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ORIGINAL CONTRIBUTIONS

Bariatric Surgery Impact on Reproductive Hormones, Semen Analysis, and Sperm DNA Fragmentation in Men with Severe Obesity: Prospective Study

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Abstract

Purpose Growing evidence in the literature suggests that obesity is capable of altering reproductive hormone levels and male fertility. Effects on classic semen parameters and sperm DNA fragmentation (SDF), however, have not been properly established. Additionally, the impact of bariatric surgery (BS) on those parameters is still controversial.

Materials and Methods In Phase 1, 42 patients with obesity and 32 fertile controls were submitted to reproductive hormone evaluation, semen analysis, and SDF testing. In Phase 2, patients with obesity were submitted to BS or clinical follow-up and were invited to 6-month revaluation.

Results Phase 1: Men with obesity have higher levels of estradiol, LH, and FSH and lower levels of total testosterone (TT) when compared with eutrophic fertile men. Additionally, they present worse semen parameters, with reduction in ejaculated volume and sperm concentration, worse sperm motility and morphology, and higher SDF. Phase 2: 32 patients returned to revaluation. Eighteen were submitted to BS (group S) and 14 were not submitted to any specific therapeutic regimen (group NS). In group S, TT more than doubled after surgery (294.5 to 604 ng/dL, $p < 0.0001$). Worsening of sperm concentration and total ejaculated sperm count were also noticed, and 2 patients became azoospermic after BS. SDF, however, improved after the procedure. No changes in the variables studied were observed in non-operated patients.

Conclusion In this prospective study, we have found that BS results in improvements in reproductive hormone levels and SDF after 6-month follow-up. Sperm concentration, however, reduced after the procedure.

Keywords Male infertility · Obesity · Bariatric surgery · Gonadal steroid hormones · Semen analysis · Sperm DNA fragmentation

Introduction

According to the World Health Organization (WHO), approximately 80 million people throughout the world are affected by infertility, corresponding to 15% of the couples in reproductive age, independently of their ethnical or social origins [1–3]. Some authors have reported a global tendency of decline in semen concentration in the last decades [4–9]. In parallel to this reduction, the world prevalence of obesity has duplicated since 1980, reaching more than 600 million adults suffering from obesity in 2014 [10]. Consequently, the hypothesis that both findings are related comes forward.

Effects of obesity in female infertility have been extensively reported in the literature [11]. Women with obesity are more

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prone to irregular menses cycles, due to pituitary and gonadal hormone profile changes. Women with obesity produce lower-quality embryos, and assisted reproduction techniques success rates are inversely related to female body mass index (BMI) [12]. The relationship between obesity and male infertility, however, has not been completely established.

Several mechanisms were proposed regarding the association between obesity and male infertility. High aromatase activity, an enzyme highly expressed in the adipose tissue, may result in increased testosterone conversion to estradiol [13]. Leptin, hormone chronically elevated in patients with obesity, can reduce testosterone levels [14]. Insulin resistance is associated with lower sex hormone-binding globulin (SHBG) levels, which ultimately leads to lower testosterone levels [15, 16]. Additionally, sleep apnea reduces the nocturnal testosterone and LH peak [17]. These findings disrupt the hypothalamic-pituitary-gonadal axis and explain the hormone profile frequently associated with male obesity of hyperestrogenic hypogonadotropic hypogonadism [18, 19]. Moreover, physical mechanisms such as scrotal lipomatosis and sedentarism may increase testicular temperatures and reactive oxygen species production, leading to worsening of semen parameters [20, 21].

Among all obesity treatment modalities, bariatric surgery is the one that shows better outcomes in terms of weight loss, type II diabetes remission, and long-term effectiveness and should be considered a therapeutic option in patients with BMI over 40 kg/m^2 or in patients with BMI over 35 kg/m^2 and serious comorbidities [22–24]. Some studies suggest that bariatric surgery may improve sex hormone profile in men with obesity [25–27]. Effects of obesity and bariatric surgery on semen parameters, however, remain controversial [25, 28–31]. Moreover, only one study assessed the impact of bariatric surgery on sperm DNA fragmentation [31].

In order to further evaluate the effects of obesity and bariatric surgery on male reproductive hormones, semen parameters, and sperm DNA fragmentation (SDF), we have conducted this study.

Material and Methods

Setting

This study was approved by the Local Ethical Committee and Institutional Review Board prior to the beginning of patient recruitment (registered number CAAE 39428414.3.0000.0068), and written informed consent was obtained from all participants by the time of their first appointment. Patients were part of the bariatric surgery program of the Obesity Division of the University of Sao Paulo Clinics Hospital (HCFMUSP). Urological evaluation, semen analysis, and sperm DNA fragmentation tests were performed in the Andrology Lab of the Human Reproductive Center of HCFMUSP.

Human Subjects and Clinical Data Collection

Recruitment took place from January 2016 to October 2018. In Phase 1, patients candidates for voluntary vasectomy were included in the control group and were considered fertile and non-obese controls, since all had previously experienced paternity at least two times and had BMI lower than 35 kg/m^2. For the obesity group, only patients with BMI over 40 kg/m^2 or 35 kg/m^2 with serious comorbidities were invited. Patients with history of illicit drug use, exposure to any environmental or occupational toxicants, use of medication with proven toxicity on fertility, exposure to radiation or heat, mumps with orchitis, sexually transmitted or systemic diseases, cryptorchidism regardless of treatment, testicular torsion, genitourinary anomalies, epididymal or vas deferens anomalies, and scrotal or inguinal surgery were excluded from the study. After exclusions, a total of 32 patients in the control group and 42 patients in the obesity group were included. At baseline, all of them were submitted to urological examination, reproductive hormone assessment, semen analysis, and evaluation of sperm DNA fragmentation.

