University of Southern Denmark

Impact of psychological stress measured in three different scales on testis function

A cross-sectional study of 1362 young men

Nordkap, Loa; Priskorn, Lærke; Bräuner, Elvira V.; Marie Hansen, Åse; Kirstine Bang, Anne; Holmboe, Stine A.; Winge, Sofia B.; Egeberg Palme, Dorte L.; Mørup, Nina; Erik Skakkebæk, Niels; Kold Jensen, Tina; Jørgensen, Niels

Published in:
Andrology

DOI:
10.1111/andr.12835

Publication date:
2020

Document version:
Accepted manuscript

Citation for pulished version (APA):
Nordkap, L., Priskorn, L., Bräuner, E. V., Marie Hansen, Å., Kirstine Bang, A., Holmboe, S. A., Winge, S. B., Egeberg Palme, D. L., Mørup, N., Erik Skakkebæk, N., Kold Jensen, T., & Jørgensen, N. (2020). Impact of psychological stress measured in three different scales on testis function: A cross-sectional study of 1362 young men. *Andrology*, *8*(6), 1674-1686. https://doi.org/10.1111/andr.12835

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use
This work is brought to you by the University of Southern Denmark.
Unless otherwise specified it has been shared according to the terms for self-archiving.
If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim.
Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 19. Sep. 2021

Title:

Impact of psychological stress measured in three different scales on testis function; A cross sectional study of 1,362 young men

Authors:

Loa Nordkap[a], Lærke Priskorn[a], Elvira V. Bräuner[a], Åse Marie Hansen[b], Anne Kirstine Bang[a], Stine A. Holmboe[a], Sofia B. Winge[a], Dorte L. Egeberg Palme[a], Nina Mørup[a], Niels Erik Skakkebæk[a], Tina Kold Jensen[a,c], Niels Jørgensen[a].

Affiliations:

a: Department of Growth and Reproduction and International Centre for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, Denmark (address: Blegdamsvej 9, 2100 Copenhagen Ø, Denmark).

b: Department of Public Health, University of Copenhagen (address: Øster Farigmagsgade 5, 1014 Copenhagen K, Denmark) and National Research Centre for the Working Environment, Copenhagen, Denmark (address: Lersø Parkallé 105, 2100 Copenhagen Ø, Denmark).

c: Department of Environmental Medicine, University of Southern Denmark (address: Winsløwsparken 17A, 5000 Odense C, Denmark).

Short title: Stress and testicular function.

Keywords: Perceived stress, stress symptoms, stressful life events, semen quality, reproductive hormones.

Corresponding author

Niels Jørgensen, MD, PhD.

Department of Growth and Reproduction, Rigshospitalet

Blegdamsvej 9, 2100 Copenhagen Ø, Denmark

Tel +45 3545 5064

Fax +45 3545 6054

Email Niels.Joergensen@regionh.dk

Disclosure statement: The authors have nothing to disclose.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ANDR.12835

This article is protected by copyright. All rights reserved

Author Manuscript

Acknowledgements: This study was supported by the Danish Council for Independent Research (grant no. DFF – 1331-00044), Nordic Research Committee (grant no. R195-A16270), Aase and Ejnar Danielsens Foundation (grant no. 10-000615), the A.P. Møller Foundation for the Advancement of Medical Science, Innovation Fund Denmark (14-2013-4), ReproUnion (grant no. 20200407), and The Research Fund of Rigshospitalet (grant no. R42-A1326). None of the sponsors had any involvement in the study design or data analysis.

ABSTRACT

Background: Studies have reported associations between psychological stress and semen quality, but most have been performed on selected populations using different stress measures. Thus, it is uncertain which stress scale best quantifies the effects of stress on testicular function.

Objective: To study the association between three different measures of stress and testicular function in young men.

Material and methods: In total, 1,362 men (median age 19 years) delivered semen and blood samples. They also answered a questionnaire including information from three stress scales: Stress Symptoms, Stressful Life Events and Perceived Stress. Various statistical analyses for associations between stress and testicular function (semen quality and reproductive hormones) were performed.

Results: Perceived Stress was negatively associated with sperm concentration, total count and motility and positively associated with serum FSH. Men with the highest scores (>30 points) had 38% (95% CI 3-84%) lower sperm concentration, 42% (95% CI 5-91%) lower total count, and 22% (95% CI 2-32%) lower proportion of motile spermatozoa than men with the lowest scores (0-10 points). For the stress symptoms score, men with highest scores (>95th percentile vs. lower) had lower sperm concentration, total sperm count, motility and serum Inhibin-B/FSH-ratio. Although men with highest stress levels were characterized by an unhealthier lifestyle, adjusting for lifestyle factors did not attenuate results suggesting that the associations between stress and testicular function were not mediated by lifestyle. Stressful Life Events were not associated with testicular function.

Discussion and Conclusion: The linear association between Perceived Stress and semen parameters and lack of dose-response association for the other two stress scales indicated that perceived stress was the most sensitive marker of stress affecting semen quality in young men. The lack of associations between Stressful Life Events and testis function confirmed that the perception of stressful events rather than the stressful event *per se* matters.

Author Manuscript

This article is protected by copyright. All rights reserved

INTRODUCTION

Infertility is a major problem worldwide, and in Denmark, 8.7% of children born in 2017 were conceived by means of fertility treatment[1]. For couples that need fertility treatment to conceive, about half of the cases are estimated to be due to a 'male factor', primarily impaired semen quality.

Semen quality may be affected by lifestyle and behavioural factors e.g. smoking, alcohol, diet and physical fitness[2-6]. To date, several studies have also associated psychological stress with impaired semen quality as previously summarized by Li *et al.* and Nordkap *et al.*[7;8] and recently updated by Bräuner *et al.*[9]. However, the studies used different methods to assess stress and most were conducted in small or selected populations of infertile men, making it difficult to compare results and distinguish between stress as a cause or a consequence of infertility. Also, most studies did not measure reproductive hormones that potentially could elucidate whether psychological stress affects the testicles directly or rather interfere with the hypothalamic-pituitary regulation of testicular function. Currently, no good objective measures of psychological stress exist, and the assessment of psychological stress is usually approached using questionnaires. Although several stress scales exist, it is not known which scale is the most sensitive measure of psychological stress capturing the potential biological negative effects on testicular function in a population of young men.

The main purpose of this study was to assess three measures of stress; namely Stress Symptoms, Stressful Life Events and Perceived Stress and their association with testicular function (semen quality and reproductive hormones) in young men from the general population.

MATERIAL AND METHODS

Study population

Men were included from a population that attended a medical examination before being considered for military service in the period from September 2012 to December 2016. This medical examination is mandatory for all young Danish men, except those suffering from severe or chronic diseases (<15%), and the included men are therefore considered representative of the general population. Men that consented to participate were given an appointment for examination at the Department of Growth and Reproduction at Rigshospitalet (Copenhagen, Denmark). Participants received a financial compensation (approximately 65 €). Inclusion of the study population and physical examinations have been described in detail in our previous study of psychological stress in Danish men from the general population[8]. However, none of the current study subjects were included in the previous study that considered 1,215 men recruited in an earlier period from April 2008 to 2012 with less comprehensive information regarding psychological stress. On the day of attendance at the department, each man underwent a physical examination, returned a completed

Author Manuscript

This article is protected by copyright. All rights reserved

questionnaire (containing information on age and previous or current diseases, known history of fertility, as well as stress related questions), and provided both blood and semen samples.

