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Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men.

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Abstract

Background A high body mass index (BMI) has been associated with reduced semen quality and male subfecundity, but no studies following obese men losing weight have yet been published. We examined semen quality and reproductive hormones among morbidly obese men and studied if weight loss improved the reproductive indicators.

Methods In this pilot cohort study, 43 men with BMI > 33 kg/m² were followed through a 14 week residential weight loss program. The participants provided semen samples and had blood samples drawn, filled in questionnaires, and had clinical examinations before and after the intervention. Conventional semen characteristics as well as sperm DNA integrity, analysed by the sperm chromatin structure assay (SCSA) were obtained. Serum levels of testosterone, estradiol, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), anti-Müllerian hormone (AMH) and inhibin B (Inh-B) were measured.

Results Participants were from 20 to 59 years of age (median = 32) with BMI ranging from 33 to 61 kg/m². At baseline, after adjustment for potential confounders, BMI was inversely associated with sperm concentration ($p = 0.02$), total sperm count ($p = 0.02$), sperm morphology ($p = 0.04$), and motile sperm ($p = 0.005$) as well as testosterone ($p = 0.04$) and Inh-B ($p = 0.04$) and positively associated to estradiol ($p < 0.005$). The median (range) percentage weight loss after the intervention was 15% (3.5 – 25.4). Weight loss was associated with an increase in total sperm count ($p = 0.02$), semen volume ($p = 0.04$), testosterone ($p = 0.02$), SHBG ($p = 0.03$) and AMH ($p = 0.02$). The group with the largest weight loss had a statistically significant increase in total sperm count [193 millions (95% CI: 45; 341)] and normal sperm morphology [4% (95% CI: 1; 7)].

Conclusion This study found obesity to be associated with poor semen quality and altered reproductive hormonal profile. Weight loss may potentially lead to improvement in semen quality. Whether the improvement is a result of the reduction in body weight per se or improved lifestyles remains unknown.

Introduction

The prevalence of overweight and obese individuals is increasing globally [1] and concern is rising over the reproductive consequences of male obesity. Male obesity has been linked to subfecundity [2-4] and a dose-response relationship between increasing BMI and subfecundity has been proposed [3]. Furthermore, male obesity has been associated with abnormal semen characteristics [5-14], although results are conflicting [15-21]. The hormonal abnormality [22-24] associated with obesity is likely to play a major role, and although controversial [25-27], previous studies have shown that the endocrine abnormalities may be reversed by weight reduction [28-33].

Several studies have focused on inhibin B (Inh-B) [34-37], and more recently also anti-Müllerian hormone (AMH), both produced almost exclusively by the Sertoli cells, as markers of spermatogenesis [38-40]. Studies have shown Inh-B to be positively associated with fecundability [41], and obesity has been shown to be associated with a decreased level of Inh-B [5,16]. However, results are conflicting [42,43], and studies on the association between obesity and AMH are lacking.

It is unclear to what extent obesity affects a man's reproductive potential. The existing studies on this subject are all cross-sectional, a limited design for deriving causal inferences. There may be a causal link between male obesity and poor semen quality, however, they may also share a common aetiological factor. Longitudinal studies investigating how weight loss affects semen quality are needed to disentangle these two hypotheses, but no such studies have yet been published. In this paper, we present results from a pilot cohort study with prospectively collected data, investigating how

obesity and weight loss affect reproductive hormones including AMH and Inh-B, conventional semen characteristics as well as sperm DNA integrity.

Methods

Study population and data collection

Data collection took place from April 2006 to April 2009. Men who participated in a residential weight loss program in Ebeltoft, Denmark were recruited to this pilot cohort study. During the data collection period, men over the age of 18, independent of their weight, were invited to participate and a total of 107 men were invited. Forty-four men (41%) accepted the invitation. Out of the 44 participants, 27 men (61%) took part in the follow-up at the end of the weight loss program. We excluded one man diagnosed with Klinefelter's syndrome, and in the analyses of semen characteristics, two men with azoospermia were excluded because azoospermia probably is not caused by obesity alone.