Phase 2 included only the patients from the obesity group. Twenty-two patients were submitted to bariatric surgery (S group), while the rest kept waiting for the surgical procedure during the length of this study (NS group). Patients were then invited to a revaluation 6 months after the surgical procedure or the initial clinical consultation; on follow-up, 18 patients from the S group and 13 patients from the NS group returned. The 13 patients from the NS group received clinical and nutritional orientation and comorbidities treatment, but were not submitted to any specific therapeutic regimen. All patients were then submitted to the same evaluation performed at baseline (Fig. 1). Individuals included in this study were analyzed according to their original group assignment (intention-to-treat analysis), and there was no contamination between groups. The 6-month window time was chosen so that the results obtained would reflect the effects of the rapid weight loss and prominent metabolic changes in the studied variables.

Obesity Assessment and Urological Evaluation

BMI was measured in both evaluations. BMI variation between assessments was calculated and reported in percentage; positive values indicate weight loss. Physical activity, used in this study as a covariate, was estimated with the application of the International Physical Activity Questionnaire – Short Form (IPAQ short version) [32]. Patients were examined by infertility specialists, and testicular size was measured with aid of the Prader orchidometer. Varicocele grade and genital anatomy were recorded for each individual.

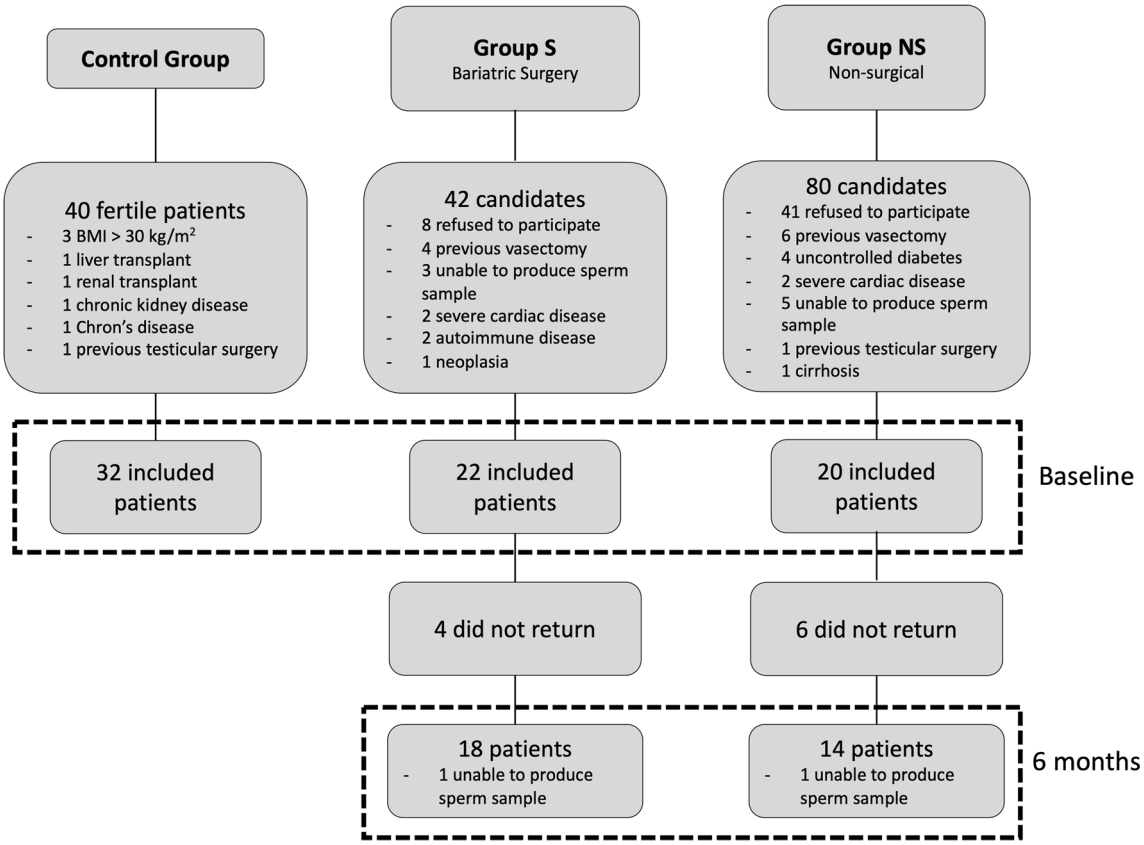

Fig. 1 Flow diagram for inclusion of patients in the study

Reproductive Hormone Measurements

Hormonal determinations were performed for all subjects, always in the morning period. Abnormal results were repeated for confirmation. FSH, LH, total testosterone (TT), estradiol (E2), and prolactin (PRL) were detected by fluoroimmunoassay using kits from Roche for electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN, USA). Intra- and inter-assay coefficients of variation were limited to 5.1% and 8.4%, respectively. Free testosterone (FT) was estimated based on SHBG and TT levels, with the Vermeulen equation [33].

Semen Analysis

Semen was collected by in-site masturbation only, with two to five sexual abstinence days. Semen analysis was performed manually, by two blinded trained specialists, according to the 2010 World Health Organization (WHO) criteria [34]. Complete semen analysis, including strict morphology and leukocyte count assessment (Endtz test), was performed [35]. The 2010 WHO reference values for sperm analysis are shown in Table 1.