In brief, the physical examination included a scrotal ultrasound scan, blood sampling for reproductive hormone analyses, and delivery of a semen sample that was analysed on site according to WHO criteria[10;11]. Furthermore, the participants completed an electronic questionnaire on lifestyle factors (smoking and drinking habits, caffeine intake, use of recreational drugs, physical fitness), general and reproductive health (use of medication, previous and current diseases including genital diseases), information on whether the mother smoked during pregnancy, fever above 38 °C (100.4 °F) for at least 24 hours within the previous three months, and stress (detailed below). Caffeine intake (mg/day the previous week) and alcohol consumption (units/recent week) were calculated as described previously[3;12].

In total, 1,388 men were examined in the study from September 2012 to December 2016. Among these, 26 were excluded: azoospermia (N=7), no semen sample available (N=6), ejaculatory duct obstruction (N=3), diagnosis of testicular cancer during the study (N=1), previous treatment of testicular cancer (N=1), previous treatment of leukaemia (N=1), only one testis in scrotum (N=2), and active use of anabolic steroids (N=5), leaving a population of 1,362 men.

<u>Stress assessment</u>

Supplementary Table 1 provides an overview of the stress scales and the questions included in these.

The Stress Symptoms Scale (N=1,362)

A 14 item Stress Symptoms Scale including both psychological and somatic stress symptoms was constructed based on two validated 4-item stress scales (the "Stress Scale" and the "Somatic Stress Scale") from the Copenhagen Psychosocial Questionnaire II (COPSOQ II)[13] and additionally six items from other COPSOQ II stress scales[13] (overviewed in Supplementary Table 1). Among the total of 14 COPSOQ II items, we performed factor analysis aiming at constructing one scale sensitive for both psychological and somatic stress symptoms. The best scale in terms of internal consistency was assessed by Cronbach's alpha and included all 14 items (Cronbach's Alpha 0.85). Since this 14-item scale assesses *stress symptoms* (rather than perceived stress), it is referred to as the Stress Symptoms Scale. COPSOQ II items, and thereby also the Stress Symptom Scale, address stress symptoms during the previous four weeks. Each item has five response categories: "All the time" (100 points), "A large part of the time" (75 points), "Part of the time" (50 points), "A small part of the time" (25 points), and "Not at all" (0 points). A mean score was calculated, giving a personal score from 0-100 points[13].

Author Manuscript

This article is protected by copyright. All rights reserved

Author Manuscript

Stressful Life Events (SLEs) (N=1,362)

All participants were asked whether they had experienced SLEs during the previous three months. Included events were chosen among the most stressful events on the Holmes and Rahe´s Social Readjustment scale[14]: "Serious disease or death of near family member or relatives", "Divorce or serious relationship problems", "Serious legal or economic problems", "Fired from job", "Serious disease of the participant", and "Other serious events (open-ended)" (Supplementary Table 1).

Cohen´s Perceived Stress Scale (PSS) (N=608)

In 2015, the 10-item Perceived Stress Scale (PSS) that assesses *perceived stress* during the last four weeks[15] was added to the questionnaire providing information from 608 men included in the years 2015 and 2016. This Danish version of the PSS has been back-translated into English and accepted by Cohen and co-workers[16]. Each item was scored on a 5-point Likert scale ranging from 0 (never) to 4 (very often). Thus, a summed score from 0-40 could be obtained by accepted methods after reversing responses (i.e., 0=4, 1=3, 2=2, 3=1, and 4=0) to the four positively stated items 4, 5, 7, and 8 and then summing across all scale items.

Physical examination

The presence of varicocele was evaluated by inspection and palpation. Varicocele was graded with the men in standing position: grade 0: no varicocele present, grade 1: only palpable during the Valsalva procedure, grade 2: palpable in the resting state, and grade 3: plainly visible[17] in accordance with our previous publications[18].

<u>Semen quality</u>

Details of the semen analysis have previously been described in detail[18]. In brief, each man provided a semen sample by masturbation in a room next to the semen laboratory. The men were instructed to abstain from ejaculation for at least 48 hours and the exact duration of ejaculation abstinence was recorded. The sample was analysed for semen volume (assessed by weighing), sperm concentration (assessed using a Bürker-Türk haemocytometer), percentage motile spermatozoa (assessed on wet preparations on a clean warm glass slide), and morphologically normal spermatozoa (assessed according to stricter criteria on Papanicolaou stained smears) as described in detail elsewhere[10;11]. Total sperm counts were calculated as semen volume x sperm concentration.

<u>Blood sampling and reproductive hormone analyses</u>

This article is protected by copyright. All rights reserved

On the day of attendance, a venous blood sample was drawn from each participant for assessment of reproductive hormones. Samples were drawn between 8 am and 1 pm (median 10:40 am). Samples were left to clot, and the serum was isolated and frozen at -20 °C until analysis. Assessments of total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone-binding globulin (SHBG) were performed by a time-resolved immunofluorometric assays (Delfia Wallac, Turku, Finland), and Inhibin-B by a two-sided enzyme immunometric assay (Inhibin-B gen II, Beckman Coulter Ltd., High Wycombe, UK). However, for men included in 2014-2016, testosterone and SHBG were measured by ELISA (Access2, Beckman Coulter Ltd., High Wycombe, UK). The hormones were analysed yearly in batches including reanalysis of a number of controls from the previous year to ensure comparability over time. Free testosterone (cFT) was calculated by the method described by Vermeulen *et al.* using a fixed albumin level at 43.0 g/L[19].

Statistics

Descriptive statistics (medians and 5-95 percentiles or frequencies) were calculated on data from the questionnaire and physical examination for the total population and stratified according to stress. Differences in basic characteristics between categories of stress symptom scores were compared by Kruskal-Wallis test for continuous variables and chi-squared test for categorical variables. Furthermore, the men were stratified according to stress symptom and perceived stress score categories and according to whether their semen parameters were above the lower WHO reference levels for sperm concentration (≥15 mill/mL), total sperm count (≥39 mill), and percentage of motile spermatozoa (≥40%)[11].

The associations between different measures of stress as the explanatory factor and testicular function (semen variables and reproductive hormones) as outcome were examined in both unadjusted (model 1) and adjusted (model 2-4) linear regression analyses. To meet regression model assumptions, semen volume, sperm concentration, and total sperm count were transformed by use of cubic root. The percentage of motile spermatozoa and reproductive hormones were transformed by use of natural logarithm. The percentage of morphologically normal spermatozoa entered the model untransformed. To ease the interpretation of the results, we calculated the percentage difference in semen parameters between men with the highest and the lowest stress levels assessed by the Perceived Stress Scale by additionally transforming semen parameters by use of natural logarithm followed by back-transformation of estimates. Effect sizes and p-values from this alternative transformation approximately approached those obtained by the superior model (better model fit) based on cubic root-transformed outcome variables.

Author Manuscript

This article is protected by copyright. All rights reserved

For the Stress Symptoms Scale as the explanatory variable in linear regression analyses, the score was first inserted as a continuous variable and secondly with the stress scores divided into three categories (<5^{th}; 5-95^{th}; and ≥95^{th} percentile) since a visual inspection of associations between stress and sperm concentration and total sperm count indicated a non-linear association such that lower counts were observed for men with the lowest and highest stress symptom scores (~<5^{th} and >95^{th} percentiles), whereas there was no difference between men with intermediate stress symptom scores. For SLEs, participants were stratified according to the number of SLEs (0 SLE; 1 SLE; >1 SLEs). For regression analyses according to the Perceived Stress scale, the score was again analyzed as both a continuous variable and with participants categorized according to their PSS score (0-10; 11-20; 21-30; and >31 points) with the group containing the median (17.9) stress score chosen as reference group (11-20 points).