The weight loss program, based on a healthy diet and daily exercise, lasted approximately 14 weeks. Before and after the weight loss program, the participants had blood samples drawn, provided semen samples and had clinical examinations. The clinical examination was performed on site by one investigator and included height- and weight measurements. Blood samples were drawn by a trained technician between 6:45 a.m. and 8:20 a.m. at baseline and between 7:00 a.m. and 10:30 a.m. after the intervention. The blood samples were transported to the hospital laboratory on dry ice, centrifuged and stored at -80°C until analysed. The participants were asked to provide the semen sample by masturbating into a plastic container after at least 48 hours of sexual abstinence. They were instructed to keep the container close to the

body, during transportation to the mobile laboratory on the weight loss centre to avoid cooling, and one trained medical laboratory technician performed all initial semen analyses within one hour after collection. Furthermore, before and after the weight loss program, the participants completed questionnaires on their reproductive experience, medical (e.g. history of diseases in the reproductive organs) and lifestyle factors (e.g. smoking status and alcohol consumption) as well as time and date of the preceding ejaculation, and spillage (if any) during semen sample collection. Finally, testis volume was measured by ultrasound of the testes at baseline by a trained person under the supervision of a medical doctor.

The men received no incentives, and participation was conditional on written informed consent. The regional ethics committee approved the study (reg. number 20060039).

Analyses of serum samples

Serum samples for testosterone, estradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were analysed at the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark by Avida Centaur (Bayer Healthcare, Leverkusen, Germany). The sex hormone-binding globulin (SHBG) concentrations were determined using IMMULITE (DPC, Koege, Denmark). Serum concentrations of AMH were measured at the Laboratory of Reproductive Biology, University Hospital of Copenhagen, University of Copenhagen, Denmark using specific ELISA kits according to the manufacturer's instructions (DSL-10-14400; Diagnostic System Laboratories Inc., Webster, TX, USA). Detection limit was 0.05 ng/ml and inter- and intra-assay variations were <10%. Serum concentrations of Inh-B were measured at the Laboratory of Reproductive Biology, University Hospital of Copenhagen,

Denmark using a specific ELISA-kit according manufactures instructions (The Oxford Bio-innovation kit; Biotech-IgG, Copenhagen, Denmark).

Analyses of semen samples

Semen volume was estimated by weight (1 g = 1 mL). Sperm concentration and sperm motility were assessed as described in 'WHO Laboratory Manual for the Examination of Human Semen-Cervical Mucus Interaction' (World Health Organization, 1999). Analysis of 96% of the samples was initiated within one hour after ejaculation, and within this time it has been shown that the sperm motility is stable [44]. Sperm morphology was assessed using the Tygerberg strict criteria [45]. The laboratory took part in the European Society for Human Reproduction and Embryology external quality control (EQC) program and control tests were in accordance with results obtained by expert examiners within the EQC program.

Sperm chromatin structure assay (SCSA)

After semen analysis, 100 µL of the raw semen sample was frozen at -80°C for later analysis of sperm DNA integrity. Sperm DNA integrity was analysed by the flow cytometric-based sperm chromatin structure assay (SCSA) at the Reproductive Medicine Centre, Skanes University Hospital, Malmö, Sweden. The details of this analysis have previously been described in detail [46,47]. In brief, the SCSA is based on the fact that damaged chromatin denatures when exposed to an acid-detergent, whereas normal double-stranded chromatin remains stable. After blue-light excitation, the SCSA measures the denaturation of sperm DNA with the dye acridine orange, which differentially stains double- and single-stranded nucleic acids. Five thousand cells were analysed by FACSort (Becton Dickinson, San Jose, CA, USA). Analysis of

the flow cytometric data was carried out using dedicated software (SCSA Soft; SCSA Diagnostics, Brookings, SD, USA.). The percentage of abnormal sperm with detectable DFI (%DFI) was calculated from the DFI frequency histogram. For the flow cytometer set-up and calibration, a reference sample was used from a normal donor ejaculate sample retrieved from the laboratory repository. The intra-laboratory coefficient of variation for DFI analysis was found to be 4.5%. One investigator blinded to the exposure and other co-variates performed the analyses.

Statistical analyses

In the cross-sectional study, three groups were formed according to BMI at baseline (1: 33.3 to 41.6 kg/m², 2: 41.7 to 46.08 kg/m² and 3: 46.1 to 60.9 kg/m²). In the longitudinal study, we calculated the percentage weight loss and formed three groups according to percentage weight loss (I: 3.5 to 12.1%, II: 12.2 to 17.1% and III: 17.2 to 25.4%).