Sperm DNA Fragmentation

The alkaline comet assay adapted from McKelvey-Martin et al. was used to measure sperm DNA fragmentation [36]. The semen sample was diluted to a concentration of 1×10^6/mL with 0.7% low melting point agarose gel (Sigma-Aldrich Co., St Louis, MO, USA); 100 μL was then added to a slide covered with 1% normal melting point agarose gel before assay. The slide was coverslipped and kept at 4 °C for 10 min. The coverslip was removed and the slide was covered

Table 1 World Health Organization reference values for human semen characteristics (2010)

	Reference
Semen volume (mL)	1.5
Sperm concentration (10^6/mL)	15
Total ejaculated volume (10^6)	39
Total motility (%)	40
Progressive motility (%)	32
Normal morphology (%)	4
Leukocyte count (10^6/mL)	< 1.0

Data from World Health Organization. Lower reference limits obtained from the lower fifth centile value

with 200 mL 0.7% low melting point agarose gel and then coverslipped and kept at 4 °C for an additional 10 min. The coverslip was again removed and the slide was submerged in lysing solution at 4 °C for 20 min. The slide was then washed with milli-Q water for 5 min, and the slide was immersed in alkaline electrophoresis solution (sodium hydroxide 300 mM and 1 mM ethylenediaminetetraacetic acid) for 20 min, leading to the unwinding of the double-stranded DNA. The slide was electrophoresed at 4 °C for 20 min, 35 V, and 200 mA. The slide was then removed from the electrophoresis solution, washed with a tris-borate buffered solution for 10 min and fixed with ethanol for 10 min. The slide was then air-dried and stained with SYBR Green II (Thermo Fischer, catalog number S-7564, EUA). An inverted fluorescence microscope (Olympus, BX51, fluorescence microscope, EUA) was used to examine the slides at × 200 magnification. All reagents, such as lysing and enzyme solutions, were prepared according to Blumer et al. [37]. To evaluate sperm DNA fragmentation, 100 spermatozoa were classified according to the intensity of DNA damage observed by the tail and nuclear intensity and divided into grades I (high DNA integrity: no DNA migration), II (low DNA fragmentation: little DNA migration), III (increased DNA fragmentation: an intense comet tail and an observed nucleus), or IV (high DNA fragmentation: an intense comet tail with no observed nucleus).

Surgical Technique

Surgery technique was decided after a multidisciplinary meeting, based on patients' comorbidities and initial weight. Surgical complications were recorded and ranked according to the Clavien-Dindo classification [38].

Roux-en-Y gastric bypass (RYGB) was performed through open or laparoscopic approach. A new gastric pouch with an estimated volume of 30–50 mL, without silicone rings, was made. The rest of the stomach, duodenum, and proximal jejunum was excluded from the flow of nutrients by the Roux-en-Y derivation, with a biliopancreatic loop of approximately 60–80 cm and a Roux limb of approximately 100–120 cm [39].

Vertical sleeve gastrectomy (VG) was performed laparoscopically, through devascularization of the greater curvature, from a point 5 cm to the pylorus up to the His angle. With the use of linear cutting staplers, a gastric tube was made, calibrated with a 32-French bougie, in order to obtain an internal lumen of approximately 3 cm. Occasionally, a hemostatic suture was performed in the stapler line [40].

Data Logging and Statistical Analysis

The clinical and laboratory data were extracted from the institutional REDcap data system [41]. Results were presented as medians (25–75% interquartile range) for continuous and count (%) for categorical variables. Numerical variables were compared by the Wilcoxon signed rank test or paired samples *t* test, when appropriate, to evaluate differences between baseline or 6-month follow-up. Categorical variables differences were assessed by chi-square or Fisher exact test. Finally, linear regression analysis was performed to evaluate if greater weight loss was associated with larger effects on numerical variables. All tests were two-tailed, and statistical significance was set at $p < 0.05$. Analyses were performed with Statistical Analysis System (SAS version 9.04; SAS Institute Inc., Cary-NC, EUA).

Results

Phase 1

Baseline characteristics of the patients enrolled are shown in Table 2. As expected, systemic hypertension and diabetes were more frequent in patients from the obesity group. Median BMI was 27.1 kg/m^2 in the control group and 45.1 kg/m^2 in the obesity group. The summary of findings on reproductive hormones and semen analysis are shown in Table 3.

Reproductive Hormones

When compared with controls, patients with obesity had higher E2 (22.0 vs. 33.3 pg/mL, $p = 0.0003$), LH (4.1 vs. 6.3 IU/L, $p = 0.0004$), and FSH levels (3.2 vs. 4.8 IU/L, $p = 0.006$). Total testosterone was lower on patients with obesity (413 vs. 272.5 ng/dL, $p = 0.0008$).

Table 2 Phase 1: baseline characteristics

	Controls ($N = 32$)	Obesity ($N = 42$)	p
Age (years)	36.5 (15.0)	40.0 (14.0)	*0.0277*
BMI (kg/m2)	27.1 (5.4)	45.1 (8.8)	*< 0.0001*
Tobacco use	5 (16%)	2 (5%)	0.2276
Alcohol use	16 (50%)	24 (57%)	0.5413
SAH	3 (9%)	26 (62%)	*< 0.0001*
Type II diabetes	0	18 (43%)	*< 0.0001*
IIEF-5 score	24 (3.5)	24 (7.0)	0.8721
Physical activity score	3581.8 (4422.3)	1058.1 (2459.0)	*0.0003*
Right testis size (cm^3)	12 (3.0)	15 (10.0)	0.1060
Left testis size (cm^3)	12 (5.0)	15 (8.0)	*0.0013*
Varicocele	12 (38%)	4 (10%)	*0.0038*

Values expressed in median (interquartile range) or *n* (%). BMI, body mass index; SAH, systemic arterial hypertension; IIEF-5, International Index of Erectile Function. *p* value < 0.05 was considered significant

Values in italics are statistically significant ($p < 0.05$)