In the adjusted linear regression analyses, a stepwise adjustment was conducted. A number of covariates closely associated to the outcome measurement were accounted for in model 2. Semen volume, sperm concentration, and total sperm count were adjusted for the ejaculation abstinence period (using splines with cutoffs at 48 and 96 hours), whereas sperm motility was adjusted for ejaculation abstinence period and duration from ejaculation until motility analysis (using splines with a cutoff at 30 minutes). Reproductive hormones were adjusted for time of day for blood sampling and year of participant inclusion. We also evaluated the impact of potential confounders (listed in Table 1) identified in the literature to be associated with semen parameters, reproductive hormones, and stress. Potential confounders were excluded by stepwise backward elimination and kept in the models if they were significant ($p<0.05$). Thus, lifestyle variables (caffeine intake (daily intake >300 mg/day), alcohol intake (≤14 or >14 units/recent week), daily use of cannabis, and daily cigarette smoking) (model 3), and also BMI (model 4) were adjusted for in the final models. Variables adjusted for in model 3 and 4 were considered potential mediators for the association between stress and testicular function. Fit of the regression models was evaluated by visually inspecting the residual plots to address linear regression model assumptions of normal distribution and homoscedasticity. Reliability analyses were used for calculation of Cronbach's alpha values for the stress scales (Supplementary Table 1). In total, 23 men had missing values (maximum two items per scale).

In sub-analyses both unadjusted and adjusted models were repeated after excluding men with previous or current andrological disorders (testicular torsion, congenital cryptorchidism, grade 2 or 3 varicocele, sexually transmitted diseases, epididymitis, orchitis, fever >38 °C within the last 3 months). Furthermore, analyses of the stress symptom score were repeated in the sub-population with available information on perceived stress.

Author Manuscript

This article is protected by copyright. All rights reserved

Statistical analyses were completed using IBM SPSS Statistics version 22 and p-values <0.05 were considered statistically significant.

Ethical approval

Approval of the study was obtained from the local science ethical committee (H-KF-289428). Informed written consents were obtained from all participants.

RESULTS

The median age of the 1,362 men included in the study was 19.0 years. The scores of the three stress scales (Stress Symptoms Scale, SLE and PSS) were correlated as the group with the highest Stress Symptoms scores also had the highest level of perceived stress and SLEs (Table 1). A higher proportion of men with highest stress levels had previously been diagnosed with epididymitis, orchitis, sexually transmitted disease or testicular torsion, and reported to have had fever during the last three months (Table 1). Age and BMI did not vary according to stress levels, but men with highest stress levels smoked more often and used cannabis more often, had a higher caffeine intake and reported lower physical fitness.

Stress Symptoms Scale

Figure 1 illustrates that the proportion of men having sperm concentration, total sperm counts and motility below the WHO reference levels differed between the three stress levels. More men with highest stress levels had semen variables below the reference levels. There were no associations between stress symptoms on a continuous scale and testicular function. However, men with the highest stress symptom scores (>95th percentile) had lower sperm concentration, total sperm count, percentage of motile spermatozoa, Inhibin-B and Inhibin-B/FSH-ratio than the reference group of men with an intermediate symptoms score both in unadjusted and adjusted models (Tables 2 and 3). The observed higher FSH level was non-significant. For the men with the lowest symptoms scores (<5th percentile), the tendency for lower semen volume, total sperm count and percentage of motile spermatozoa as well as other variables shown in Table 2 were all non-significant compared to the intermediate group both in unadjusted and adjusted models. LH, testosterone and testosterone/LH-ratio did not differ between groups in any of the statistical models.

In sub-analyses of 988 men excluding the 374 men with andrological disorders or fever, the same direction of associations between highest stress symptoms and sperm concentration, total sperm count, motility, Inhibin-B and Inhibin-B/FSH-ratio were detected as for the entire population, but the effect sizes for all above mentioned outcomes were more pronounced (Table 3). For those with the lowest symptoms scores, the sub-

This article is protected by copyright. All rights reserved

analysis did not show significant differences in any parameters compared to those with intermediate stress scores (5-95th percentiles).

An additional sub-analysis that only included the 608 men who also provided results for the PSS showed the same tendencies for differences between men with highest versus lowest stress symptoms scores, although the difference for total sperm counts was no longer statistically significant.

Stressful life events (SLEs)

30% of the men (N=413) reported at least one SLE within the recent three months prior to participation in the study. "Serious disease or death of a near family member or relative" and "Other serious events" were the most commonly reported SLEs (Supplementary Table 1). Neither semen parameters nor reproductive hormones differed according to SLEs within the previous three months (Supplementary Table 2) or in the subgroup of men without andrological disorders (data not shown). Nor were any associations observed when stratifying analyses on the type of SLE reported.

Perceived stress scale (PSS)

Table 4 shows the semen and reproductive hormone levels in four PSS score categories in the group of men than answered the PSS questions (those examined in 2015-16). Figure 2 shows that a higher proportion of men had sperm concentration, total sperm count, and percentage of motile spermatozoa below the WHO reference levels with increasing PSS scores.

Statistically, the associations between PSS scores and markers of testicular function were best described as linear, and Table 5 shows that the sperm concentration, total sperm counts, and motility were lower and FSH higher in men with increasing PSS scores both in unadjusted and adjusted models. Similar associations were observed when excluding men with known andrological disorders except that the association between PSS and reduced motility became only borderline significant. No associations were found for LH, testosterone and testosterone/LH-ratio (Table 5). The PSS associations with semen quality were equivalent to a 38% (95% confidence interval [95% CI]: 3-84%) lower sperm concentration ($p=0.029$), 42% (95% CI: 5-91%) lower total sperm count ($p=0.016$), and 22% (95% CI: 2-32) lower proportion of motile spermatozoa ($p=0.030$) in men with the highest PSS score (>30 points) compared with those with the lowest score (0-10 points).

DISCUSSION

In this cross-sectional study of men unselected with regards to stress, semen quality and fertility status, we detected that the highest self-reported stress levels assessed by different scales were associated with

This article is protected by copyright. All rights reserved

impaired semen quality concomitantly with changes of serum reproductive hormones reflecting spermatogenesis. The results did not indicate that the associations were mediated by unhealthy lifestyle factors in men with high stress levels. Of the three investigated stress scales, perceived stress (PSS), assessed by Cohen´s Perceived Stress scale, appeared more sensitive in capturing the association with semen quality/testicular function than stress symptom scores or reported SLEs in this population of young men. The Stress Symptoms Scale only detected lower testicular function among those with the very highest stress levels, and although SLEs are believed to be the most objective stress marker, these were not related to testicular function in this population.