Outcome variables included reproductive hormones (testosterone, estradiol, FSH, LH, SHBG, AMH and Inh-B as well as the calculated free androgen index (FAI), the free testosterone/free estradiol ratio and LH/free testosterone ratio), conventional semen characteristics (semen volume, sperm concentration, total sperm count, sperm motility and sperm morphology) and DFI. In the longitudinal study, the outcome variables included the differences in the parameters mentioned above.

For each of the outcome variables, crude median, 25th, and 75th percentiles were calculated. We performed multiple linear regression analyses with BMI and percentage weight loss as the main determinants. Low BMI/percentage weight loss

was considered the reference category. When we tested for trend, BMI and percentage weight loss was entered as a continuous explanatory variable.

In the cross-sectional study, data on the semen characteristics, as well as LH, FSH, AMH, Inh-B, the free testosterone/free estradiol ratio, LH/free testosterone ratio and testis volume were transformed logarithmically to obtain an approximate linear distribution of residuals, whereas no transformations were used on data in the longitudinal study. In the longitudinal study, differences in semen characteristics and reproductive hormones from baseline to follow-up were calculated by subtracting the second sample value from the first sample value, thus a positive difference corresponds to a rise in the characteristics from baseline to follow-up.

A priori, we decided which covariates that potentially should be included in the models, and due to the sample size, we based the selection on a 5% change-in-estimate principle [48]. In the cross-sectional study, the following potential confounders were considered for the regression analyses (see table 1): smoking (yes or no), history of diseases in reproductive organs (cryptorchidism, testicular cancer, surgery in urogenital organs, orchitis and chlamydia infection combined into one variable, present, not present or unknown), season of blood- or semen sampling (April to September or October to March) and age at blood- or semen sampling (continuous). For the analyses on semen characteristics, we also considered the period of abstinence time (< 48 hours, 2 – 5 days or > 5 days), spillage at semen sampling (yes or no) and for analysis of motility also minutes from ejaculation to analysis (continuous). Furthermore, for the regression analyses of reproductive hormones we also considered recent fever.

In the longitudinal study, the following potential confounders were considered (see table 2): differences in smoking status (no difference, smoker at the first sample, but not at the second sample or smoker at the second sample, but not at the first sample) and difference in season (no difference in season, September - April at the first sample and March - October at the second sample or March - October at the first sample and September - April at the second sample). In the semen analyses, the differences in spillage (no difference, spillage at the first sample and not at the second sample or spillage at the second sample and not at the first sample) and the differences in abstinence time (days) were additionally considered, and for analysis of motility, the differences in minutes from sampling to analysis. In the statistical analyses on semen volume and total sperm count, the men reporting spillage were excluded from the analyses.

We performed sub-analyses to check consistency of our results, using differences in BMI as the explanatory variable instead of weight loss in percent. Finally, due to the low number of participants in the analyses of semen volume and total sperm count after exclusion of participants with spillage, we performed two sub-analyses with all participants included and adjusted for spillage instead. In one model, we adjusted for the covariates by using the difference (e.g. difference in spillage) from baseline to follow up, as described above. Additionally, we fitted a model with total sperm count at follow-up as a function of the weight loss, controlling for total sperm count at baseline as well as the other covariates (spillage, abstinence time and season).

The statistical analyses were performed by using Stata 11 software (Stata Corporation, Cillege Station, TX). A two-tailed *P* value of < 0.05 was considered statistically significant.

Results

The median (range) age was 32 (20– 59) years. The median (range) BMI was 44 (33 – 61) kg/m². In table 1, the semen characteristics and reproductive hormone levels at baseline according to BMI are presented. After adjustment for potential confounders, BMI was inversely associated with sperm concentration, total sperm count, normal sperm morphology, and motile sperm. The group with the highest BMI had a 71% (95% CI: -6; 92) lower sperm concentration and 68% (95% CI: 14; 88) lower total sperm count than the group with the lowest BMI. For semen volume and DFI, no statistically significant trends were observed, however, the median DFI tended to increase with higher levels of BMI. Furthermore, BMI was negatively associated with testosterone and Inh-B and positively associated with estradiol at baseline. The calculated FAI and free testosterone/free estradiol ratio were lower at higher levels of BMI. The data indicated a higher level of SHBG with higher levels of BMI, although not statistically significant. There was no difference in testis volume in the groups (Table 1).

