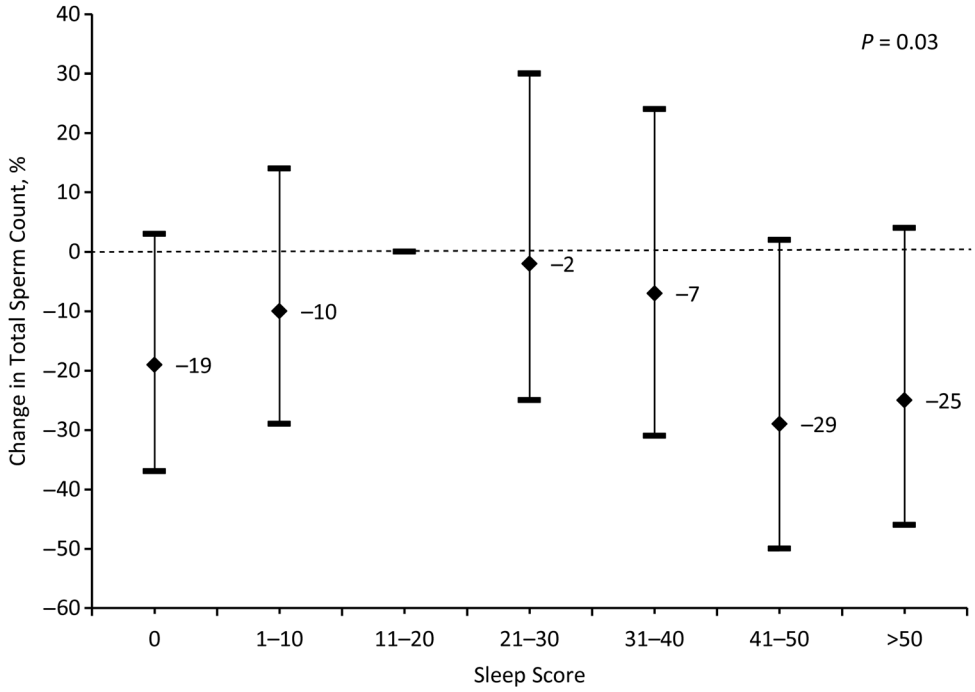

Figure 2. Adjusted (for period of abstinence, alcohol intake, smoking, and age) change in total sperm count (%) according to sleep score (reference score: 11–20) among young Danish men ($n = 838$), January 2008–June 2011. The P value refers to the linear trend from the reference sleep score to the highest score (>50).

found no associations with serum reproductive hormone levels. However, sleep disturbances may affect nocturnal testosterone rhythm (19) without affecting serum testosterone levels. In addition, sleep disturbances may be associated with lifestyle factors and high-risk behavior, since the men with sleep disturbances were more often from lower social classes and had more often had sexually transmitted diseases (i.e., may have engaged in unprotected intercourse). We adjusted for smoking, BMI, caffeine and alcohol intake, exercise habits, social class, and sexually transmitted diseases, but residual confounding may still have been present. Sleep disturbances may also be related to occupational exposures (e.g., shift work and chemical exposures), which we did not obtain information about. However, 78% of the men were still attending school.

Depression may be related to both sleep disturbances and semen quality and thereby may have confounded the analyses. However, the participants were young, and only 21 men reported being diagnosed with depression. Exclusion of these men with depression strengthened the findings, suggesting that the association with sleep disturbances was even stronger among men without depression. However, we only obtained information on physician-diagnosed depression and we recognize that there is a considerable amount of undiagnosed depression, which may explain the findings. In addition, sleep disturbances may be associated with psychological stress, which has been implicated as a cause of idiopathic infertility in both men and women, with the majority of studies having been conducted among women (24). Most studies have been conducted in infertile populations, making it difficult to differentiate between stress as a cause of infertility and stress as a consequence of infertility. In addition, the availability of a wide range of different tools with which to assess stress and other psychological conditions makes comparison across studies difficult (25–36). Some studies have examined the relationship between different types of stress and/or anxiety and semen quality, whereas others have addressed psychological personality factors and stress coping styles. Three studies have examined the effect of stressful life events (33, 34, 36). Most investigators have reported an association between higher stress levels and lower semen quality, although 1 prospective study found no association but did observe a decreased pregnancy rate during high-stress cycles (25). However, none of these studies reported data on sleeping disturbances, which may be an independent, sensitive stress marker.