We observed a negative linear association between PSS score and semen quality, and men with the lowest perceived stress had the best testicular function. A negative linear association between PSS score and semen quality has been described previously in a study of men from the general population[20] whereas a study of infertile men did not find any association[21]. Explanations for the disparities for the associations using the Stress Symptom Scale and the PSS in the present study may rely on differences between the scales; the Stress Symptoms Scale measures stress symptoms whereas the PSS measures the perception of stressful situations[13;15]. Also, the items in the Stress Symptoms Scale were developed to measure work-related stress whereas the PSS was developed to measure general stress[13;15]. The items in the Stress Symptoms Scale have been validated in populations that were older than our population, whereas the PSS has mostly been validated in younger cohorts such as college students more comparable to our population. Therefore, the PSS may be superior to the Stress Symptoms Scale in a population of young men as the one included in the present study.

We did not find associations between SLEs and semen quality contrary to three previous studies[20;22;23]. In one study, death of a family member within the last three months, reported by 12 men, was associated with decreased motility[23]. Another study detected that experiencing two SLEs within the recent three months were associated with lower sperm concentration and total sperm count[22], and a third study found lower sperm motility and morphology in 12 men who had experienced at least two SLEs within the past year[20]. Overall, results from previous studies detecting mainly associations in small sub-samples, along with our results, does not provide strong evidence for associations between SLEs and semen quality. However, we cannot exclude that our study population had not experienced what might be considered “real serious SLE’s” yet due to their young ages.

Participants with the highest stress levels had a more unhealthy lifestyle in accordance with previous studies[24], and these unhealthy lifestyle factors have been linked with decreased semen quality. Nevertheless,

Author Manuscript

This article is protected by copyright. All rights reserved

controlling for these factors did not attenuate our results suggesting that they were neither important confounders nor mediators for the studied associations. We cannot make final conclusions on the direction of the associations due to the cross-sectional nature of the study, but the results support the hypothesis that stress *per se* has a negative effect on testicular function. We consider it less likely that a reduction in spermatogenesis – that was not known by the men – will induce stress. It is important to decide whether interventions should be directed towards stress reduction or unhealthy lifestyle habits such as smoking, marihuana use, caffeine intake, physical inactivity etc. If the unhealthy lifestyle at least partly is a consequence of stress, it may be rational to prevent or treat stress rather than trying to get a stressed man to quit unhealthy habits. However, intervention studies will be needed to elucidate this.

We can only speculate about the possible biological explanation for the association between high stress levels and impaired spermatogenesis. Studies of rodents have shown that stress-induced increased levels of glucocorticoids can induce apoptosis of germ cells and dysfunction of the germinal epithelium[25;26]. Furthermore, we have previously detected that the glucocorticoid receptor and genetic variants of this may be involved in regulation of human testicular function[27]. Thus, direct effects of stress may be mediated via increased levels of glucocorticoids.

Serum FSH and Inhibin-B are markers of spermatogenesis[28;29]. FSH was increased in men with higher stress scores thus corroborating the findings of lower sperm counts in this group of men, and indicating a primary testicular reduction of the capacity for spermatogenesis[28]. If the direct effects of stress on the testicles were fully compensated by increased FSH, unchanged sperm counts and Inhibin-B levels would be expected. We did, however, not detect such a complete compensation potentially indicating also a hypothalamic-pituitary effect of stress. The Leydig cell function assessed by LH, testosterone and the ratio testosterone/LH was not associated with any of the stress measures.

The strengths of this study were that we included a large cohort of men from the general population and obtained thorough information on general and reproductive health and lifestyle factors. Additionally, we assessed serum markers of testicular function. We also assessed stress by various scales to explore if the same overall interpretation could be reached independently of scale, and we included SLEs as a more objective stress marker. In addition, the men were unaware of their reproductive function when answering the stress questions. Limitations were that we only used the PSS in the most recently included men. However, we have no reason to consider these men as substantially different from the men that were included earlier. Another limitation is that we did not include information on coping style and social support. Cronbach´s alpha values were lower in our population compared with reported values from validation cohorts, which may be

Author Manuscript

This article is protected by copyright. All rights reserved

explained by differences in populations regarding age, sex, and life circumstances. Both the Stress Symptoms Scale and the PSS assess psychological stress within the previous four weeks, which may not be optimal taking into account the length of the spermatogenic cycle of approximately 72 days[30]. However, we expect that psychological stress reported for the previous four weeks would represent long-term psychological stress also relevant for spermatogenic cycle. Furthermore, stress assessed by the PSS score has been associated with other outcomes related to chronic stress such as BMI and metabolic syndrome implicating that the PSS score is representative of long-term stress extending beyond the four weeks considered in the scoring[31;32]. The PSS scores in our study were similar to scores reported by 4,676 Danish men and 960 US men[33;34], supporting that men included in our study represented the general population with regard to stress levels. In addition, although we adjusted for many potential confounders, we cannot exclude residual confounding from for example health behaviour. Finally, it should be mentioned that our statistical approach had an exploratory character. Thus, our findings ought to be tested in separate and independent studies.

CONCLUSION

In this study of young men from the general population, we found that high self-reported stress levels assessed by different scales were associated with impaired testicular function; especially sperm concentration, total sperm count and motility. Of three investigated stress scales, the assessment of perceived stress was more sensitive than the assessment of stress symptoms or SLEs in relation to testicular function. Associations with serum markers of spermatogenesis supported the findings, and indicated that stress primarily exerts direct testicular effects, but indirect effects at the hypothalamic-pituitary level could not be excluded. The association between stress and testis function did not seem to be mediated by an unhealthier lifestyle in men with high stress levels. We suggest that intervention studies should be designed to accomplish proof-of-concept and potentially include objective stress markers.

Contributors

Substantial contributions to conception and design: LN, ÅMH, TKJ, NES, and NJ. Data acquisition: LN, LP, AKB, SAH, SBW, DLEP, and NM. Data analysis: LN. Data interpretation: All authors. Drafting the manuscript: LN, EVB and NJ. Revising manuscript critically for important intellectual content: All authors. Final approval of the manuscript: All authors.

Funding

This study was supported by the Danish Ministry of Health, Danish Council for Independent Research (grant no. DFF – 1331-00044), Nordic Research Committee (grant no. R195-A16270), Aase and Ejnar Danielsens

Author Manuscript

This article is protected by copyright. All rights reserved

Foundation (grant no. 10-000615), the A.P. Møller Foundation for the Advancement of Medical Science, Innovation Fund Denmark (14-2013-4), ReproUnion (grant no. 20200407), and The Research Fund of Rigshospitalet (grant no. R42-A1326). None of the sponsors had any involvement in the study design or data analysis.

Legends to Figures

Figure 1:

The proportion of young men with sperm concentration, total sperm count, and percentage of motile spermatozoa below and above the WHO lower reference levels according to stress levels on the Stress Symptoms scale. P-values for equal distribution between the three stress levels.

Figure 2:

The proportion of young men having sperm concentration, total sperm count, and percentage of motile spermatozoa below and above the WHO lower reference levels according to stress levels on the Perceived Stress Scale. P-values for equal distribution between the three stress levels.

Reference List

(1) Danish Health Authorities. Assisteret reproduktion 2017 (Assisted Reproduction 2017). 2019 Feb 20.

(2) Jensen TK, Jorgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B et al. Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: A cross-sectional study of 1,770 young men from the general population in five European countries. American Journal of Epidemiology 2004;159(1):49-58.

(3) Jensen TK, Gottschau M, Madsen JO, Andersson AM, Lassen TH, Skakkebaek NE et al. Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. Bmj Open 2014;4(9):e005462.