Following the weight loss program, the median (range) weight loss was 22 (4; 39) kg, corresponding to a median percentage weight loss on 15%, ranging from 3.5% to 25.4%. In table 2, the adjusted mean (95% CI) differences in semen characteristics and reproductive hormone levels according to weight loss in percent are presented. After adjustment, the percentage weight loss was positively associated with an increase in total sperm count and semen volume. The group with the largest weight loss had a statistically significant increase in both total sperm count [193 millions (95% CI: 45; 341)] and morphology [4% (95% CI: 1; 7)]. We observed no difference in DFI from baseline to follow-up. When using the differences in BMI instead of

percentage weight difference as the explanatory variable, the direction and magnitude of the associations were essentially unchanged. Additionally, the percentage weight loss was associated with an increase in testosterone, SHBG and AMH, and FAI and the free testosterone/free estradiol ratio tended to increase with increasing weight loss in percent.

Finally, the results from the sub-analyses with semen volume and total sperm count with all participants were in the same direction, however, attenuated as expected, and p-values were no longer below 0.05.

Discussion

The study showed that a high BMI at baseline was associated with low values of total sperm count, sperm concentration, normal sperm morphology, and motile sperm. Weight loss was associated with an increase in total sperm count and semen volume among men who participated in a 14-week weight loss program. Additionally, the weight loss was associated with an increase in testosterone, SHBG and AMH, and FAI improved significantly in the group with the largest weight reduction. Weight loss did not decrease serum estradiol levels.

As far as we know, this is the first cohort study investigating the association between weight loss and semen quality. Thus the results are unchallenged and further research is necessary to disclose the matter further. Our results indicate that there is a causal inverse association between BMI and semen quality, and that it may be possible to improve semen quality by a weight reduction. However, we cannot exclude that changes in lifestyle, diet or exercise caused the observed improvement in semen quality, rather than the reduction in weight per se.

Despite conflicting results [15-21], previous studies (all cross-sectional) have mainly shown low sperm concentration among overweight and obese men [5,8,9,11,12,49], similar to what we find. Considering the well-established association between male obesity and altered reproductive hormonal profile, and the fact that testosterone is required in large concentrations to maintain spermatogenesis, it is reasonable to consider obesity to also affect semen quality. Thus we believe that the inverse association between BMI and semen quality is not a chance finding.

The hormonal profile among obese men evaluated in this study was characterized by abnormalities in the sex hormones, and weight loss improved some of the hormone levels, however, they were not normalized. It should be noted that the men were severely obese at baseline and remained overweight or obese after the weight loss program. This could explain why we did not observe a larger improvement in the hormonal parameters. The previous published studies, reporting improvement or normalization of the reproductive hormones, were on less obese men than in this present study.

Inh-B and AMH are produced almost exclusively by the Sertoli cells and have been proposed as markers of spermatogenesis. Inh-B have been found to be significantly lower in men with testicular dysfunction [34-36] and AMH to be significantly lower in subfertile men [38-40]. Therefore, we expected both hormones to be negatively associated with BMI, but this was only seen for Inh-B, as previously reported [16]. In this present study we compared severely obese men, all with BMI above 30 kg/m² when entering the study and the AMH levels among these men might be lower than normal weight men, which could explain why we see no difference when comparing the two groups with the most obese men with the least obese men. Tüttelmann *et al.*

[43] showed that, among men with a median BMI of 25.7 kg/m², the median (range) concentration of AMH was 6.3 ng/mL (1.8; 26.8), higher than among the men in our study where the median (range) AMH concentration was 3.3 ng/mL (0.2; 10.7). Furthermore, we hypothesized that Inh-B and AMH would improve by weight loss but only AMH increased significantly.

The major strength of this study is the successful weight loss program, providing prospectively collected data, which adds new important information to the existing cross-sectional studies. The risk of misclassification of the outcome variables is limited and most likely non-differential, since analyses of semen and blood samples were performed blinded to the exposure variables. Misclassification of the exposure variables is unlikely since anthropometric measurements were obtained on-site by one investigator and do not depend on self-reports. From the questionnaires, data were available on the main factors that we think affect semen quality, such as abstinence time and diseases of the reproductive organs. However, confounding from other unknown factors is possible and our findings may also be due to chance, since the sample size is small.