Table 3 Phase 1: summary of findings on reproductive hormones and semen parameters

	Controls ($N = 32$)	Obesity ($N = 42$)	
Hormone evaluation			
Estradiol (pg/mL)	22.0 (17.9)	33.3 (16.7)	*0.0003*
LH (IU/L)	4.1 (2.2)	6.3 (3.5)	*0.0004*
FSH (IU/L)	3.2 (1.6)	4.8 (3.5)	*0.0060*
Total testosterone (ng/dL)	413.0 (135.0)	272.5 (242.0)	*0.0008*
Sperm analysis			
Oligospermia	0	14 (36)	*0.0009*
Severe oligospermia	0	9 (23)	*0.0036*
Azoospermia	0	3 (8)	0.2491
Ejaculated volume (mL)	2.5 (1.5)	1.5 (1.0)	*< 0.0001*
Concentration (10^6/mL)	82 (45.0)	43.0 (86.5)	*0.0183*
Total ejac. sperm (10^6)	205.2 (217.0)	82.5 (167.0)	*0.0002*
Total motility (%)	72.0 (20.0)	54.5 (32.0)	*0.0004*
Progressive motility (%)	54.0 (21.0)	27.5 (29.0)	*< 0.0001*
Normal morphology (%)	3.0 (3.0)	2.0 (3.0)	*0.0098*
Sperm DNA fragmentation			
Class I (%)	54.5 (38.0)	21.0 (24.0)	*< 0.0001*
Class II (%)	15.0 (14.0)	31.0 (29.0)	*0.0003*
Class III (%)	17.0 (19.0)	28.0 (26.0)	*0.0059*
Class IV (%)	4 (5.0)	8.0 (18.0)	0.3086

Values expressed in median (interquartile range) or *n* (%). *p* value < 0.05 was considered significant
Values in italics are statistically significant ($p < 0.05$)

Semen Analysis

Overall, patients in the obesity group had worse semen parameters than controls. Three patients with obesity were found to be azoospermic, and those were unaware of this diagnosis prior to this study. Patients with obesity had lower ejaculated volume (2.5 vs. 1.5 mL, $p < 0.0001$), sperm concentration (82 vs. 43 10^6/mL, $p = 0.0183$), total sperm count (205.2 vs. 82.5×10^6, $p = 0.0002$), progressive and total motility (54 vs. 27.5%, $p < 0.0001$ and 72 vs. 54.5%, $p = 0.0004$, respectively), and normal morphology rate (3.0 vs. 2.0%, $p = 0.0098$). Additionally, patients with obesity showed higher SDF and were more likely to be oligospermic (sperm concentration lower than 15×10^6/mL) and severe oligospermic (sperm concentration lower than 5×10^6/mL) than controls.

Phase 2

Ten patients initially enrolled did not show up to revaluation. At the end of the study, group S and group NS were composed of 18 and 14 patients, respectively. Clinical outcomes are shown in Table 4. Reproductive hormones and semen analysis results are shown in Tables 5 and 6, respectively.

Bariatric Surgery

Of the 18 patients from the S group that returned, 15 were submitted to RYGB and 3 patients to laparoscopic VG. Median of hospital stay was 4 days. Only 2 patients had minor complications during the first 30 days after surgery, both classified as Clavien I.

Clinical Outcomes

Median BMI decrease in operated patients was 11.6 kg/m^2 ($p < 0.0001$). Prevalence of systemic arterial hypertension and type II diabetes also reduced in the S group. Operated patients were more active after surgery, but this was not statistically significant. No statistical differences were observed in the NS group. No changes were observed in testicular size.

Reproductive Hormones

TT and FT dramatically changed in operated patients. While median FT increased approximately 33% ($p = 0.0026$), and median TT more than doubled after surgery (294.5 to

Table 4 Phase 2: demographic and physical examination characteristics

	Group S bariatric surgery ($N = 18$)			Group NS non-surgical ($N = 14$)		
	Baseline	6 months	p value	Baseline	6 months	p value
Age (years)	39.0 (16.0)			41.5 (9.0)		
BMI (kg/m2)	43.9 (11.6)	32.3 (5.5)	*< 0.0001*	43.9 (8.0)	45.0 (10.0)	0.3743
Tobacco use	0	1 (5.6)	1	1 (7.1)	3 (21.4)	0.5956
Alcohol use	10 (55.6)	3 (16.7)	*0.0151*	7 (50.0)	5 (35.7)	0.4450
SAH	10 (55.6)	3 (16.7)	*0.0151*	10 (71.4)	10 (71.4)	1
Type II diabetes	7 (38.9)	2 (11.1)	0.1212	7 (50.0)	6 (42.9)	0.7047
IIEF-5 score	22.5 (9.0)	22.5 (7.0)	0.7632	23.0 (9.0)	21.5 (10.0)	0.8359
Physical activity score	1492 (3528)	1928 (2563)	0.2522	1091 (1365)	1250 (2628)	0.7344
Right testis size (cm^3)	17.5 (8.0)	20 (8.0)	0.5625	12.0 (5.0)	13.5 (8.0)	0.1953
Left testis size (cm^3)	17.5 (8.0)	20 (8.0)	0.3750	13.5 (8.0)	15.0 (8.0)	1

Values expressed in median (interquartile range) or n (%). BMI, body mass index; SAH, systemic arterial hypertension; IIEF-5, International Index of Erectile Function. p value < 0.05 was considered significant

Values in italics are statistically significant ($p < 0.05$)

604 ng/dL, $p < 0.0001$). FSH, prolactin, and SHBG levels were also statistically different between evaluations. Hormone levels in the NS group, however, were not different between time points.