(4) Nassan FL, Jensen TK, Priskorn L, Halldorsson TI, Chavarro JE, Jorgensen N. Association of Dietary Patterns With Testicular Function in Young Danish Men. JAMA Netw Open 2020 February 5;3(2):e1921610.

(5) Priskorn L, Jensen TK, Bang AK, Nordkap L, Joensen UN, Lassen TH et al. Is Sedentary Lifestyle Associated With Testicular Function? A Cross-Sectional Study of 1,210 Men. Am J Epidemiol 2016 August 15;184(4):284-94.

This article is protected by copyright. All rights reserved

(6) Gianfrilli D, Ferlin A, Isidori AM, Garolla A, Maggi M, Pivonello R et al. Risk behaviours and alcohol in adolescence are negatively associated with testicular volume: results from the Amico-Andrologo survey. Andrology 2019 November;7(6):769-77.

(7) Li Y, Lin H, Li Y, Cao J. Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. Fertil Steril 2011 January;95(1):116-23.

(8) Nordkap L, Jensen TK, Hansen AM, Lassen TH, Bang AK, Joensen UN et al. Psychological stress and testicular function: a cross-sectional study of 1,215 Danish men. Fertil Steril 2016 January;105(1):174-87.

(9) Brauner EV, Nordkap L, Priskorn L, Hansen AM, Bang AK, Holmboe SA et al. Psychological stress, stressful life events, male factor infertility, and testicular function: a cross-sectional study. Fertil Steril 2020 March 9.

(10) Jorgensen N, Joensen UN, Jensen TK, Jensen MB, Almstrup K, Olesen IA et al. Human semen quality in the new millennium: a prospective cross-sectional population-based study of 4867 men. Bmj Open 2012;2(4).

(11) World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. 2010.

(12) Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M. Effects of caffeine on human health. Food Addit Contam 2003 January;20(1):1-30.

(13) Pejtersen JH, Kristensen TS, Borg V, Bjorner JB. The second version of the Copenhagen Psychosocial Questionnaire. Scand J Public Health 2010 February;38(3 Suppl):8-24.

(14) Holmes TH, Rahe RH. The Social Readjustment Rating Scale. J Psychosom Res 1967 August;11(2):213-8.

(15) Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983 December;24(4):385-96.

(16) Olsen LR, Mortensen EL, Bech P. Prevalence of major depression and stress indicators in the Danish general population. Acta Psychiatr Scand 2004 February;109(2):96-103.

(17) Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G et al. European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol 2012 August;62(2):324-32.

(18) Nordkap L, Jensen TK, Hansen AM, Lassen TH, Bang AK, Joensen UN et al. Psychological stress and testicular function: a cross-sectional study of 1,215 Danish men. Fertil Steril 2016 January;105(1):174-87.

(19) Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999 October;84(10):3666-72.

Author Manuscript

This article is protected by copyright. All rights reserved

(20) Janevic T, Kahn LG, Landsbergis P, Cirillo PM, Cohn BA, Liu X et al. Effects of work and life stress on semen quality. Fertil Steril 2014 August;102(2):530-8.

(21) Jurewicz J, Radwan M, Merecz-Kot D, Sobala W, Ligocka D, Radwan P et al. Occupational, life stress and family functioning: does it affect semen quality? Ann Hum Biol 2014 May;41(3):220-8.

(22) Gollenberg AL, Liu F, Brazil C, Drobnis EZ, Guzick D, Overstreet JW et al. Semen quality in fertile men in relation to psychosocial stress. Fertil Steril 2010 March 1;93(4):1104-11.

(23) Fenster L, Katz DF, Wyrobek AJ, Pieper C, Rempel DM, Oman D et al. Effects of psychological stress on human semen quality. J Androl 1997 March;18(2):194-202.

(24) Hatch SL, Dohrenwend BP. Distribution of traumatic and other stressful life events by race/ethnicity, gender, SES and age: a review of the research. Am J Community Psychol 2007 December;40(3-4):313-32.

(25) Yazawa H, Sasagawa I, Ishigooka M, Nakada T. Effect of immobilization stress on testicular germ cell apoptosis in rats. Hum Reprod 1999 July;14(7):1806-10.

(26) Silva EJ, Vendramini V, Restelli A, Bertolla RP, Kempinas WG, Avellar MC. Impact of adrenalectomy and dexamethasone treatment on testicular morphology and sperm parameters in rats: insights into the adrenal control of male reproduction. Andrology 2014 November;2(6):835-46.

(27) Nordkap L, Almstrup K, Nielsen JE, Bang AK, Priskorn L, Krause M et al. Possible involvement of the glucocorticoid receptor (NR3C1) and selected NR3C1 gene variants in regulation of human testicular function. Andrology 2017 November;5(6):1105-14.

(28) Andersson AM, Jorgensen N, Frydelund-Larsen L, Rajpert-De Meyts E, Skakkebaek NE. Impaired Leydig cell function in infertile men: A study of 357 idiopathic infertile men and 318 proven fertile controls. Journal of Clinical Endocrinology & Metabolism 2004;89(7):3161-7.

(29) Jorgensen N, Liu F, Andersson AM, Vierula M, Irvine DS, Auger J et al. 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. Fertility and Sterility 2010;94(6):2128-34.

(30) Amann RP. The cycle of the seminiferous epithelium in humans: a need to revisit? J Androl 2008 September;29(5):469-87.

(31) Janczura M, Bochenek G, Nowobilski R, Dropinski J, Kotula-Horowitz K, Laskowicz B et al. The Relationship of Metabolic Syndrome with Stress, Coronary Heart Disease and Pulmonary Function--An Occupational Cohort-Based Study. PLoS One 2015;10(8):e0133750.

(32) Carson TL, Desmond R, Hardy S, Townsend S, Ard JD, Meneses K et al. A study of the relationship between food group recommendations and perceived stress: findings from black women in the Deep South. J Obes 2015;2015:203164.

Author Manuscript

This article is protected by copyright. All rights reserved

(33) Nielsen L, Curtis T, Kristensen TS, Rod NN. What characterizes persons with high levels of perceived stress in Denmark? A national representative study. Scand J Public Health 2008 June;36(4):369-79.

(34) Spacapan S, Oskamp S. The Social Psychology of Health. In: Cohen S, Williamson GM, editors. Perceived Stress in a Probability Sample of the United States.Newbury Park, CA: Sage.: Scientific Research; 1988. p. 31-67.

Author Manuscript

This article is protected by copyright. All rights reserved

Author Manuscript

Table 1: Basic description of 1,362 study participants and stratified according to psychological stress assessed by the Stress Symptom Scale. Values are medians (5; 95 percentiles) or percentages.