The major limitation in this study is the limited sample size, resulting in wide confidence intervals, and the results must therefore be interpreted with caution. The participation rate (41%) is low, and leaves open the possibility of selection among participants. However, to cause bias away from the null, selection has to be related to both semen quality and BMI, and the participation rate of men with poor semen quality and high BMI must be higher. We have no reason to suspect participation to be associated with the exposure and the risk of differential participation and selection bias is limited, although it is possible as a chance phenomenon. Furthermore, loss to

follow-up leaves room for selection bias, if attrition is dependent on the change in semen quality as well as related to the weight loss. Therefore, we examined if those who dropped out of the study systematically differed from those who remained in the sample. The two groups were found to have similar weight, BMI and reproductive hormones at baseline. Sperm concentration and total sperm count were lower among loss to follow-up men than among those who remained and the direction of this selection bias could be both away and toward the null.

Finally, the follow-up period was on average 103 days (ranging from 86 to 111 days), and spermatogenesis takes approximately 64 days [50]. Thus the follow-up period in the present study should be able to detect changes on the early stages of spermatogenesis, although a longer follow-up period would be desirable.

Thirty-four percent of the men had sperm concentrations below the World Health Organization (2010) referent level of 15 million/ml when entering the study. The median (p25, 75) sperm concentration of all participants at baseline was 25 (12, 64) million/ml and 19 (8, 33) million/ml among the most obese men. Since fecundity increases with sperm concentrations up to approximately 40 million/mL [51], some may have problems fathering a child.

Conclusions

To conclude on this pilot cohort study, we observed that the altered androgen profile tended to improve following weight loss and that weight loss may potentially lead to improvement in semen quality, although we can not conclude this to be a result of the reduction in body weight per se. The observation has biologic plausibility, but the

findings should be replicated in a larger cohort with longer follow-up time including a wider range of BMI levels.

Competing interests

None declared.

Authors' contributions

LBH contributed to analysis and interpretation and drafted the manuscript. AMT contributed to study design, acquisition of data, analysis and interpretation of data. ASA contributed to study design, acquisition of data and interpretation of data. JO contributed to analysis and interpretation of data. JPB contributed to study design and analysis and interpretation of data. CYA contributed to acquisition of data and interpretation of data. MB contributed to acquisition of data and interpretation of the data. EHE contributed to acquisition of data and interpretation of data. MLH contributed to analysis and interpretation of data. EE contributed to study design, acquisition of data and interpretation of data. CHRH contributed to study design, acquisition of data, analysis and interpretation of data. All authors read and approved the final manuscript.

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Table 1. Semen characteristics and reproductive hormone levels at baseline according to body mass index (BMI)

