Semen quality and reproductive hormones changes in men with severe obesity and after weight reduction

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Abstract: Background: Obesity has been associated with reduced semen quality and male subfecundity, but the mechanisms that explain these relations are not fully understood and the effects of weight loss are not well clarified. We examine semen quality and reproductive hormones among obese men and studied if it will be improved by weight reduction. **Methods:** This study was conducted on 35 obese males and 20 healthy non obese male subjects of matched age as a control group. Obese subjects were prescribed a hypocaloric dietary program and daily exercise, lasted approximately 20 weeks. Before and after weight loss program, the obese subjects had blood samples drawn, provided semen samples and had clinical examinations, while healthy control subjects were tested once. Semen samples were analyzed for conventional semen parameters. Serum levels of testosterone, estradiol (E2), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and inhibin-B (inh-B) were measured. Free androgenic index (FAI) was calculated. **Results:** Participants were from 22 to 51 years of age (median=37) with body mass index (BMI) ranging from33.5 to 52.0 kg/m^2**.** We found decreased levels of serum testosterone, SHBG, inh-B, and increased levels of E2, LH and FSH in obese subjects at base line compared to controls (P<0.001). Also, obese males have low values of total sperm count, sperm concentration, normal sperm morphology, and motile sperm compared to controls. In addition, we found strong inverse associations of BMI with serum levels of testosterone(r=-0.894), SHBG (r=-0.968), FAI (r=-0.887) and inh-B (r=-0.923) as well as sperm concentrations (r=-0.872), total sperm count (r=-0,826), sperm motility (r=-0.943), semen volume (r=-0.530) and a positive association with serum estradiol (r=0.914) (P=0.000). Weight loss was associated with an increase in total sperm count and sperm concentrations. Additionally, the weight loss was associated with an increase in testosterone, SHBG and inhibin-B. Weight loss also significantly decreases serum estradiol levels. **Conclusion:** This study found that obesity is associated with changes in reproductive hormone levels that negatively influence male reproductive potential as assessed by poor semen quality. Weight loss leads to improvement in reproductive hormonal profile and semen quality.

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Key words: Obesity, Body mass index, reproductive hormone, semen quality.

Introduction:

Obesity is rapidly increasing worldwide [(1)]. Excess weight is not only linked to increased risk of chronic disease, but has also been shown to increase risk of reproductive problems [(2)]. Several studies have shown that women with excess body weight are more likely to have fertility problems. However, it is less clear whether men also experience reduced fertility with excess weight [(3)].

Similar to women, a sex hormone imbalance may affect reproduction in men, and excess weight can affect male hormone levels. A significantly reduced testosterone to estradiol ratio has been observed among overweight or obese men (body mass index; BMI >25) when compared with men with lower BMI [(4)]. Men with higher BMI have also exhibited altered quantity and quality of semen [(5)], although results are conflicting, previous studies have shown that the endocrine abnormalities may be reversed by weight reduction [(6)].

Several studies have focused on inhibin B (Inh-B) [(7)], a dimeric glycoprotein, member of the Transforming Growth factor-β (TGF-β) superfamily, is produced and secreted in males, almost exclusively, by Sertoli cells [(8)]. Inh-B main action seems to be inhibition of Follicle Stimulating Hormone (FSH) synthesis and secretion through a negative feedback mechanism [(9)]. On the other hand, FSH induces Inh-B secretion [(10)]. Inh-B is considered as a marker of spermatogenesis, as there is evidence that there is a significant positive correlation between basal serum Inh-B levels and sperm concentration [(11)]. Obesity has been shown to be associated with a decreased

level of Inh-B [12], however, results are conflicting [13].

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 etiological 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 [13]. The aim of this study is to investigate how obesity affects reproductive hormones including Inh-B and semen quality as well as to evaluate changes in these parameters after weight reduction.

Subjects and methods:

Participants:

The investigated subjects in this study were randomly withdrawn from the Outpatient Clinics of Fertility and Endocrinology, Mansoura University Hospitals, Egypt. They included 35 infertile obese males, (mean age, 36.2±6.19 years and BMI 41.9±5.29 kg/m^2) and 20 healthy fertile non obese male subjects of matched age (mean age, 34.5±6.61 years and BMI 26.7±2.58 kg/m^2) selected from the hospital employee, as a control group. All participants gave written informed consent to participate in the study and the investigations conformed to the principles outlined in the Declaration of Helsinki. The study protocol was approved by local ethics committee of the hospital.