	Participants with data	Total population	Stress Symptom Scale (N=1,362)			p-value[a]
			Lowest (<5th percentile) N = 93 (6.8%)	Intermediate (5-95th percentile) N = 1,188 (87.2%)	Highest (>95th percentile) N = 81 (6.0%)	
Stress assessment						
Stress Symptoms Scale (0-100 points)	1,362	17 (4; 43)	2 (0; 4)	18 (5; 36)	46 (43; 66)	-
Perceived stress scale (0-40 points)	608	12 (4; 23)	9 (2; 17)	11 (4; 20)	21 (13; 31)	<0.0001
Any stressful life events last three months (%)	1,362	30.3	14.0	29.2	65.4	<0.0001
≥ 2 Stressful life events last three months (%)	1,362	5.7	3.2	5.2	16.0	<0.0001
Age and anthropometrical variables						
Age (years)	1,362	19.0 (18.4; 22.2)	18.9 (18.4; 22.2)	19.0 (18.4; 22.1)	19.0 (18.4; 27.6)	0.19
Weight (kg)	1,352	74 (58; 94)	71 (60; 105)	74 (58; 93)	72 (60; 90)	0.27
Height (cm)	1,351	182 (172; 194)	183 (172; 194)	182 (172; 194)	182 (172; 194)	0.68
BMI (kg/m²)	1,342	22 (18; 28)	22 (19; 29)	22 (18; 28)	21 (18; 27)	0.26
Variables important for outcome assessment						
Time from ejaculation until motility analysis (min)	1,326	30 (10; 70)	28 (9; 72)	30 (11; 70)	30 (15; 75)	0.31
Time of day for blood sampling	1,361	10:44 (9:19; 12:16)	10:42 (9:28; 12:11)	10:45 (9:19; 12:18)	10:32 (9:21; 12:20)	0.44
Ejaculation abstinence (hours)	1,350	61 (37; 133)	65 (36; 160)	61 (37; 132)	61 (36; 112)	0.21
Data related to andrological health and semen quality						
Varicocele (grade 2 or 3) (%)	1,338	11.9	7.8	11.6	18.2	0.11
Congenital maldescended testis (%)	1,338	2.3	3.2	2.3	1.2	0.68
STD[b], epididymitis, or orchitis previously (%)	1,356	6.5	4.3	6.3	12.5	0.061
Testicular torsion (%)	1,362	1.3	0.0	1.2	4.9	0.008
Fever > 38 °C (>24h) within the last 3 months (%)	1,361	10.4	4.3	10.3	18.5	0.009
Exposure to mother´s smoking in utero (%)	1,349	12.9	6.5	13.5	13.8	0.27
Lifestyle						
Smoking cigarettes daily (%)	1,361	19.7	12.9	19.6	28.4	0.037
Smoking hash/pot/marihuana daily (%)	1,362	2.3	1.1	2.0	7.4	0.005
Alcohol > 14 units recent week (%)	1,356	22.4	21.7	22.7	19.8	0.81
Caffeine intake >300 mg/day (the previous week)[c] (%)	1,362	14.9	9.7	14.6	25.9	0.007
Used medicine[d] (%)	1,362	18.6	14.0	18.9	18.5	0.50
Good/very good self-rated physical fitness (%)	1,360	57.2	67.7	59.9	50.6	0.18

[a] Kruskal-Wallis test used for continuous variables and chi-squared test for categorical variables.

[b] Sexually transmitted diseases (chlamydia, gonorrhea, or syphilis)

[c] Calculated from reported intake of coffee, tea, cocoa, chocolate, energy drink, and cola.

[d] >3 consecutive days within the last three months

This article is protected by copyright. All rights reserved

Table 2: Semen parameters and serum reproductive hormones of study participants (N=1,362) stratified according to psychological stress assessed by the Stress Symptom S
Values are medians (5; 95 percentiles).

	Participants with data	Total population	Stress Symptom Scale (N=1,362) Lowest (<5th percentile) N=93 (6.8%)	Intermediate (5-95th percentile) N=1,188 (87.2%)	Highest (>95th percentile) N=81 (6.0%)
Semen parameters					
Semen volume (mL)	1,357	3.2 (1.3; 6.1)	3.0 (1.4; 7.4)	3.3 (1.3; 6.0)	3.2 (1.0; 6.2)
Sperm concentration (mill/mL)	1,357	41 (3; 44)	42 (2; 213)	41 (4; 144)	34 (1; 117)
Total sperm count (mill)	1,357	126 (7; 452)	118 (3; 574)	127 (11; 443)	105 (1; 437)
Motile spermatozoa (%)	1,348	69 (32; 88)	67 (38; 85)	69 (33; 88)	65 (22; 85)
Morphologically normal (%)	1,349	6.5 (0.5; 15.0)	5.5 (0.5; 14.3)	6.5 (0.5; 15.0)	6.8 (1.5; 16.5)
Reproductive hormones					
Inhibin B (pg/mL)	1,351	174 (87; 286)	179 (79; 291)	176 (87; 287)	153 (86; 250)
FSH (U/L)	1,353	2.7 (1.0; 6.8)	2.9 (1.0; 7.2)	2.7 (1.0; 6.6)	3.1 (1.2; 8.1)
Inhibin B/FSH	1,351	66 (16; 243)	68 (14; 244)	68 (16; 249)	53 (15; 182)
Testosterone (nmol/L)	1,357	21 (12; 33)	20 (12; 34)	21 (12; 33)	23 (14; 34)
LH (U/L)	1,353	3.4 (1.6; 6.6)	3.7 (1.6; 6.9)	3.3 (1.6; 6.6)	3.6 (1.6; 6.2)
Testosterone/LH	1,353	6.2 (2.9; 12.8)	5.3 (2.5; 12.5)	6.3 (2.9; 13.0)	6.5 (3.2; 12.5)

Author Manuscript

This article is protected by copyright. All rights reserved

Table 3: Beta-estimates and 95% CIs from linear regression analyses of the transformed outcomes (semen parameters and reproductive hormones) and psychological stress assessed by the Stress Symptom Scale amongst men with the highest stress levels (>95th percentile). Men with intermediate stress levels (5-95th percentile) served as reference population.