Parameter	Body mass index (BMI), kg/m ²			Test for trend*
	33.3 – 41.6 (n = 14) [#]	41.7 – 46.08 (n = 14) [#]	46.1 – 60.9 (n = 15) [#]	P-value
Sperm concentration (millions/ml)				
Median (p25, 75)	54 (25, 102)	24 (4, 55)	19 (8, 33)	0.03
Adjusted back-transformed median (95% CI) ^{a, b, d}	18 (3, 111)	4 (1, 28)	5 (1, 39)	0.02
Semen volume (ml)				
Median (p25, 75)	2.9 (2.2, 4.0)	3.5 (2.2, 5.8)	3.3 (2.4, 4.0)	0.92
Adjusted back-transformed median (95% CI) ^{a, b, c, d, e}	1.7 (0.8, 3.5)	2.6 (1.3, 5.4)	1.7 (0.7, 4.1)	0.74
Total sperm count (millions)				
Median (p25, 75)	209 (62, 230)	93 (11, 204)	46 (22, 76)	0.03
Adjusted back-transformed median (95% CI) ^{a, e}	70 (32, 156)	31 (11, 90)	23 (9, 56)	0.02
Normal sperm morphology (%)				
Median (p25, 75)	9 (6, 11)	5 (2, 13)	5 (1, 9)	0.28
Adjusted back-transformed median (95% CI) ^{a, c, d, e}	10 (0, 244)	7 (0, 103)	2 (0, 61)	0.04
Motile sperm (%)				
Median (p25, 75)	73 (64, 77)	57 (43, 71)	55 (40, 67)	0.06
Adjusted back-transformed median (95% CI) ^h	59 (21, 163)	46 (16, 132)	19 (7, 51)	0.005
DNA fragmentation index, DFI (%)				
Median (p25, 75)	10 (7, 18)	16 (12, 32)	18 (12, 23)	0.23
Adjusted back-transformed median (95% CI) ^{a, b, d, e, f}	9 (4, 19)	12 (6, 25)	10 (4, 24)	0.70
Testosterone (nmol/L)				
Median (p25, 75)	9.2 (7.8, 11.4)	8.0 (6.4, 11.0)	7.0 (6.0, 8.0)	0.005
Adjusted mean (95% CI) ^{b, d, e, g}	8.7 (5.3, 12.2)	9.1 (6.0, 12.2)	6.3 (2.6, 10.1)	0.04
Estradiol (nmol/L)				
Median (p25, 75)	0.10 (0.09, 0.15)	0.15 (0.14, 0.17)	0.19 (0.16, 0.23)	<0.005
Adjusted mean (95% CI) ^{b, d, e, g}	0.11 (0.07, 0.16)	0.13 (0.09, 0.17)	0.18 (0.13, 0.23)	<0.005
SHBG (nmol/L)				
Median (p25, 75)	18.0 (12.4, 22.7)	17.4 (14.7, 25.0)	22.8 (15.2, 27.5)	0.62
Adjusted mean (95% CI) ^{b, d, e, g}	20.5 (13.0, 27.9)	21.5 (14.9, 28.1)	24.2 (16.1, 32.3)	0.07
FSH (IU/L)				
Median (p25, 75)	2.8 (2.6, 3.7)	4.5 (2.2, 5.9)	3.2 (2.2, 3.4)	0.36
Adjusted back-transformed median (95% CI) ^{b, d, e, g}	2.8 (1.7, 4.6)	3.9 (2.5, 6.2)	2.2 (1.3, 3.9)	0.30
LH (IU/L)				
Median (p25, 75)	3.6 (2.9, 4.6)	4.9 (3.7, 6.8)	3.9 (2.8, 5.2)	0.86
Adjusted back-transformed median (95% CI) ^{b, d, e, g}	3.1 (2.0, 4.8)	4.7 (3.1, 7.0)	2.9 (1.8, 4.8)	0.60
Inhibin B (pg/ml)				
Median (p25, 75)	160 (141, 220)	123 (117, 170)	120 (86, 171)	0.004
Adjusted back-transformed median (95% CI) ^{d, e}	156 (94, 257)	128 (84, 195)	110 (64, 188)	0.04
AMH (ng/ml)				
Median (p25, 75)	3.6 (3.1, 4.3)	2.9 (1.8, 4.0)	3.3 (2.2, 4.9)	0.60
Adjusted back-transformed median (95% CI) ^{b, d, e, g}	2.8 (1.7, 4.7)	2.3 (1.5, 3.7)	2.5 (1.4, 4.3)	0.68
Free androgen index (FAI)				
Median (p25, 75)	59.1 (43.2, 75.8)	45.3 (38.9, 62.8)	33.4 (28.7, 44.0)	0.008
Adjusted back-transformed median (95% CI) ^{b, d, e, g}	55.0 (36.3, 73.6)	46.3 (29.7, 62.8)	28.5 (8.3, 48.7)	<0.005
Free testosterone/free estradiol ratio				
Median (p25, 75)	95.2 (76.8, 108.4)	56.2 (45.8, 82.8)	35.6 (32.0, 56.1)	<0.005
Adjusted median (95% CI) ^{b, d, g}	69.4 (45.7, 105.2)	59.5 (40.0, 88.3)	32.5 (20.8, 51.0)	<0.005
LH/free testosterone ratio				
Median (p25, 75)	0.07 (0.06, 0.09)	0.10 (0.08, 0.11)	0.10 (0.08, 0.17)	0.005
Adjusted back-transformed median (95% CI) ^{b, d, e, g}	0.07 (0.04, 0.10)	0.11 (0.07, 0.17)	0.11 (0.06, 0.18)	0.009
Testis volume (ml)				
Median (p25, 75)	13.5 (11.0, 14.0)	10.0 (8.0, 17.5)	12.0 (10.0, 15.0)	0.80
Adjusted back-transformed median (95% CI) ^{a, d, e}	8.5 (4.0, 18.5)	8.0 (4.0, 16.0)	10.0 (4.0, 24.0)	0.98