The participants had primary idiopathic infertility since marriage (> 3 years) and clinically normal epididymis and ductus deferens. None of them gave a past history of genital infection or trauma; chronic systemic disease (hepatorenal, cardiovascular and musculoskeletal, uncontrolled diabetes mellitus, uncontrolled hypertension, anemia or fever); gentic abnormalities; long-term medications therapy (methotrexate, colchicine, cimetidine, spironolactone) or chronic exposure to chemicals. One male with azoospermia was also excluded because azoospermia probably is not caused by obesity alone.

All maternal partners of these men (35 women) had no inducing factor for infertility (pelvic, genital, endocrinal and inflammatory diseases or chronic medications intake) and had normal menstrual and secondary sex characters (no hirsutism, acne or clitoromegally) as well as normal reproductive investigations for ovulation, ovarian, tubal and uterine clinical status (transvaginal ultrasonography, endometrial biopsy and uterosalpingography).

Study design:

Obese subjects were prescribed a hypocaloric dietary program and daily exercise, lasted approximately 20 weeks. Before and after weight loss program, the obese subjects had blood samples drawn, provided semen samples and had clinical examinations. Healthy control non obese subjects were tested once. Subjects did not complete the dietary program were excluded.

All participants were interviewed to obtain a thorough history (e.g. history of reproductive experience, disease in the reproductive organs and lifestyle factors as smoking) and each underwent clinical examination that documented the following parameters: pulse, blood pressure, temperature, cardiac examination, chest and abdominal examination. Anthropometric measurements included body weight, which was measured to the nearest 1.0 kg, and height, which was measured to the nearest 1.0 cm. Body mass index (BMI) was calculated as weight (kg) /height (m^2). Finally, testis volume was measured by the ultrasound.

Ten milliliters of peripheral venous blood was obtained from all participants (obese and controls) in the morning after 12 hours of overnight fasting. Each blood sample was left to coagulate for 30 minutes, and then centrifuged at 3000 rpm for 15 minutes to separate serum. Serum aliquots were immediately labeled and stored at –70°C until laboratory investigations were performed.

Semen samples were collected from all male participants by masturbation after an abstinence period of 3–4 days. After liquefaction (within 1.0 hour from collection), each semen sample was subjected for analysis.

Hormonal analysis:

Serum samples for testosterone, estradiol (E_2), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin were analyzed by an electrochemiluminescent immunoassay (Elecsys 2010, Roche Diagnostics, Germany). The sex hormone-binding globulin (SHBG) concentrations were determined using solid phase, 2 site chemiluminescent immunometric assay. [IMMULITE 1000: Diagnostic Products Corporation (DPC), Los Angeles, CA, USA]. Serum concentrations of Inh-B were measured by an enzymatically amplified tow-site sandwich- type immunoassay (DSL-10-84100 ACTIVE Inhibin –B ELISA kit, Diagnostic Systems Laboratories Inc., Texas, USA). Inter-assay and intra-assay coefficients of variation for inh-B were 6.2% and 3.5%, respectively. Assay sensitivity was 7 pg/ml. Free androgen index (FAI) was calculated as: testosterone/SHBG X 100 [14].

Semen samples analysis:

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 the samples was initiated within one hour after ejaculation, and within this time it has been shown that the sperm motility is stable [15]. Sperm morphology was assessed using the Tygerberg strict criteria [16].

Statistical Analysis:

Statistical analyses were performed using SPSS software version 15 (SPSS Inc. Chicago, IL, USA). Data are expressed as means ± SD. Unpaired samples t test was used to compare the mean values in 2 groups. The relationships between variables were assessed using univariate linear regression analysis and Pearson's correlation coefficient. A P value <0.05 was accepted as having statistical significance.

Results:

Table (1): Shows the clinical and semen analysis data of obese male (before and after weight loss) and controls. Following weight loss program, the median (range) weight loss was 20 (6-31) kg, corresponding to a median percentage weight loss of 16.3% (4.2%-23.3%). There were significant lower levels of sperm concentrations, total sperm count and sperm motility in obese male before and after weight loss than controls (P<0.001). Also there was significant lower sperm concentrations and total sperm count in obese male before weight loss compared to after weight loss (P=0.029 and 0.047, respectively).

