EARLY EVALUATION OF HORMONAL, SEMINAL AND FUNCTIONAL SPERM PROFILE OF OBESE PATIENTS UNDERGOING BARIATRIC

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ABSTRACT: **Background:** Obesity is being considered a global epidemic. This condition is associated to diabetes, cardiac dysfunctions and fertility problems. The treatment indicated to obese people (degree 3) is the bariatric surgery which in long term improves some physiological alterations including male reproductive aspects; but the early consequences of BMI reduction in male fertile potential still unknown. **Objectives:** To verify the early weight loss effects resulting from bariatric surgery in obese men in sexual hormonal

profile, seminal parameters, sperm functional features and seminal lipid peroxidation levels **Materials and methods:** Patients who had a bariatric surgery indication made available blood and seminal sample to two analysis moments: before the surgery and 90 days after the procedure. The obtained blood samples were used to the hormonal profile analysis and, with the seminal sample, the conventional seminal analysis was performed, besides sperm DNA integrity evaluation, sperm mitochondrial activity analysis and evaluation of lipid peroxidation. **Results:** After 90 days of the surgery, the body weight and consequently the BMI considerably decreased; moreover, the total testosterone levels improved and estradiol was decreased. In relation to semen analysis, just the round cell parameter showed significant results with amount reduced. No significant results were found in the sperm DNA integrity, sperm mitochondrial activity and lipid peroxidation levels. Lastly, correlation analysis showed a positive correlation between BMI and estradiol and BMI and round cells. **Discussion and Conclusion:** Knowing the moment when the overweight loss begins to improve the fertile potential of obese men supports a better and organized clinical management for his reproductive treatment. Thus, we concluded that the early weight loss resulting from the bariatric surgery is already sufficient to positively modify the hormonal profile and the seminal quality of the obese man; however, it is not enough to improve the sperm functional quality.
KEYWORDS: Infertility; obesity; bariatric surgery; semen analysis; hormonal profile.

INTRODUCTION

Obesity is defined as the excessive accumulation of adipose tissue that can reach levels harmful to health[1]. This condition is diagnosed by Body Mass Index (weight (Kg)/ height (m)^2 - BMI) and individuals with results equal or above 30 kg/m^2 are classified as obese [2]. Moreover, obesity can be branched into three degrees: degree 1 which results in a BMI between 30 to 34,9 kg/m^2; degree 2, BMI between 35 to 39,9 kg/m^2 and degree 3 to BMI equal or superior to 40 kg/m^2 [3].

This condition is being considered a global epidemic. In 2016 more than half a billion adults worldwide were classified as obese[4]; in 2030, considering the secular tendency, there is a projection that this value will exceed 1 billion adults affected by this disease[5]. The increase in the number of obese individuals is accompanied by the growing of the consequences accrue of this condition, amongst them: diabetes[6], vascular and cardiac dysfunctions[7], cancer[8] and fertility problems[9,10]. Regarding the male fertility, obesity modifies the reproductive hormonal profile[11] and decreases seminal quality impairing the sperm functional aspects and altering the seminal plasma components important to the fertilizing moment[12,13].

The bariatric surgery is the treatment indicated to people diagnosed with obesity degree 3 [14]. According to the American Society for Metabolic and Bariatric Surgery, in 2017, 228,000 surgeries were performed in the USA [15]. This treatment leads to weight loss, and consequently a BMI reduction through restriction in the amount of food that the stomach can receive [16].

After the first months of the surgical procedure weight loss can already be observed indicating the effectiveness of this treatment[17–19]. It has already been demonstrated that in long term (over one year) such slimming conduct improves diabetes signals, hyperlipidemia and hypertension in these individuals [20]; in addition, increased testosterone levels, raised sperm concentration [21], changes in cellular viability [22] and improved sexual performance can be observed [23,24]. However, it is yet not known if the early BMI reduction is already capable of softening obesity effects over the male fertile potential, considering that spermatogenesis takes around 60 to 70 days to occur completely [25].

Thus, the purpose of this study was to verify the early weight loss effects resulting from bariatric surgery in obese men in sexual hormonal profile, seminal parameters, sperm functional features and seminal lipid peroxidation levels.