Our participation rate was approximately 30%, which is higher than participation rates in other population-based studies of semen quality (37, 38). In addition, the majority of the young men included in the study had no knowledge of their own fertility potential (93%), and this is unlikely to have affected their motivation to participate. Therefore, these men were not experiencing additional distress due to failed attempts at conception, which may have been the case in most previous studies of infertile populations. We were therefore better able to distinguish stress as a cause or consequence of infertility than investigators in previous studies. Additionally, since our goal was to compare semen quality

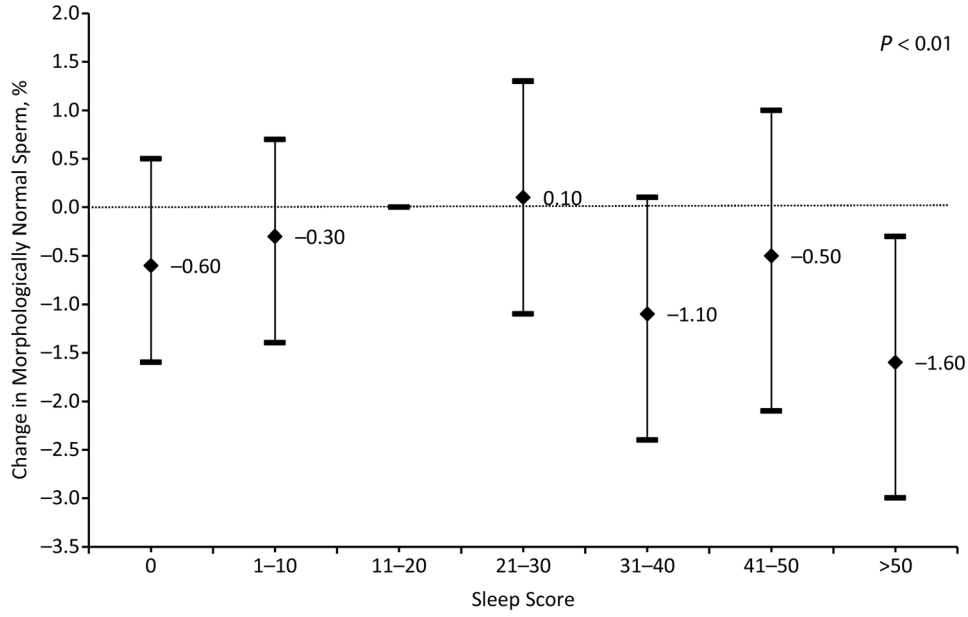

Figure 3. Adjusted (for period of abstinence, alcohol intake, smoking, and age) change in the percentage of morphologically normal spermatozoa according to sleep score (reference score: 11–20) among young Danish men ($n = 838$), January 2008–June 2011. The *P* value refers to the linear trend from the reference sleep score to the highest score (>50).

among men with different sleep scores rather than to estimate population semen parameters, whether or not the men were representative of the general population is of secondary importance. The mean sleep score was 19.5 (standard deviation, 17.4), which is marginally lower than in a previous Danish study among 3,517 employees aged 20–59 years (21.3 (standard deviation, 19.0)) (15). The men in our study were younger, however, and many were students and therefore expected to have lower scores. However, in a recent study using the Bergen Insomnia Scale (Dr. Åse Marie Hansen, National Research Centre for the Working Environment (Copenhagen, Denmark), unpublished data), investigators reported that young Danish men aged 18–24 years do not have fewer sleeping disturbances than older men (http://www.arbejdsmiljoforskning.dk).

Our study was cross-sectional and therefore reverse causation is possible, although it is unlikely because the men were unaware of their semen quality when they responded to the sleep questions. The men were asked to reported sleep disturbances occurring within the 4 weeks before completion of the questionnaire. If this differed from their general level of sleep disturbances, misclassification of exposure may have occurred, but since they responded to the questionnaire before they delivered their semen sample, this nondifferential misclassification is likely to have resulted in underestimation of the effect of sleep on reproductive health.

It is well known that interobserver variability in semen analysis exists and is particularly high for morphology and motility assessment, which may help explain the lack of an association of sleep with motility. However, all analyses were performed blinded, and the same technicians assessed all morphology slides. We obtained only 1 semen sample for each man, and intraindividual variability exists, as well as a circadian rhythm in hormone production (however, blood samples were mostly taken in the mornings, and we adjusted sampling times to 8:00 AM), which may have introduced nondifferential misclassification, thereby underestimating the potential effects.

We found an inverse U-shaped association between self-reported sleep disturbances, semen quality, and testis size. The study was cross-sectional, however, and therefore we cannot exclude the possibility of reverse causation or residual confounding from unhealthier lifestyle and health behavior. However, given the facts that approximately 20% of all young men may have reduced semen quality and that sleep disturbances are common and increasing in industrialized countries, the results of this study may have important public health implications. In future studies, researchers should attempt not only to elucidate the biological explanations but also to prospectively assess whether semen quality improves after interventions aimed at improving sleeping patterns.

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