There were significant higher FSH, LH, E2 and significant lower testosterone, SHBG, FAI and inh–B in obese male before and after weight loss than controls. After weight loss there were significant increase in serum testosterone and inh-B (P<0.05) than before weight loss **(Table 2).**

Table 3&4: show correlation coefficient between different studied parameters. There were significant positive correlations between BMI and FSH, LH, E2. While, significant negative correlations were found between BMI and serum testosterone **(figure 1)**, SHBG, inh-B, FAI, semen volume, sperm concentrations **(figure 2)**, total sperm count and sperm motility.

Several clinical and hormonal variables were entered in multiple regression analysis to detect relation to BMI. Only the SHBG showed significant association with a P value of 0.001 **(Table 5).**

Table 1: Clinical and semen parameters of obese male (before and after weight loss) versus controls

Parameter	Obese male (n=35)		Controls (n=20)	P value		
	Before weight loss	After weight loss		P1	P2	P3
Age (year)	36.2±6.19	-	34.5±6.61	0.350	-	-
Weight (kg)	110.0±20.7	95.1±18.6	84.5±12.8	0.000	0.009	0.009
BMI (kg/m^2)	41.9±5.29	36.9±4.82	26.7±2.58	0.000	0.000	0.000
Semen volume (ml)	2.22±1.10	2.34±0.95	2.44±1.06	0.460	0.710	0.620
Sperm conc. (millions/ml)	39.4±20.0	51.4±24.8	76.2±27.6	0.000	0.001	0.029
Total sperm count (milli/ejac)	97.5±76.8	137±86.9	190±108	0.000	0.054	0.047
Sperm motility (%)	51.3±14.2	52.3±13.3	67.1±11.5	0.000	0.000	0.77
Abnormal sperm (%)	16.6±7.2	16.1±6.76	15.6±7.58	0.63	0.81	0.76

Significant P<0.05 P1 value: control vs before weight loss P2 value: control vs after weight loss
P3 value: before weight loss vs after weight loss

Table 2: Reproductive hormonal changes of obese male (before and after weight loss) versus controls

Parameter	Obese male (n=35)		Controls (n=20)	P value		
	Before weight loss	After weight loss		P1	P2	P3
FSH (mIU/ml)	5.89±1.59	4.90±1.83	3.39±1.37	0.000	0.002	0.019
LH (mIU/ml)	5.44±1.56	4.82±1.90	4.05±1.93	0.005	0.16	0.14
Testosterone (nmol/L)	8.46±2.68	9.91±2.28	14.4±5.50	0.000	0.000	0.017
Estradiol (pmol/L)	120.0±32.1	106.0±37.2	81.2±27.1	0.000	0.014	0.089
Inhibin-B (pg/ml)	126.0±19.5	143.0±34.8	179.0±31.2	0.000	0.000	0.020
SHBG (nmol/L)	20.5±4.92	23.3±5.63	25.6±3.83	0.000	0.068	0.035
Free androgen index	47.3±27.5	50.9±20.3	102.0±56.4	0.000	0.000	0.50
Prolactine (uIU/ml)	156.0±51.0	156.0±61.1	152.0±48.7	0.75	0.79	0.99

Significant P<0.05 P1 value: control vs before weight loss P2 value: control vs after weight loss
P3 value: before weight loss vs after weight loss

Table 3: Correlation of age and BMI with different reproductive hormones

		Age	BMI	FSH	LH	Testo	E2	Inhib	SHBG	FAI
BMI	r	0.092	-	0.900**	0.802**	-0.894-**	0.914**	-0.923-**	-0.968**	-0.887-**
	p	0.597		0.000	0.000	0.000	0.000	0.000	0.000	0.000
Testosterone	r	-0.046-	-0.894-**	-0.895-**	-0.768-**	-	-0.897-**	0.919**	0.898**	0.969**
	p	0.792	0.000	0.000	0.000		0.000	0.000	0.000	0.000
E2	r	-0.081-	0.914**	0.907**	0.851**	-0.897-**	-	-0.935-**	0.920**	-0.947-**
	p	0.645	0.000	0.000	0.000	0.000		0.000	0.000	0.000
Inhibin-B	r	0.005	-0.923-**	-0.895-**	-0.851-**	0.919**	-0.935-**	-	0.917**	0.923**
	p	0.978	0.000	0.000	0.000	0.000	0.000		0.000	0.000
SHBG	r	0.004	-0.968**	0.906**	0.849**	0.898**	0.920**	0.917**	-	-0.906-**
	p	0.983	0.000	0.000	0.000	0.000	0.000	0.000		0.000

Significant P<0.05

Table 4: Correlation of BMI, age and reproductive hormones with different semen parameters