MATERIALS AND METHODS

Study design

This study was approved by the Institutional Review Board. Twenty-two men were included, who had a bariatric surgery indication by the Gastrointestinal Surgery Outpatient Clinic. The inclusion criteria were, as follows: age between 20 and 55 years old and enough BMI value to undergo the surgical procedure (BMI ≥40 kg/m^2 or BMI ≥ 35 kg/m^2 associated with a comorbidity); those men who had performed vasectomy surgery were excluded.

After the inclusion and exclusion criteria analysis, 10 patients were excluded from our study and the final number of included patients was 12 men. Thus, after the Informed Consent Form signature, each participant collected blood and seminal samples in two analysis moments: before the surgery (T0) and 90 days after the procedure (T1); in these moments weight and height of each patients were al measured which in turn were used to calculate BMI. Therefore, each patient represented his own control. The obtained blood samples were used to the hormonal profile analysis and, with the seminal sample, the conventional seminal analysis was performed, besides sperm DNA integrity evaluation, sperm mitochondrial activity analysis and evaluation of lipid peroxidation (Fig. 1).

Surgical Procedure

The Roux-en-Y gastric bypass surgery was the bariatric surgery performed in the studied patients. This technique can be done by laparotomy or laparoscopy. Such procedure consists in the proximal gastric pouch formation resulting in a Y-shaped stomach that has direct exit to the small intestine. Clips are introduced during the surgery with the purpose of performing the separation of the proximal stomach from its other parts [26].

Hormonal profile analysis

The peripheral blood samples were collected between 7 and 8 am respecting fasting food period of 12 hours. The dosed hormones were the following: Total Testosterone (TT), Free Testosterone (FT), Luteinizing Hormone (LH), Stimulating Follicle Hormone (FSH), Estradiol (E2) and Prolactin (PRL). The blood sample was centrifuged and the obtained serum was stored at -80 ºC until the moment of the analysis. Then, an automatized system (Modular E, Roche Diagnostics GmbH) was applied utilizing the chemiluminescent immunoassay technique with the corresponding commercial kit.

Semen analysis

The seminal sample was collected after 2 to 5 days of ejaculatory abstinence period. The collection occurred by masturbation in appropriate room attached to the andrology laboratory of the Human Reproduction Section. After sample liquefaction time the semen analysis was performed according to World Health Organization (WHO) recommendation[27] and the sperm morphology was evaluated by the Kruger's criteria [28]. The reminiscent seminal volume was divided in aliquots to be used in the sperm functional analysis and seminal lipid peroxidation analysis.

Sperm DNA integrity assessment

For the sperm DNA integrity evaluation, the Sperm Chromatin Dispersion (SCD) test was performed, according to what was proposed by Fernández *et al*. [29].

Aliquots of fresh semen were diluted in 1% Low Melting Point Agarose (LMPA) (GE Healthcare, Amersham, England) to obtain sperm concentrations ranging between 5 and 10 million / mL. After, 50 µL of the solution were deposited into a microscopy slide (26x76mm, Precision Glass Line, China) previously prepared with 1000µL Normal Melting Point Agarose (NMPA) (GE Healthcare, Amersham, England) in 1% TBE (0.089 M Tris, 0.089 M borate and 0.002 M Na2 EDTA). The slides were covered with a coverslip (24x60 mm) and stored at 4° C for 4 minutes to solidify. Then, the coverslips were removed and slides were immersed in freshly prepared solution of denaturation acid (0.08 N HCl) for 7 minutes at 22°C in the dark. Denaturation was then completed and proteins were removed by the lyses neutralization solution 1 (0.4 M Tris, 0.8 M DTT, 1% SDS and 50 mM EDTA pH 7.5) for 10 minutes at room temperature followed by incubation with lyses neutralization solution 2 (0.4 M Tris, 2 M NaCl, and 1% SDS, pH 7.5) for 5 minutes at room temperature. The slides were washed with TBE (0.09 M Tris-borate and 0.002 M EDTA, pH 7.5) for 2 minutes, sequentially dehydrated in baths of 70% ethanol, 90% and 100% (2 min each) and air dried.

The samples were flushed with panoptic hematological staining reagent (Laborclin Laboratory Products Ltd.) and the cells were observed in light microscopy. Cells that

presented an intact DNA, a halo formation around the sperm head could be observed, and, in cells with fragmented DNA the halo expansion was not viewed. So, a total of 200 sperm were evaluated and classified according to the observed halo around its sperm head: A) big halo; B) middle halo; C) little halo and D) halo absence.

