Acrosome reaction is impaired in spermatozoa of obese men: a preliminary study

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Objective: To compare spontaneous (Sp-AR) and P-induced acrosome reaction (AR) in spermatozoa of obese and lean subjects.
Setting: Bariatric unit at a university hospital.
Design: Prospective, observational study.
Patient(s): Twenty-three obese (mean ± SD body mass index [BMI], 44.3 ± 5.9 kg/m^2) and 25 age-matched lean (BMI, 24.2 ± 3.0 kg/m^2) subjects.
Intervention(s): None.
Main Outcome Measure(s): Spontaneous and P-induced AR in spermatozoa of obese and lean subjects.
Result(s): A statistically significant difference was found between obese and lean cohorts in total T and calculated free T, E_2, glycated hemoglobin, and high-density lipoproteins, whereas among the routine semen parameters analyzed, only immotile sperm percentage and ejaculate volume differed significantly. Spermatozoa of obese (n = 13) vs. lean men (n = 19) showed a higher Sp-AR (17.9% ± 7.2% vs. 8.3% ± 4.2%), which resulted in a reduced ability to respond to P evaluated as the AR-after-P-challenge parameter (3.5% ± 3.2% vs. 17.6% ± 9.2%). Multivariate analysis adjusted for age revealed a significant correlation between BMI, waist, E_2, and glycated hemoglobin with both Sp-AR (age-adjusted $r = 0.654$, $r = 0.711$, $r = 0.369$, and $r = 0.644$, respectively) and AR-after-P-challenge (age-adjusted $r = -0.570$, $r = -0.635$, $r = -0.507$, and $r = -0.563$, respectively). A significant difference in sperm cholesterol content was reported between obese and lean men (29.8 ± 19.5 vs. 19.1 ± 14.6 ng/μg of proteins).
Conclusion(s): Sperm AR is impaired in obese men, showing reduced response to P and elevated Sp-AR, associated with altered circulating levels of E_2 and sperm cholesterol content. (Fertil Steril® 2014;102:1274–81. ©2014 by American Society for Reproductive Medicine.)
Key Words: Acrosome reaction, obese, semen analysis, sex hormones, estradiol, cholesterol

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Recent data suggest that male obesity is associated with a high prevalence of secondary hypogonadism (1, 2), which is an endocrine condition characterized by decreased circulating levels of T in the presence of low or normal gonadotropins (3). However, reduced fertility and impaired semen quality in male obesity are still controversial (4–8). Significant differences in the cohorts of subjects analyzed may justify these conflicting results. In particular, the obese groups often consisted of subjects with a body mass index (BMI) mainly defining a mild obesity grade (30–35 kg/m^2), or the number of the enrolled obese were often limited compared with the lean and overweight subjects. Finally, the obese cohorts were often biased by being selected in infertility centers. In addition, conventional quantitative sperm parameter analysis is insufficient to predict sperm fertilization potential. In fact, functional ability of

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spermatozoa to undergo pivotal processes required for fertilization, such as acrosome reaction (AR) or zona pellucida (ZP) binding, cannot be predicted on the basis of the routine semen analysis (9).

In this scenario, the present study investigated the functional aspect of AR in spermatozoa obtained in a cohort of morbidly obese men recruited in a bariatric surgery center compared with age-matched lean subjects, in addition to the evaluation of sex steroid hormones and semen parameters. Acrosome reaction is an exocytotic process that is physiologically stimulated by a rapid influx of calcium in the sperm head induced by ZP binding or by the high levels of P present in the follicular/tubal fluid and cumulus oophorus surrounding the oocyte (10, 11). This process results in the release of the enzymes contained in the acrosomal vesicle of the sperm head, which enables spermatozoa to pass the ZP glycoprotein. To enable fertilization, AR must occur with a specific timing, when the sperm is close to the oocyte. If occurring too early or in the wrong place, acrosome-reacted spermatozoa may be no longer able to fertilize.

MATERIALS AND METHODS

Patients

Twenty-three obese men were recruited from July 1 to December 31, 2013 among the consecutive patients attending the Metabolic and Bariatric Surgery Unit of Careggi University Hospital in Florence (Italy), with BMI >36 kg/m^2 and indication for bariatric surgery as inclusion criteria only.