		Age	BMI	FSH	LH	Testo	E2	Inhib	SHBG	FAI
Semen volume	r	0.084	-0.530-**	-0.481-**	-0.489-**	0.356*	-0.491-**	0.518**	0.471**	0.382*
	p	0.631	0.001	0.003	0.003	0.036	0.003	0.001	0.004	0.024
Sperm Conc.	r	-0.126-	-0.872-**	-0.827-**	-0.764-**	0.844**	-0.868-**	0.850**	0.880**	0.877**
	p	0.471	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
T. sperm count	r	-0.059-	-0.826-**	-0.779-**	-0.743-**	0.716**	-0.829-**	0.827**	0.798**	0.766**
	p	0.735	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sperm motility	r	-0.056-	-0.943-**	-0.877-**	-0.770-**	0.888**	-0.868-**	0.880**	0.936**	0.884**
	p	0.748	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Significant P<0.05

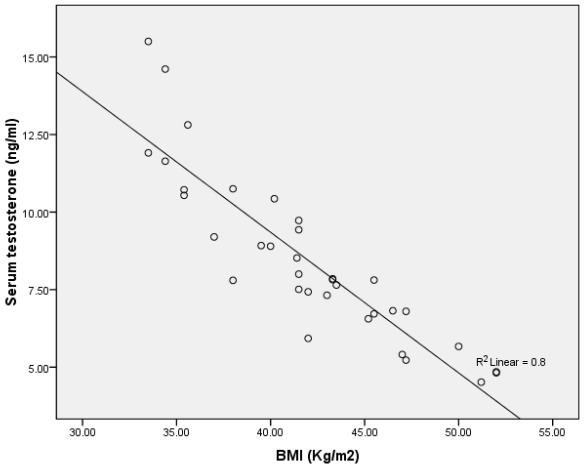

Figure 1: Correlation between serum testosterone and BMI

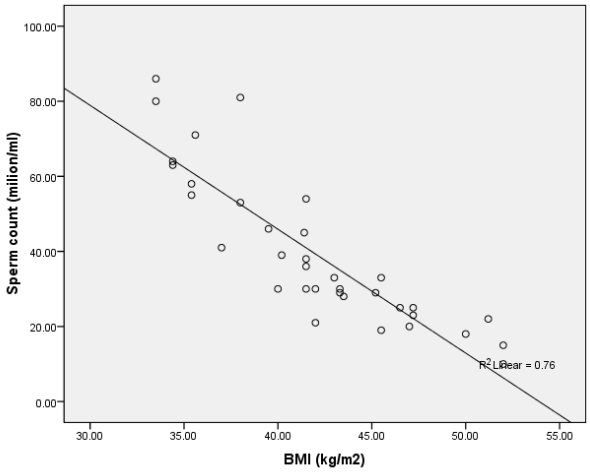

Figure 2: Correlation between sperm concentration and BMI

Table 5: Linear regression analysis of the independent factors affecting male reproduction

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
Constant	29.187	8.043		3.629	0.002
FSH	0.587	0.439	0.176	1.336	0.196
LH	-0.584-	0.349	-0.172-	-1.671-	0.110
Testosterone	-0.258-	0.422	-0.131-	-0.610-	0.548
E2	0.049	0.027	0.296	1.815	0.084
Inhibin-B	-0.039-	0.041	-0.146-	-0.954-	0.351
Prolactin	0.001	0.005	0.005	0.117	0.908
SHBG	0.603	0.154	0.561	3.926	0.001
FAI	0.071	0.045	0.367	1.560	0.134
Semen volume	-0.394-	0.467	-0.082-	-0.844-	0.408
Sperm conc.	-0.003-	0.050	-0.013-	-0.068-	0.946
T. sperm count	0.003	0.015	0.039	0.173	0.864
Sperm motility	-0.073-	0.045	-0.196-	-1.629-	0.118
Age	0.070	0.037	0.082	1.905	0.071

Dependent Variable: BMI Significant P<0.05

Discussion:

Potential effects of increased body mass index in men on male fertility have not been subjected to the same degree of research as female obesity. There is growing evidence over the years that suggest a trend towards deterioration in semen quality in relation to obesity [17]. Several hypotheses have been proposed, and male obesity was suggested as a strong factor. Several studies have linked male obesity to poor semen quality and male infertility [18,19]. The mechanisms that explain the relation between obesity and male infertility are not fully understood. Higher DNA fragmentation indexes in obese males [20], increased oxidative stress [21], and hormonal imbalance [22] have been suggested as possible mechanisms of obesity-associated sub-fertility. The aim of this study was to investigate the effect of male obesity on semen quality and hormonal milieu including inh-B and to evaluate the changes in these parameters after weight reduction.