Sperm mitochondrial activity

The sperm mitochondrial activity was evaluated by the 3,3' diaminobenzidine (DAB) coloring, according to what was proposed by Hrudka [30]. This technique is based on the DAB oxidation by the cytochrome c oxidase enzyme; the reagent is polymerized and deposited in the mitochondrial sheath arranged along the sperm middle piece.

An aliquot of fresh semen was added to a solution containing 1mg/mL DAB in PBS (137mm NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.4 mM KH2PO4) in a ratio of 1:1 to 1:3 and incubated for 1 hour in a water bath at 37° C in the dark. After the incubation period, two smears were prepared on microscope slides with 10µL. After drying, the slides were fixed in 10% formaldehyde for 10 minutes.

Two hundred sperm were evaluated by phase contrast microscopy (Olympus BX51) in 100x magnification and were classified in 4 classes: class 1 (100% mitochondria stained), class 2 (more than 50% of mitochondria stained), class 3 (less than 50% of mitochondria stained) and class 4 (no mitochondria stained).

Evaluation of lipid peroxidation

The lipid peroxidation levels of the seminal sample were evaluated by the Thiobarbituric Acid Reactive Substances (TBARS) method, proposed by Ohkawa *et al.*[31]. This protocol performed in greatest temperatures and low pH is based in a reaction between two thiobarbituric acid (TBA) molecules with one malondialdehyde (MDA) molecule resulting in the MDA $(TBA)_2$ complex formation.

To precipitate proteins, 500µL of seminal plasma and 1000µL of a 10% solution (v:v) of trichloroacetic acid (TCA 10%) were mixed and centrifuged (16.000 x g for 15 min at 15ºC). After centrifugation, 500µL of the supernatant and 500µL of 1% (v:v) thiobarbituric acid (TBA, 1%), in 0.05N sodium hydroxide in glass tubes were placed into a boiling water bath (100ºC) for 10 min, and subsequently cooled in an ice bath (0ºC) to stop the chemical reaction.

The TBARS was then quantified using a spectrophotometer at a wavelength of 532nm. A standard curve was previously prepared with a standard solution of malondialdehyde for the results comparison. The results were described in TBARS nanograms/mL of semen.

Statistical analysis

The statistical analysis was performed in the SPSS 18.0 software. In order to verify the data normality, the Kolmogorov-Smirnov test was applied; in the variables with normal distribution, the parametric paired Student's t test was performed, and in the variables without normal distribution, the non-parametric paired Wilcoxon test was applied. For those variables that showed statistical significance, a correlation existence evaluation was performed by the Pearson test and when the correlation was found, a linear regression was applied.

The results were considered significant when the $p<0,05$.

RESULTS

The results of age, weight, BMI and hormonal profile of the patients, before and after the surgery are in Table 1. As expected, after 90 days of the surgery, the body weight ($p < 0,0001$) and consequently the BMI ($p < 0,0001$) considerably decreased.

In relation to hormonal profile of these men, we can observe that after the surgery, the blood TT levels increased ($p = 0,025$) and the amount of E2 decreased ($p < 0,0001$). No significant results were found for blood hormone levels FT ($p = 0,101$), PRL ($p = 0,188$), FSH ($p = 0,804$) e LH ($p = 0,055$).

When we compare the seminal sample parameters before and after the weight loss surgery, a significant result was found in the round cell parameter ($p = 0,025$) which reduced with the BMI reduction. No other variable analyzed in this context showed a significant result (Table 2).

In the sperm DNA integrity (SCD) analysis, sperm mitochondrial activity (DAB) analysis and seminal lipid peroxidation levels (TBARS) evaluation, no significant results were found when comparing samples before and after surgery (Table 2).

Lastly, the correlation analysis was applied among the following variables that showed significant results: BMI, TT, E2 and round cells. Thus, the statistic showed the presence of a median effect correlation between BMI and E2 ($R2 = 0.601$ and $p < 0.0001$) and a weak correlation between BMI and round cells ($R2 = 0.197$ and $p = 0.030$).