Semen Analysis

One patient from each group was not able to produce a semen sample on follow-up. Therefore, 17 and 13 patients were included in groups S and NS, respectively, for this analysis.

Individuals submitted to bariatric surgery presented a reduction in sperm concentration ($p = 0.0022$) and on total ejaculated sperm count ($p = 0.0017$). Moreover, 2 patients in group S developed azoospermia at the end of the follow-up period. These patients had initial semen concentrations of 0.1 and 82 million/mL. Additionally, the prevalence of oligospermic patients (concentration lower than 15 million/mL) and of severe oligospermic patients (concentration lower than 5 million/mL) was also higher after surgery.

Sperm DNA Fragmentation

The percentage of class I sperm (no DNA migration, high DNA integrity) increased after surgery ($p = 0.0049$), while the percentage of class III sperm (intense comet tail and an observed nucleus, increased DNA fragmentation) decreased ($p = 0.0155$), suggesting an improvement on sperm chromatin integrity. Once again, no differences were observed in the NS group.

Linear Regression

Afterwards, we applied linear regression tests to determine if the magnitude of weight loss was related to the size of the

Table 5 Phase 2: variation of reproductive hormone blood levels between time points

	Group S bariatric surgery			Group NS non-surgical		
	Baseline	6 months	p value	Baseline	6 months	p value
Hormone evaluation	$N = 18$			$N = 14$		
Estradiol (pg/mL)	33.3 (20.4)	34.0 (13.7)	0.6800	29.2 (10.0)	22.5 (28.9)	0.1988
LH (IU/L)	5.8 (4.0)	6.5 (3.9)	0.5604	6.3 (2.0)	7.2 (4.4)	0.1238
FSH (IU/L)	4.1 (3.6)	5.1 (4.6)	*0.0002*	6.0 (4.3)	6.0 (4.0)	0.2603
Prolactin (ng/mL)	9.0 (4.5)	6.8 (3.4)	*0.0024*	8.5 (6.2)	10.4 (5.8)	0.1909
SHBG (nmol/L)	35.5 (17.2)	68.5 (43.0)	*< 0.0001*	32.6 (16.2)	29.5 (22.3)	0.5313
Total testosterone (ng/dL)	294.5 (205.5)	604.0 (343.0)	*< 0.0001*	231.5 (216.0)	200.5 (216.0)	0.4562
Free testosterone (pmol/L)	198.5 (116.5)	264.0 (110.0)	*0.0026*	162.0 (79.0)	145.0 (84.0)	0.7861

Values expressed in median (interquartile range) or n (%). p value < 0.05 was considered significant

Values in italics are statistically significant ($p < 0.05$)

Table 6 Phase 2: variation of semen parameters and sperm DNA fragmentation between time points

	Group S bariatric surgery			Group NS non-surgical		
	Baseline	6 months	*p* value	Baseline	6 months	*p* value
Sperm analysis		*N* = 17			*N* = 13	
Abstinence						
< 2 days	1 (5.9)	3 (17.7)		2 (16.7)	0	
2 to 5 days	9 (52.9)	11 (54.7)	0.2524	8 (66.7)	11 (84.6)	0.4671
> 5 days	7 (41.2)	3 (17.7)		2 (16.7)	2 (16.7)	
Oligospermia	2 (11.8)	7 (41.2)	0.1175	7 (53.9)	7 (53.9)	1
Severe oligospermia	1 (5.9)	5 (29.4)	0.1748	4 (30.8)	3 (23.1)	1
Azoospermia	0	2 (11.8)	0.4848	1 (7.7)	1 (7.7)	1
Leukocyte count	2 (12.5)	5 (29.4)	0.3983	1 (7.7)	2 (18.2)	0.5761
Ejaculated volume (mL)	1.5 (0.8)	1.2 (1.0)	0.3826	1.0 (1.7)	1.0 (1.3)	0.6470
Concentration (10^6/mL)	72.5 (110.0)	47.0 (67.3)	*0.0022*	14.0 (26.5)	13.0 (65.3)	0.2744
Total ejac. sperm (10^6)	122.8 (133.5)	17.0 (80.7)	*0.0017*	22.0 (28.8)	13.0 (66.6)	0.2334
Total motility (%)	64.5 (25.0)	52.0 (29.0)	0.1926	41 (40.5)	43.5 (25.5)	0.1592
Progressive motility (%)	39.0 (29.0)	31.0 (32.0)	0.2187	18.5 (25.5)	18.0 (26.5)	0.5091
Normal morphology (%)	3.0 (2.0)	2.0 (3.0)	0.9143	0.0 (2.5)	1.0 (3.0)	0.3750
Sperm DNA fragmentation						
Class I (%)	12.5 (19.0)	30.5 (33.0)	*0.0049*	25.5 (21.0)	26.5 (36.0)	0.7969
Class II (%)	33.5 (18.5)	29.0 (16.5)	0.4505	36.0 (34.0)	31.0 (18.0)	0.3624
Class III (%)	41.0 (30.5)	23.0 (15.5)	*0.0155*	27.0 (9.0)	16.5 (19.5)	0.6289
Class IV (%)	7.0 (16.0)	5.5 (21.5)	0.8441	7.0 (18.0)	8.5 (19.5)	0.5586

Values expressed in median (interquartile range) or *n* (%). *p* value <0.05 was considered significant

Values in italics are statistically significant ($p < 0.05$)

effects observed in the variables studied. For this analysis, BMI loss in percentage was calculated (Fig. 2).

Weight loss is strongly correlated to higher blood levels of TT, FT, and SHBG, with an adjusted R^2 value of 0.7031, 0.3020, and 0.4701. The adjusted R^2 value indicates the amount of change in the numerical variable attributable to BMI variation, and higher values indicate a stronger correlation. In this model, for example, a negative variation in BMI of 10% resulted in an elevation of the predicted value of TT of 4.55 nmol/L. Statistically significant linear regression models were also obtained for total ejaculated sperm count and FSH levels.