The present study was conducted on 35 infertile obese males and 20 fertile healthy non obese male subjects of matched age as a control group. Obese subjects were prescribed a hypocaloric dietary program and daily exercise, lasted approximately 20 weeks.

Our study showed decreased levels of serum testosterone, SHBG, inh-B, FAI and increased serum levels of E2, LH and FSH. Also, we found strong inverse associations of BMI with serum levels of testosterone, FAI and inhibin-B and a positive association with serum estradiol levels. These associations are well documented effects of excess body weight on these hormones. Excess adiposity leads to increased aromatization of androgens in the adipose tissue leading to higher circulating estradiol levels [23]. Hyperinsulinemia, secondary to obesity-related insulin resistance, decreases SHBG production in the liver [24]. Low testosterone levels are thought to be the result of decreased SHBG binding capacity [25], direct action of leptin and other adipocyte-derived hormones on Leydig cells [26,27] and, in morbidly obese men, impaired functioning of the hypothalamic-pituitary-testicular (HPT) axis [28] possibly as a result of enhanced negative feedback on gonadotropin secretion by estradiol [23]. Overweight and obesity have been related to lower testosterone and SHBG levels and higher estradiol levels in multiple studies [18,29,30] and body weight has been found to explain a greater proportion of the variability in testosterone levels than age and lifestyle practices

(30). Further, testosterone increases after weight loss in massively obese men (28,31). Our findings regarding inhibin-B levels are in agreement with other previous reports of the relationship between body weight and inhibin B in adult men (18,29,32). Moreover, in a study among severely obese men who underwent gastroplasty, inhibin B levels increased after surgery among the men with the greatest amount of weight loss (on average 50 kg or 16.9 kg/m^2) (31). The observed lower testosterone:LH ratio among the most obese men also suggests decreased Leydig cell function among these men and is consistent with a report of impaired LH-stimulated testosterone production among morbidly obese men (33). The consistency of these findings across studies and the reversibility of this pattern following weight loss suggest a causal role of increased body weight on the hormonal pattern described above.

We also found positive associations between BMI and gonadotropin levels which were more marked among men with abnormal semen analysis results. In men with an intact HPT axis lower levels of testosterone and inhibin-B, as those observed with increasing levels of body weight, would be expected to result in higher levels of LH and FSH, respectively (23). Our findings suggest that excess body weight can lead to an impairment of the feedback regulation of the HPT axis, particularly among men who eventually develop semen quality abnormalities. However, several studies have reported no relation between excess body weight and gonadotropin levels (18,29,30,34).

Our study showed that, obese males have 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 sperm concentrations among men who participated in a 20-week weight loss program. Additionally, the weight loss was associated with an increase in testosterone, SHBG and inhibin-B. Weight loss also significantly decreases serum estradiol levels. 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 (13).

Despite conflicting results (12,,29,34,35,36), previous studies have mainly shown low sperm concentration among overweight and obese men (4,18,22,37,38). 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.

Others have reported that increased adiposity is related to decreased fertility (39,40,41) and negatively affects nearly every semen analysis parameter including concentration (4,18,42), ejaculate volume (43), total sperm count (18,43,44), motile count (43) and progressive motility (23), similar to what we find. The most consistent positive finding across studies has been lower sperm concentration among overweight and obese men compared to normal weight men. This has been reported by previous studies(4,18,42,44,) while other studies (29,34) did not find this association.

However, Chavarro et al., (2010) did not observe statistically significant differences in sperm concentration, sperm morphology or sperm motility across levels of BMI. Only ejaculate volume was significantly lower in overweight and obese men relative to normal weight men. In addition, total sperm count was significantly lower in the group of most obese men (BMI $\geq$ 35 kg/m^2). Furthermore, they found that overweight men had a slightly higher total progressive sperm count than normal weight men (23).

In conclusion, we observed relationships between body weight and reproductive hormone levels as well as standard semen analysis in obese men when compared to normal weight men. Also, we have shown that in obese men a 20-week weight loss intervention resulted in improvements in reproductive function. Additional research is needed to examine long-term effects of weight loss on reproductive functions. Further investigation is required to identify the mechanisms by which weight loss improve reproductive function in overweight and obese male.

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