	Total population N=1,269 β (95% CI)	Men *without* reproductive disorders[1] or fever N=988 β (95% CI)
Semen volume (ml), cubic root		
Model 1	-0.01 (-0.06; 0.04)	-0.04 (-0.11; 0.03)
Model 2a	-0.01 (-0.06; 0.04)	-0.04 (-0.10; 0.03)
Model 3	-0.02 (-0.06; 0.04)	-0.04 (-0.11; 0.02)
Model 4	-0.02 (-0.06; 0.03)	-0.04 (-0.11; 0.03)
Sperm concentration (mill/ml), cubic root		
Model 1	-0.36 (-0.62; -0.11)*	-0.53 (-0.88; -0.19)*
Model 2a	-0.35 (-0.59; -0.10)*	-0.52 (-0.85; -0.18)*
Model 3	-0.35 (-0.60; -0.09)*	-0.52 (-0.86; -0.16)*
Model 4	-0.30 (-0.54; -0.04)*	-0.47 (-0.81; -0.11)*
Total sperm count (mill), cubic root		
Model 1	-0.54 (-0.93; -0.16)*	-0.84 (-1.36; -0.32)*
Model 2a	-0.51 (-0.88; -0.14)*	-0.80 (-1.30; -0.31)*
Model 3	-0.52 (-0.90; -0.14)*	-0.81 (-1.33; -0.29)*
Model 4	-0.45 (-0.82; -0.07)*	-0.73 (-1.24; -0.22)*
Motility (%), natural logarithm		
Model 1	-0.20 (-0.39; -0.01)*	-0.35 (-0.55; -0.10)*
Model 2a	-0.20 (-0.39; -0.01)*	-0.33 (-0.58; -0.10)*
Model 3	-0.21 (-0.40; -0.02)*	-0.34 (-0.60; -0.10)*
Model 4	-0.17 (-0.36; 0.02)	-0.30 (-0.55; -0.04)*
Morphology (%), natural logarithm		
Model 1	0.60 (-0.44; 1.63)	-0.15 (-1.56; 1.26)
Model 2a	0.48 (-0.56; 1.52)	-0.18 (-1.59; 1.24)
Model 3	0.61 (-0.43; 1.65)	-0.17 (-1.59; 1.25)
Model 4	0.66 (-0.41; 1.72)	-0.05 (-1.50; 1.39)
Inhibin B (pg/ml), natural logarithm		
Model 1	-0.11 (-0.20; -0.03)*	-0.17 (-0.29; -0.05)*
Model 2b	-0.11 (-0.20; -0.03)*	-0.17 (-0.29; -0.05)*
Model 3	-0.12 (-0.20; -0.03)*	-0.16 (-0.28; -0.04)*
Model 4	-0.13 (-0.22; -0.05)*	-0.19 (-0.31; -0.07)*
FSH (iu/l), natural logarithm		
Model 1	0.10 (-0.03; 0.23)	0.15 (-0.02; 0.33)
Model 2b	0.10 (-0.03; 0.23)	0.14 (-0.04; 0.31)
Model 3	0.10 (-0.04; 0.23)	0.15 (-0.04; 0.31)
Model 4	0.11 (-0.03; 0.23)	0.18 (0.002; 0.35)*
Inhibin B/FSH, natural logarithm		
Model 1	-0.22 (-0.41; -0.02)*	-0.32 (-0.58; -0.06)*
Model 2b	-0.21 (-0.41; -0.02)*	-0.31 (-0.57; -0.05)*
Model 3	-0.21 (-0.40; -0.02)*	-0.30 (-0.55; -0.32)*
Model 4	-0.25 (-0.41; -0.02)*	-0.36 (-0.62; -0.10)*
Testosterone (nmol/l), natural logarithm		
Model 1	0.07 (-0.001; 0.133)	0.03 (-0.06; 0.12)
Model 2b	0.07 (-0.001; 0.13)	0.03 (-0.06; 0.12)
Model 3	0.06 (-0.007; 0.13)	0.03 (-0.06; 0.12)
Model 4	0.06 (-0.02; 0.12)	0.02 (-0.07; 0.11)
LH (iu/l), natural logarithm		
Model 1	0.03 (-0.07; 0.13)	0.04 (-0.17; 0.10)
Model 2b	0.03 (-0.07; 0.13)	-0.03 (-0.17; 0.10)
Model 3	0.03 (-0.07; 0.13)	-0.03 (-0.16; 0.11)
Model 4	0.04 (-0.06; 0.14)	-0.01 (-0.15; 0.12)
Testosterone/LH, natural logarithm		
Model 1	0.04 (-0.07; 0.14)	0.07 (-0.07; 0.21)
Model 2b	0.01 (-0.09; 0.11)	0.05 (-0.09; 0.17)
Model 3	-0.003 (-0.11; 0.10)	0.04 (-0.11; 0.18)
Model 4	-0.02 (-0.12; 0.01)	0.02 (-0.12; 0.16)

[1] Varicocele, cryptorchidism, sexually trasmitted diseases, epididymitis, and testicular torsion.

* $p < 0.05$

Model 1: Unadjusted.

Model 2a: Model 1 further adjusted for ejaculation abstinence period and for motility also duration from ejaculation to assessment.

Model 2b: Model 1 further adjusted for hour of day of blood sampling and year of examination.

Model 3: Model 2a/2b furhter adjusted for lifestyle (caffeine, cigarette smoking, use of marihuana, alcohol).

Model 4: Model 3 additionally adjusted for BMI.

Author Manuscript

This article is protected by copyright. All rights reserved

Author Manuscript

This article is protected by copyright. All rights reserved

Table 4: Semen parameters and levels of reproductive hormones of study participants (N=608) stratified according to stress assessed by the PSS score. Values are medians (5; 95 percentiles).

			PSS score			
	Participants with data	Total population with PSS data	0-10 points N=177	11-20 points N=159	21-30 points N=141	>30 points N=131
Semen parameters						
Semen volume (ml)	603	3.2 (1.3; 6.1)	3.2 (1.2; 6.3)	3.4 (1.5; 6.1)	3.2 (1.5; 5.8)	3.0 (1.1; 5.4)
Sperm concentration (mill/ml)	603	39 (2; 147)	42 (5; 171)	37 (3; 114)	40 (1; 175)	32 (1; 127)
Total sperm count (mill)	603	113 (7; 144)	116 (15; 552)	113 (7; 443)	129 (4; 458)	101 (2; 368)
Motile spermatozoa (%)	599	71 (32; 88)	72 (44; 89)	73 (37; 88)	70 (24; 89)	68 (22; 87)
Morphologically normal (%)	600	6.0 (0.5; 14)	6.0 (1.0; 14.3)	6.5 (0.5; 14.5)	6.5 (0.5; 14.0)	5.5 (0.3; 14.0)
Reproductive hormones						
Inhibin B (pg/ml)	605	182 (101; 302)	188 (96; 306)	184 (92; 315)	176 (104; 279)	180 (97; 277)
FSH (iu/l)	605	2.8 (1.0; 6.6)	2.5 (0.9; 6.8)	2.8 (1.0; 6.4)	2.9 (1.2; 5.8)	3.0 (1.0; 8.2)
Inhibin B/FSH	605	66 (17; 253)	75 (15; 297)	66 (18; 276)	60 (23; 191)	64 (14; 232)
Testosterone (nmol/l)	608	23 (14; 35)	22 (13; 34)	23 (13; 35)	22 (14; 34)	24 (14; 37)
LH (iu/l)	605	3.3 (1.6; 6.4)	3.2 (1.6; 6.6)	3.5 (1.6; 6.5)	3.2 (1.4; 6.6)	3.3 (1.6; 6.0)
Testosterone/LH	605	6.9 (3.1; 13.8)	7.1 (3.0; 13.9)	7.0 (2.8; 14.5)	7.2 (2.9; 13.3)	6.5 (3.8; 14.5)

Author Manuscript

This article is protected by copyright. All rights reserved

Table 5: Beta-estimates and 95% CIs from regression analyses of the transformed outcomes (semen parameters and reproductive hormones) for linear trends between preceived stress scores and the outcomes.