Additional Analysis

In order to determine if surgery type had any effect on the results shown previously, we separately analyzed the results. The small sample size of patients submitted to VG reduces the power of statistical analysis; therefore, data should be analyzed with care. Results are shown in Table 7. We still observed a postoperative increase in total and free testosterone and reduction in sperm concentration and total ejaculated sperm count, in both groups.

Discussion

The influence of obesity over reproductive hormones and semen parameters has been extensively studied. In 2010, a meta-analysis conducted by MacDonald et al., including 18 articles and approximately 15,000 patients, concluded that higher BMI is strongly related to lower TT and SHBG levels. E2 and FT levels, however, have a weaker association. In our data, we observed higher E2, LH, and FSH levels and markedly lower total testosterone in patients suffering from severe obesity.

Regarding semen parameters, results are less clear. Most studies that tried to address this issue included in the analysis patients from infertility clinics, which reduce the potential for generalization of results. In 2010 Paasch et al., in a case-cohort study with 2157 patients, found that BMI was inversely correlated to total sperm count, only in patients between 20 and 30 years old [42]. Patients in this study, however, were all attending a fertility clinic. In 2008, a study from Aggerholm et al. included more than 2000 men from 8 European countries, with no previously known infertility issues, and could not identify changes in sperm parameters in overweight men or in men suffering from obesity [43]. In this article, however, only 8% of the included patients were suffering from obesity, and patients with obesity were only classified in a single group of patients with BMI over 30 kg/m^2. In the same manner, the meta-analysis from MacDonald et al. contained five articles that included all patients suffering from obesity in only one group, and the compilation of results did not show any variations in sperm parameters [18]. Additionally, two of the included studies contained patients seeking fertility care. Once again, the same strategy was applied in the meta-analysis published by Campbell et al. in 2015, and no clinical significant differences were found for conventional semen parameters between patients with a BMI less than 25 and more than 30 kg/m^2 [44]. This simplification of obesity classification

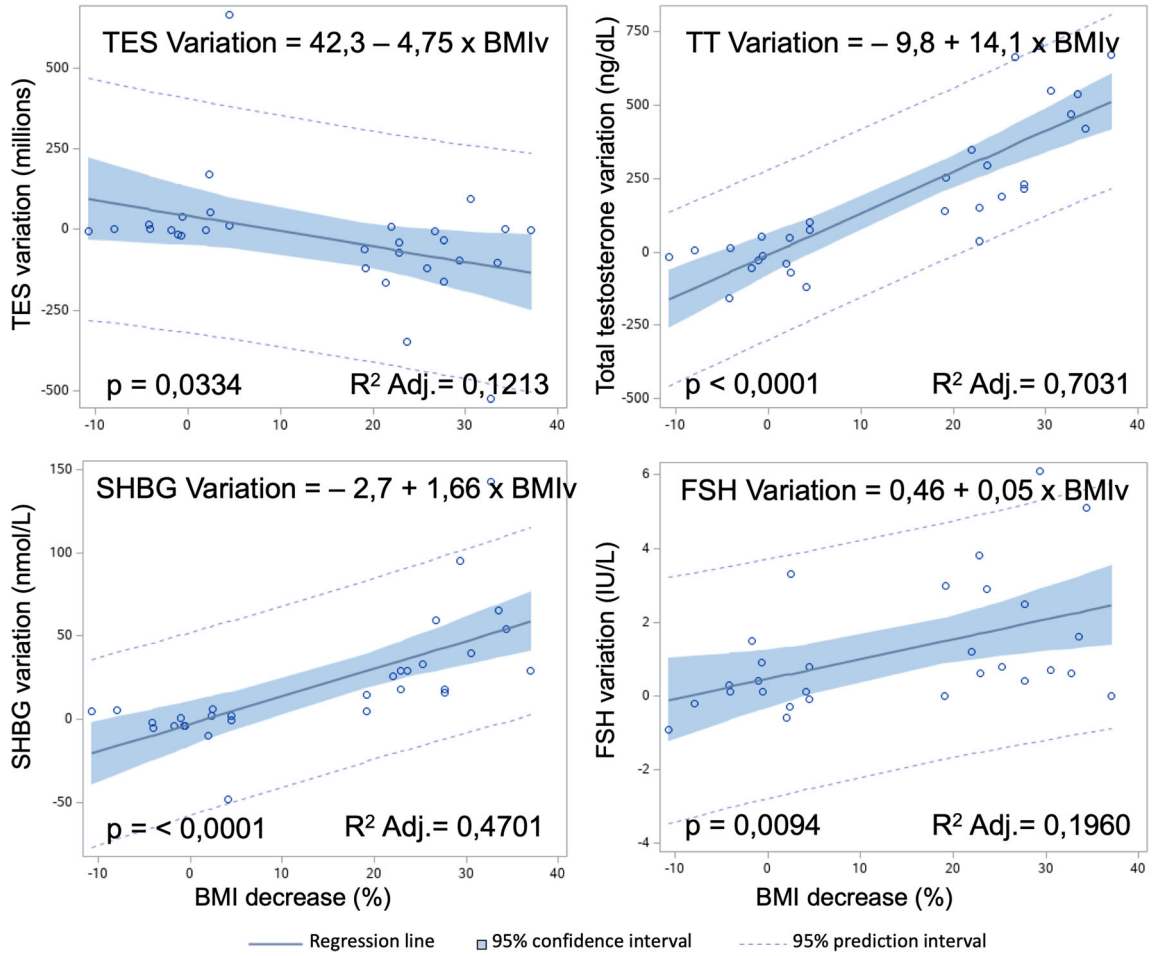

Fig. 2 Linear regression charts. The linear regression line represent the variation of the analyzed variables acording to the BMI changes. Area between dotted lines represents the predicted value of the variable according to the BMI level, with 95% confidence intervals. BMIv, body mass index variation (%); TES, total ejaculated sperm

limits the ability of these studies to determine the effects of severe obesity in semen parameters, which is probably more harmful to testicular function.