Twenty-five lean, aged-matched subjects were recruited among volunteers (70%) or subjects undergoing seminal analysis for couple infertility (30%) at the Andrology Center of Careggi University Hospital in Florence, with 20 kg/m^2 $\leq$ BMI $<$ 25 kg/m^2 and age as inclusion criteria only.

The presence of tumors and overt endocrine diseases, such as Cushing disease or thyroidisms, as well as medical treatments for hypogonadism, were the only exclusion criteria in both cohorts.

The rate of agreement to the study was 88% and 70% in the obese and lean cohorts approached, respectively.

Some potential confounding factors, such as smoking, alcohol consumption, and physical activity, were evaluated and did not significantly differ between the groups.

All patients enrolled gave signed, informed consent after receiving written and oral information on the study. The project was approved by the local ethics committee and institutional review board (protocol no. 83/13).

Anthropometric and Biochemical Measurements

The anthropometric measures height, weight, and waist circumference were measured in all subjects.

Biochemical tests were performed on blood samples collected after overnight fasting. Levels of the sex hormones serum total T (TT), FSH, LH, sex hormone–binding globulin (SHBG), and E_2 were measured by immunoassay (Immulite 2000; M-Medical System) on a sample of venous blood drawn in the morning between 8:00 AM and 10:00 AM (2, 12). The analytic sensitivity of the assays was 0.5 nM for TT, 55 pM for E_2, 0.1 mIU/mL for FSH, 0.05 mIU/mL for LH, and 0.02 nM for SHBG. Calculated free T (cFT) was evaluated as previously reported (13). Total T/E_2 was used as an indirect index of the aromatase activity.

Serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride were measured using a colorimetric assay (Siemens Healthcare). Low-density lipoprotein (LDL) cholesterol was determined by Friedewald's formula for serum triglyceride levels $<$400 mg/dL.

Glycated hemoglobin (HbA1c) measurement was performed on the whole-blood samples by high-performance liquid chromatography ion exchange chromatography on a VARIANT II instrument (Biorad Laboratories). The HbA1c values were used for diagnosis of diabetes at the 6.5% threshold (14, 15).

Semen Analysis

Human semen was collected by masturbation according to the World Health Organization procedures (16) the same day of blood sampling or within the following week. Semen parameters were assessed by a standard analysis (16). Sperm morphology and motility were evaluated by optical microscopy. Sperm morphology was evaluated by determining the percentage of normal and abnormal forms after Diff-Quick staining, scoring at least 100 spermatozoa per slide. Sperm motility was evaluated by determining the percentage of progressive motile, nonprogressive motile, and immotile spermatozoa by scoring at least 200 sperm per slide.

Based on the 5th percentile reference values of the distribution of seminal parameters of a fertile male population, taken as reference value (16), the pathologic conditions of azoospermia (total absence of spermatozoa in the ejaculate), oligozoospermia (total number of spermatozoa in the ejaculate $<39 \times 10^6$), and asthenozoospermia (percentage of progressive spermatozoa in the ejaculate $<$32%) were evaluated in the two cohorts of subjects.

Evaluation of AR

Spermatozoa were selected from seminal plasma by a 1-hour capacitating swim-up procedure in human tubal fluid–10% human serum albumin. Selected spermatozoa were diluted 10^7/mL and further capacitated for additional 2 hours and then stimulated or not (controls) for 1 hour with P (10 μM). Samples were then resuspended in 0.5 mL of hyposmotic swelling medium for 30 minutes at 37°C, fixed in 50 μL of ice-cold methanol, and layered on slides. After staining with fluorescent lectin (Sigma-Aldrich) for 1 hour, slides were observed under a fluorescence microscope (Axiolab A1 FL, Carl Zeiss), and AR was evaluated on a total of 100 viable spermatozoa per slide, characterized by a curly tail after incubation with hyposmotic swelling medium. The difference between the percentage of P-induced AR (P-AR) and spontaneous AR (Sp-AR) in control samples (ARPC, AR after P challenge) is considered as the net percentage of spermatozoa in the population capable of responding to P stimulation (ARPC = %P-AR − %Sp-AR [17]). This parameter has been extensively used for evaluation of the fertilizing potential of a sperm population to be used in IVF (17, 18).