	Total population N=608 β (95% CI)	Men *without* reproductive disorders[1] or fever N=429 β (95% CI)
Semen volume (ml), cubic root		
Model 1	-0.001 (-0.004; 0.002)	-0.002 (-0.006; 0.002)
Model 2a	-0.001 (-0.004; 0.002)	-0.001 (-0.005; 0.002)
Model 3	-0.001 (-0.003; 0.003)	-0.002 (-0.005; 0.002)
Model 4	0.000 (-0.003; 0.003)	-0.001 (-0.004; 0.003)
Sperm concentration (mill/ml), cubic root		
Model 1	-0.020 (-0.036; -0.004)*	-0.021 (0.041; 0.002)*
Model 2a	-0.019 (-0.035; -0.003)*	-0.019 (-0.038; 0.0001)
Model 3	-0.018 (-0.034; -0.002)*	-0.021 (-0.040; -0.001)*
Model 4	-0.016 (-0.030; 0.000)*	-0.018 (-0.038; 0.002)
Total sperm count (mill), cubic root		
Model 1	-0.034 (-0.058; -0.010)*	-0.041 (-0.07; -0.012)*
Model 2a	-0.032 (-0.055; -0.009)*	-0.034 (-0.062; -0.006)*
Model 3	-0.030 (-0.054; -0.005)*	-0.037 (-0.067; -0.008)*
Model 4	-0.025 (-0.049; -0.002)*	-0.030 (-0.058; -0.0003)*
Motility (%), natural logarithm		
Model 1	-0.015 (-0.026; -0.003)*	-0.013 (-0.026; 0.001)
Model 2a	-0.014 (-0.026; -0.002)*	-0.010 (-0.023; 0.004)
Model 3	-0.016 (-0.027; -0.004)*	-0.015 (-0.028; -0.001)*
Model 4	-0.013 (-0.025; -0.001)*	-0.010 (-0.024; 0.003)
Morphology (%), natural logarithm		
Model 1	-0.02 (-0.08; 0.04)	-0.04 (-0.11; 0.03)
Model 2a	-0.02 (-0.08; 0.04)	-0.04 (-0.11; 0.03)
Model 3	-0.03 (-0.08; 0.03)	-0.05 (-0.13; 0.01)
Model 4	-0.02 (-0.08; 0.04)	-0.05 (-0.12; 0.02)
Inhibin B (pg/ml), natural logarithm		
Model 1	-0.002 (-0.007; 0.003)	-0.003 (-0.009; 0.004)
Model 2b	-0.002 (-0.007; 0.003)	-0.003 (-0.009; 0.003)
Model 3	-0.002 (-0.007; 0.003)	-0.002 (-0.008; 0.005)
Model 4	-0.002 (-0.007; 0.003)	-0.002 (-0.008; 0.005)
FSH (iu/l), natural logarithm		
Model 1	0.009 (0.001; 0.02)*	0.010 (0.0003; 0.02)*
Model 2b	0.009 (0.001; 0.02)*	0.010 (0.001; 0.02)*
Model 3	0.008 (0.001; 0.02)*	0.010 (-0.001; 0.019)
Model 4	0.009 (0.001 0.017)*	0.011 (0.000; 0.020)*
Inhibin B/FSH, natural logarithm		
Model 1	-0.01 (0.02; 0.001)	-0.01 (-0.03; 0.002)
Model 2b	-0.01 (-0.02; 0.001)	-0.01 (-0.02; 0.001)
Model 3	-0.010 (-0.021; 0.002)	-0.010 (-0.024; 0.004)
Model 4	-0.011 (-0.022; 0.001)	-0.01 (-0.03; 0.003)
Testosterone (nmol/l), natural logarithm		
Model 1	0.004 (-0.00011; 0.008)	0.003 (-0.002; 0.007)
Model 2b	0.004 (0.0001; 0.008)*	0.003 (-0.002; 0.007)
Model 3	0.003 (-0.001; 0.007)	0.002 (-0.003; 0.007)
Model 4	0.003 (-0.0004; 0.007)	0.003 (-0.002; 0.007)
LH (iu/l), natural logarithm		
Model 1	0.003 (-0.003; 0.009)	0.001 (-0.007; 0.008)
Model 2b	0.003 (-0.003; 0.009)	0.0004 (-0.0007; 0.008)
Model 3	0.003 (-0.003; 0.009)	0.001 (-0.006; 0.009)
Model 4	0.003 (-0.003; 0.009)	0.001 (-0.007; 0.009)
Testosterone/LH, natural logarithm		
Model 1	0.001 (-0.006; 0.007)	0.002 (-0.007; 0.0010)
Model 2b	-0.0003 (-0.007; 0.006)	0.001 (-0.007; 0.009)
Model 3	-0.001 (-0.007; 0.006)	0.0002 (-0.008; 0.009)
Model 4	-0.0005 (-0.007; 0.006)	0.001 (-0.008; 0.009)

[1] Varicocele, cryptorchidism, sexually trasmitted diseases, epididymitis, and testicular torsion.

* $p < 0.05$

Model 1: Unadjusted.

Model 2a: Model 1 further adjusted for ejaculation abstinence period.

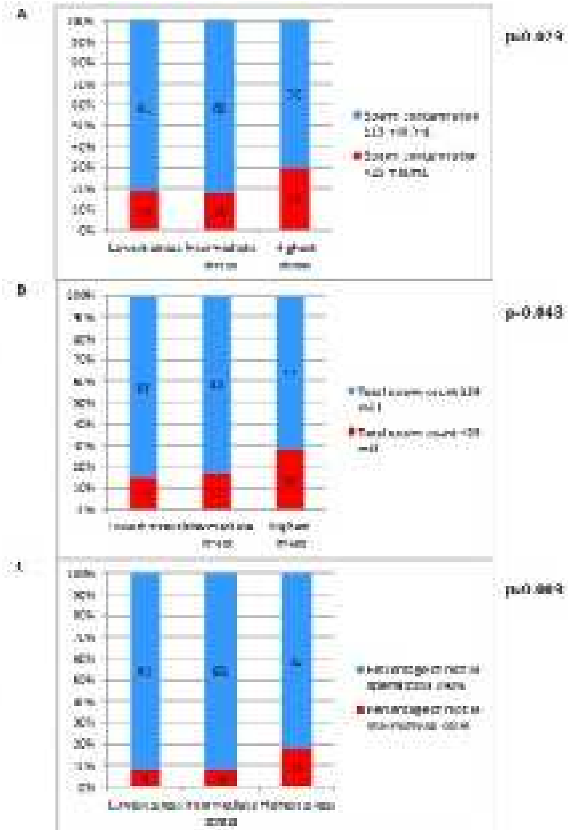
Model 2b: Model 1 further adjusted for hour of day of blood sampling.

Model 3: Model 2a/2b furhter adjusted for lifestyle (caffeine, cigarette smoking, use of marihuana, alcohol).

Model 4: Model 3 additionally adjusted for BMI.

Author Manuscript

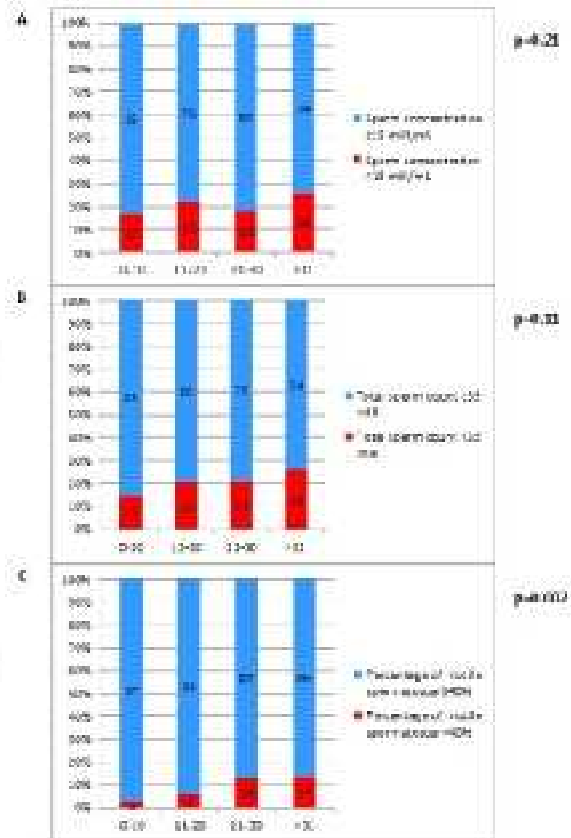
Author Manuscript



andr_12835_f1.jpg

This article is protected by copyright. All rights reserved

Author Manuscript



andr_12835_f2.jpg

This article is protected by copyright. All rights reserved