In 2013, Sermondade et al. published a new meta-analysis with the use of some methodological differences [45]. This time the authors included complete data from more than 13,000 patients, coming from 20 studies, in opposition to the previous meta-analysis that included only the final conclusions from the analyzed studies. Authors concluded that azoospermia and oligospermia were more frequent in patients suffering from obesity, observing higher rates in patients with morbid obesity (odds ratio 1.31 and 1.97, respectively). Posteriorly, Eisenberg at al. in 2014 studied the association between BMI and seminal parameters, in a cohort of 500 couples trying to establish natural conception [46]. Once again, authors found that patients with obesity had 19 times more chance of being oligospermic when compared with men with normal BMI.

The well-known limitations of conventional semen analysis lead to specialists to resort to additional functional tests to identify the fertility potential of individuals, such as the sperm DNA fragmentation test and evaluation of sperm acrosome reaction. In 2014, Samavat et al. showed that sperm acrosome reaction is impaired in patients with obesity, which could lead to reduced fertilization rates [47]. Fewer studies, however, tried to establish the association between obesity and SDF. The concentration of fat tissue in the pubic area, scrotal lipomatosis, and sedentarism have all been related to higher scrotal temperature, resulting in higher oxygen reactive species (ROS) production [48]. Additionally, the chronic pro-inflammatory state present in patients with obesity, related mainly to adipokines production, will also lead to an increase of free radicals and disruption of the balance between ROS and antioxidant capacity [49]. While studies from Kort et al. and Fariello et al. suggest a positive relation between elevated BMI and SDF, Bandel et al. did not find any association between both in patients from 4 European cohorts [50–52]. In the latter study, however, all patients were 18 years old, which may have contributed to the absence of identified changes. Lastly, the absence of concordance between studies on sperm

Table 7 Phase 2: variation of hormones and semen parameters divided by surgical type

	Roux-en-Y gastric bypass			Vertical gastrectomy		
	Baseline	6 months	*p* value	Baseline	6 months	*p* value
Hormone evaluation		*N* = 15			*N* = 3	
Estradiol (pg/mL)	42.9 (24.5)	34.4 (13.2)	0.1747	26.2 (33.7)	46.2 (40.8)	0.5078
LH (IU/L)	5.3 (3.5)	6.5 (4.2)	0.6745	7.9 (4.4)	7.1 (5.2)	0.2662
FSH (IU/L)	3.9 (1.2)	5.1 (4.6)	*0.0005*	5.2 (6.2)	6.1 (9.1)	1.000
Prolactin (ng/mL)	10.6 (4.9)	6.8 (4.0)	*0.0049*	7.6 (2.3)	6.2 (1.1)	0.5000
SHBG (nmol/L)	35.5 (12.6)	68.8 (43.0)	*0.0002*	31.2 (21.8)	60.3 (52.2)	0.2500
Total testosterone (ng/dL)	312 (187)	556 (416)	*< 0.0001*	127 (191)	765 (213)	*0.0484*
Free testosterone (pmol/L)	202 (93)	262 (113)	*0.0075*	102 (151)	291 (230)	0.1228
Sperm analysis		*N* = 14			*N* = 3	
Oligospermia	1 (7.1)	5 (35.7)	0.1647	1 (33.3)	2 (66.6)	1.000
Severe oligospermia	1 (7.1)	4 (28.6)	0.3529	0	1 (33.3)	1.000
Azoospermia	0	2 (14.2)	0.4815	0	0	–
Ejaculated volume (mL)	1.5 (1.3)	1.3 (1.0)	0.3096	1.2 (1.1)	1.0 (1.6)	1.000
Concentration (10^6/mL)	77.0 (101.0)	48.5 (67.3)	*0.0056*	15.0 (345.5)	8.5 (214.4)	0.2500
Total ejac. sperm (10^6)	130.0 (130.0)	28.6 (94.3)	*0.0052*	22.5 (429.8)	17.0 (85.4)	0.2500
Total motility (%)	68.0 (26.0)	57.5 (22.5)	0.6873	61.0 (30.0)	12.0 (18.0)	0.1407
Progressive motility (%)	38.0 (35.0)	34.5 (31.5)	0.5960	40.0 (29.0)	12.0 (18.0)	0.1278
Normal morphology (%)	3.0 (3.0)	2.5 (2.5)	1.000	2.0 (3.0)	0 (4.0)	1.000
Sperm DNA fragmentation						
Class I (%)	16.6 (19.0)	36.0 (32.0)	*0.0181*	9.0 (19.0)	24.0 (29.0)	0.2500
Class II (%)	35.0 (15.0)	27.0 (20.0)	0.5270	27.0 (21.0)	32.0 (20.0)	0.7302
Class III (%)	38.0 (26.0)	18.0 (14.0)	*0.0063*	48.0 (43.0)	26.0 (28.0)	1.0000
Class IV (%)	6.0 (14.0)	5.0 (24.0)	0.9873	19.0 (32.0)	19.0 (18.0)	1.0000

Values expressed in median (interquartile range) or *n* (%). *p* value < 0.05 was considered significant. Care is advised in the interpretation of the results due to the small sample of patients submitted to vertical sleeve gastrectomy

Values in italics are statistically significant ($p < 0.05$)

chromatin integrity suggests that there may be still some unidentified factors that may be present in part of the patients with obesity, such as comorbidities, sedentarism, and dietary patterns, leading to higher DNA damage in some of them [53].