DISCUSSION

The obesity interferes in the male fertile potential through a multifactorial cause. The accumulation of adipose tissue observed in these individuals is able to promote a gonadal pituitary hypothalamus axis modification leading to a high conversion of androgens in estrogens [32]. This tissue is capable of releasing pro inflammatory proteins resulting in a low-grade systemic inflammation [33,34]. Besides that, the tissue accumulation in the region of the testicles increases the local temperature changing its physiological function [35]. All these

processes act in synergy harming sperm production and/or altering sperm function [12,13].

The bariatric surgery, more specifically the Roux-en-Y gastric by-pass (RYGB) methodology, consists in the volume stomach reduction in a smaller organ which is then directly linked to the jejunum [36]. This technique with a restrictive and non-absorbing purpose, leads to about 70 to 80% of the overweight loss of the individual reaching their weight loss peak within 1 to 2 years after the procedure [37,38].

Wittgrove and your team followed, for a period of 3 to 30 months, patients who were submitted to the RYGB surgery. Three months after the procedure, the patients presented an average early weight loss of 45% [39]. In our study, we can notice that within 3 months of post-surgical period there was an average early loss of 22,7% of the patient's weight confirming the effectiveness of the treatment for obese individuals.

The weight loss of these patients contributed to the alteration in the TT and E2 hormone levels, as noted by Samavat *et al.* [22,40]. The adipose tissue releases acting enzymes in the conversion of androgens in estrogens promoting changes in the levels of these hormones in the obese individuals [40,41]; thus, the early decrease in the amount of this tissue resulting from surgical treatment has already been shown to be sufficient to inhibit enzymatic action by causing testosterone levels to increase and estradiol to decrease.

Besides the endocrine change, the amount of adipose tissue is capable of modifying the seminal quality mainly interfering in the sperm concentration, motility and sperm morphology and the decrease of the adiposity can contribute to the improvement of these aspects [42,43]. Just 3 months after the surgery, and with the occurrence of a complete spermatogenesis, the resulting weight loss was capable of beginning seminal parameter modifications generating a reduction in the number of round cells. These cells can represent the presence of immature sperm in the ejaculate [44] resulting probably from the testicular heating caused by the adipose tissue excess in the scrotal region [45]. This result shows us that early weight loss reaches the adipose tissue excess in the testicular region mitigating the physiological effects that warming brings on sperm production.

The testicular heating along with other factors, such as inflammatory state coming from adipose tissue excess, can together increase the cellular metabolism modifying the microenvironment and hemodynamic of the testicles creating the Oxidative Stress (OE) (imbalance between the production of reactive oxygen species and antioxidants absorbing the species [46]) [47,48]. This stress is extremely harmful to the sperm once this cell contains a membrane rich in polyunsaturated fatty acids; this membrane composition is susceptible to damage, such as the lipid peroxidation (LP). The LP is the resulting process of the interaction between the OE and such fatty acids that results in the sperm membrane alteration which may lead to a decreased activity of its mitochondria and fragmentation of its DNA [49–52].

We did not achieve significant results in the analysis related to DNA fragmentation, sperm mitochondrial activity and seminal lipid peroxidation levels. This fact can suggest that early weight loss in obese patients is not enough to interfere in OE and its consequences,

since there is still some amount of adipose tissue. After 6 months of surgery, Samavat et al. also did not observe results related to sperm DNA [22].

The main purpose of this study was to verify if the early weight loss resulting from just 3 months of post-surgical period which is already able to generate effects on the sperm production and sex hormones. With our results indicating that improvements can already be seen, clinical management for men in reproductive age can be better organized and planned. However, we must report that the limitation of our analyzes is the reduced number of study individuals, since such recruitment is difficult because it requires sample collection before and after a surgical technique and, in addition, this procedure is more performed in women, as seen in the USA [53].

Thus, we concluded that the early weight loss resulting from the bariatric surgery is already sufficient to positively modify the hormonal profile and the seminal quality of the obese man; however, it is not enough to improve the sperm functional quality.

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DISCLOSURES

The authors have no conflict of interest to disclose

AUTHOR CONTRIBUTION STATEMENT

C.F.L.: conception and design of the study, acquisition of samples for analysis, interpretation of data and drafting of the article; R.C.C.: data analysis, revision and submission final article; C.S.: data analysis and revision of the article; C.R.A.C.: interpretation of date and revision of the article; D.M.S.: acquisition of samples and revision of the article; E.M.C.J: patient screening and realized the bariatric surgery; R.F.: conception and design of the study and drafting of the article.