p, percentile; CI, confidence interval. *Trends were tested by Spearman's rank correlation test and multiple linear regression analyses with BMI entered as a continuous explanatory variable. #This number of participants (n) relates to the hormone parameters except for AMH. The numbers in the groups for the following variables are: sperm concentration n=13, n=14, n=14; semen volume n=13, n=8, n=9; total sperm count n=13, n=7, n=10; morphology n=12, n=14, n=14; motility n=13, n=14, n=14; DFI n=11, n=14, n=14; testis volume: n=5, n=9, n=7, AMH n=13, n=13, n=15. The medians are adjusted for the following: abstinence time (a), current smoking (b), season (c), diseases in the reproductive organs (d), age (e), spillage at semen sampling (f) fever (g) and minutes from ejaculation to start of semen analysis (h).

Table 2. Differences in semen characteristics and reproductive hormone levels according to weight loss.

	Weight loss in percent (%)			Test for trend*
	3.5 – 12.1 (n = 10 [#])	12.2 – 17.1 (n = 10 [#])	17.2 – 25.4 (n = 10 [#])	P-value
Adjusted mean (95% CI) differences in semen and hormone levels				
Sperm concentration (millions/ml) ^{a, c, d}	-11 (-49, 27)	19 (-23, 61)	17 (-24, 58)	0.33
Semen volume (ml) ^c	-1.0 (-2.3, 0.3)	1.5 (-0.4, 3.5)	1.3 (-0.9, 3.4)	0.04
Total sperm count (millions) ^{a, c}	-41 (-147, 65)	232 (77, 387)	193 (45, 341)	0.02
Normal sperm morphology (%) ^{a, b, c}	0 (-2, 4)	1 (-3, 4)	4 (1, 7)	0.16
Motile sperm (%) ^{a, c, d, e}	-2 (-15, 11)	4 (-10, 18)	11 (-3, 25)	0.22
DFI (%) ^{a, b, c, d}	7 (-2, 17)	-1 (-11, 9)	0 (-10, 10)	0.96
Testosterone (nmol/L) ^{a, b}	0.7 (-1.1, 2.5)	3.3 (1.4, 5.2)	3.7 (2.0, 5.4)	0.02
Estradiol (nmol/L)	-0.03 (-0.05, 0)	-0.02 (-0.05, 0)	-0.01 (-0.03, 0.01)	0.93
SHBG (nmol/L) ^{a, b}	1.7 (-2.2, 5.5)	5.0 (1.0, 9.0)	5.0 (1.4, 8.5)	0.03
FSH (iu/L) ^a	0.1 (-0.3, 0.6)	0.3 (-0.3, 0.8)	0.1 (-0.3, 0.6)	0.95
LH (iu/L) ^{a, b}	0.7 (-0.6, 2.0)	1.2 (-0.1, 2.6)	0.3 (-0.9, 1.5)	0.85
Inhibin B (pg/ml) ^{a, b}	-30.1 (-51.7, -8.4)	-22.3 (-44.8, 0.2)	-13.6 (-33.6, 6.4)	0.34
AMH (ng/ml) ^{a, b}	-0.29 (-0.65, 0.07)	-0.02 (-0.42, 0.38)	0.24 (-0.09, 0.59)	0.02
Free androgen index (FAI) ^{a, b}	-3.7 (-13.3, 6.0)	3.5 (-6.5, 13.6)	6.5 (-2.4, 15.4)	0.43
Free testosterone/free estradiol ratio ^a	15.0 (0.5, 29.4)	38.3 (22.1, 54.4)	25.7 (11.4, 40.0)	0.18

CI, confidence interval. *Trends were tested by multiple regression analyses with weight loss in percent entered as a continuous explanatory variable. #This number of participants (n) relates to the differences in hormone parameters, except for AMH. The numbers in the groups for the following variables are: sperm concentration n=9, n=9, n=9; semen volume n=7, n=4, n=4; total sperm count n=6, n=4, n=4; morphology n=9, n=9, n=9; motility n=8, n=9, n=9, DFI n=8, n=9, n=9 and AMH n=10, n=9, n=10

The means are adjusted for the following: difference in season (a), difference in smoking status (b), difference in abstinence time (c), difference in spillage at semen sampling (d) and difference in minutes from ejaculation to start of semen analysis (e).