On behalf of bariatric surgery, data currently available supports that the procedures can bring sex hormone levels to normality. A meta-analysis published by Lee et al. in 2018 showed that bariatric surgery elevates TT, FT, FSH, LH, and SHBG and can reduce PRL and E2 levels [54]. The greatest limitation of this analysis consists of the large heterogeneity of the studies included, since there are great design variabilities, different populations and surgical techniques, and several articles contained small samples. Those limitations are compatible with the difficulties involved in the conduction of randomized controlled studies including surgical interventions. Results presented here add more body to those conclusions. Here we have shown that bariatric surgery can result in dramatic changes in the reproductive hormones profile of patients with obesity, in a relatively short follow-up. Moreover, patients that experienced greater weight loss had bigger postoperative changes in TT levels, in a clear "dose-dependent" effect. These results are probably long-lasting. In 2018, Pham et al. published results from 5 years of post-bariatric follow-up [55]. In this study, TT and FT were 80 and 50% elevated in operated patients, and those effects were also more pronounced in patients that were able to keep a weight loss greater than 15% of the initial weight.

The expected improvement of sperm parameters following the results observed on reproductive hormones, however, was not observed here. Patients submitted to surgery presented important reduction in sperm concentration (72.5 to 47 millions/mL, $p = 0.0022$) and in total ejaculated sperm count (122.8 to 17 millions/mL, $p = 0.0017$). Linear regression also suggests that the intensity of this reduction was also correlated with the percentage of weight loss. This is still a controversial matter in the literature. In 2016, El Bardisi et al. reported improvement of sperm parameters in oligospermic patients submitted to VG and return of sperm to the ejaculate of six

previously azoospermic patients [28]. Nevertheless, a meta-analysis published by Lee et al. in 2018 that included 3 retrospective studies could not identify postoperative semen changes [28, 31, 54, 56]. Sub-analysis containing only patients submitted to RYGB from two case series suggests a tendency to worsening of semen parameters after surgery [29, 30]. Considering that the population included in the present study is mostly composed of patients submitted to RYGB, we can deduce that the metabolic and nutritional changes secondary to this procedure are more harmful to spermatogenesis than those caused by other techniques and may be more important than the benefits of weight loss. More studies containing larger samples of patients, however, are needed to verify this hypothesis.

Few studies so far tried to identify SDF variations after bariatric surgery. In 2017, Samavat et al., in a study with a similar design to this one, were not able to find differences in sperm SDF 6 months after RYGB, measured by the TUNEL technique [31]. Recently, a study conducted by Carette et al. at the same time as ours and published in 2019 reached results very similar to the results we present here [57]. In this study, 46 patients submitted to bariatric surgery (RYGB or VG) presented in 6-month to 1-year follow-up reduction in sperm concentration and improvement of SDF. Our results suggest that despite the negative variation on classic sperm analysis parameters, sperm chromatin integrity improves after bariatric surgery.

Results presented here are probably secondary to the metabolic changes induced by bariatric surgery and to its evolution with time. In 2009, Leichman et al. showed that, on the first three postoperative months, patients already experience lowering of fasting glucose and of insulin levels and increase in serum leptin and normalization of insulin resistance [58]. It is possible to conclude, therefore, that a large amount of the metabolic changes of bariatric procedures happens before the major weight loss, even in the first few postoperative weeks or days [59]. Insulin resistance can reduce SHBG levels, increasing the percentage of free E2. The negative feedback exerted by E2 reduces FSH and LH levels and, consequently, TT [15]. Probably, the hormonal profile presented by the operated patients in this study is secondary to those early phenomena. Classic semen parameters, however, did not follow those improvements. First, a complete spermatogenesis cycle can take up to 74 days [60]. The 6-month follow-up may have been insufficient to result in positive changes in semen analysis. Additionally, the improvement in reproductive hormone levels may have been counterbalanced by nutritional deficiencies and by the massive weight loss experienced by the subjects. Regarding the reduction in SDF, the important weight loss may have resulted in an improvement of the chronic pro-inflammatory state caused by obesity, with ROS reduction. Moreover, better temperature regulation of scrotal temperature may have also reduced sperm chromatin damage. Future studies with seminal ROS evaluation, characterization of those variations over time, and correlation with systemic inflammatory markers may help establish this physiopathology.

This study has limitations. A selection bias, inherent to all non-randomized studies, may limit generalization of results obtained. However, randomized controlled studies with surgical procedures are ethically debatable and are becoming increasingly rarer. Moreover, the fact that all patients were waiting for bariatric surgery at the beginning of this study and are all originated from the same healthcare division reduces the probability of selection bias. Additionally, only one sperm sample was obtained from each subject in each of the study time points. Despite possible variations in semen analysis collected from the same subject in different occasions, previous studies have shown that two sperm samples are not superior to only one in research studies [61, 62].

In conclusion, this study suggests that some of the deep detrimental changes caused by severe obesity over the reproductive health of individuals may be improved after bariatric surgery, in a 6-month follow-up. Patients experience an increase in TT, FT, FSH, and SHBG and reduction in prolactin levels. While improvement in sperm DNA fragmentation was observed, classic semen parameters may be impaired after surgery. Further studies are necessary to establish if those changes are stable over time and if long-term weight regain may influence those findings. Our recommendation is that infertility counseling with a urologist prior to bariatric surgery should be mandatory to all male patients with reproductive intentions and fertility preservation procedures such as sperm freezing should be discussed.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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