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Figure legend

Figure 1. Study design

Tables

Age (years)	T0	T1	p
	34,5; 7,8		
Weight (kg)	139,3; 23,0	107,7; 14,1	<0,0001*
BMI (kg/m2)	43,7; 7,2	33,5; 5,1	<0,0001*
TT (ng/dl)	230,0; 91,8	325,6; 128,3	0,025*
FT (ng/mL)	6,1; 1,9	7,4; 2,5	0,101
E2 (pg/mL)	32,5; 10,1	23,3; 10,9	<0,0001*
PRL (ng/mL)	5,1; 2,2	5,7; 2,5	0,188
FSH (mUI/mL)	4,5; 1,8	4.4; 1,6	0,804
LH (mUI/mL)	4,7; 1,0	5,6; 1,7	0,055

Data presented in mean and standard deviation; * significance difference – Student's t paired and Wilcoxon paired test

Table 1 Age, anthropometric analysis and hormonal profile of patients before (T0) and 90 days (T1) after Roux-en-Y Gastric Bypass Surgery.

	T0	T1	p
Volume (mL)	2,2; 0,7	2,3; 0,7	0,731
Motility PR (%)	50,4; 18,0	51,1; 13,9	0,797
Motility NP (%)	4,3; 2,9	6,0; 2,7	0,081
Immotility (%)	41,0; 20,5	38,6; 17,7	0,343
Concentration (x10^6/mL)	50,1; 77,1	62,6; 92,6	0,086
Total count (x10^6)	86,9; 112,4	123,5; 156,5	0,056
Morphology (%)	4,2; 3,9	5,0; 3,5	0,288
Round cells (x10^6/mL)	4,7; 8,9	1,7; 1,8	0,025*
Neutrophils (x10^6/mL)	3,9; 8,5	1,0; 1,5	0,13
SCD A+B (%)	45,5; 16,9	48,3; 17,4	0,198
SCD C+D (%)	46,1; 17,1	43,3; 16,0	0,198
DAB I (%)	2,3; 5,0	0,9; 2,3	0,144
DAB II (%)	30,5; 17,8	34,0; 19,6	0,364
DAB III (%)	40,7; 18,4	35,0; 16,1	0,137
DAB IV (%)	18,0; 10,7	21,6; 13,3	0,133
TBARS (ng/mL)	286,1; 46,9	272,8; 37,3	0,328

Data presented in mean and standard deviation; * significance difference – Student's t paired and Wilcoxon paired test motility PR, total progressive motility; motility NP, non-progressive motility.

SCD – evaluation tests for sperm DNA; DAB – evaluation test for sperm mitochondrial activity; TBARS - seminal test for lipid peroxidation

Table 2 Semen analysis, sperm DNA integrity (SCD), sperm mitochondrial activity (DAB) and TBARS analysis of patients before (T0) and 90 days (T1) after Roux-en-Y Gastric Bypass Surgery.

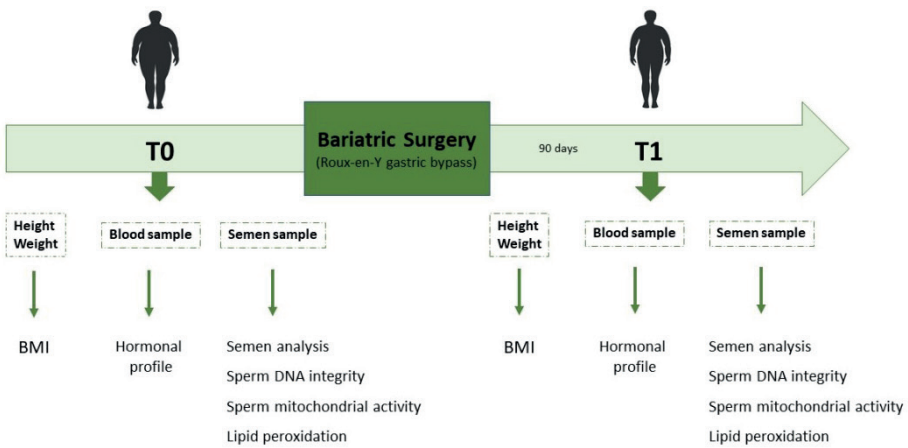

Figure 1. Study design

338x190mm (96 x 96 DPI)