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ARTICLE

Does being overweight affect seminal variables in fertile men?

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Abstract The effect of being overweight on seminal variables was assesed in 165 fertile men. Participants were divided into three groups: fertile men with normal body mass index (BMI) (18.5–24.9 kg/m^2), fertile overweight men (BMI 25–29.9 kg/m^2) and fertile obese men (BMI >30 kg/m^2). Medical history was taken, a clinical examination conducted. Semen analysis was undertaken and BMI measured. Seminal reactive oxygen species (ROS) was estimated by chemiluminescent assay, sperm vitality by the hypo-osmotic swelling test and sperm DNA fragmentation by propidium iodide staining with flowcytometry. Fertile obese men had significantly lower sperm concentration, progressive sperm motility and sperm normal morphology, with significantly higher seminal ROS and sperm DNA fragmentation compared with fertile normal-weight men and overweight men (all $P < 0.05$). BMI was negatively correlated with sperm concentration ($r = –0.091$; $P = 0.014$), progressive sperm motility ($r = –0.697$; $P = 0.001$), normal sperm morphology ($r = –0.510$; $P = 0.001$), sperm vitality ($r = –0.586$; $P = 0.001$), but positively correlated with sperm DNA fragmentation percentage ($r = 0.799$; $P = 0.001$) and seminal ROS ($r = 0.673$; $P = 0.001$). Increased BMI was found to affect semen parameters negatively even in fertile men.

KEYWORDS: BMI, male infertility, obesity, oxidative stress, semen, sperm DNA fragmentation

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Introduction

Obesity has become a worldwide epidemic, with the world's overweight population rapidly increasing as a result of poor diet and a sedentary lifestyle (Hammoud et al., 2012). Considerable research has been undertaken to combat and control this increasing health problem.

Body mass index (BMI) is a simple method of estimating body fat. It is defined as body mass divided by the square of the body height, and is expressed in units of kg/m^2 (Okorodudu et al., 2010). The relationship between obesity and altered sperm parameters is multi-factorial, representing an altered hypothalamic–pituitary–gonadal axis, peripheral aromatization of steroids to oestrogen, with decreased testosterone, increased oestradiol levels, decreased sex hormone-binding globulin and increased scrotal temperature (Crujeiras and Casanueva, 2015). Leisegang et al. (2014) found an association between increased seminal insulin and leptin concentrations and a negative effect on male reproductive function in obesity. In addition, the preferential accumulation of toxic substances and liposoluble endocrine disruptors in fatty tissue has been shown to amplify these alterations, correlating serum organo-chlorine levels with BMI (Katib, 2015; Magnusdottir et al., 2005; Sermondade et al., 2013).

Several studies have linked increased weight in infertile men with poor sperm quality (decreased sperm concentration, abnormal sperm morphology and reduced sperm motility) compared with men of normal weight (Hanafy et al., 2007; Hofny et al., 2010). Data on the relationship between obesity and male infertility have therefore accumulated; little attention, however, has been paid to its seminal effect in fertile men.

The sperm hypo-osmotic swelling (HOS) test, reactive oxygen species (ROS) assay and sperm DNA integrity evaluation have been shown to be helpful in testing male infertility (Hossain et al., 2010; Mostafa et al., 2009; Taha et al., 2012, 2014). An association between oxidative stress and obesity has been found (Karaouzene et al., 2011), and an association between increased sperm DNA fragmentation in obese men and poor quality of spermatogenesis has also been reported (Smit et al., 2010).

In the present study, the effect of increased BMI on semen variables was assessed in fertile men.

Materials and methods

This cross-sectional, case-controlled cohort study included 165 fertile men (men who fathered a child within the previous 12 months). Institutional Review Board approval was obtained on 12 October 2010 for 2 years, and participants provided informed consent. Participants were assigned to three groups according to their body mass index (BMI): fertile men with normal BMI (18.5–24.9 kg/m^2) ($n = 81$), fertile overweight men (BMI 25–29.9 kg/m^2) ($n = 59$), and fertile obese men (BMI >30 kg/m^2) ($n = 25$). Exclusion criteria were smoking, varicocele, leukocytospermia, chronic medical disorders and men taking antioxidants.

Medical history was taken for all participants. Each participant underwent a clinical examination and semen analysis. Semen analysis included sperm morphology assessment by the Papanicolaou method according to the World Health Organization (2010) guidelines. Other assessments included the HOS test, estimation of seminal ROS and sperm DNA fragmentation.

The HOS test

A total of 1 ml of freshly prepared hypo-osmotic medium (0.735 g sodium citrate dihydrate and 1.351 g fructose in 100 ml distilled water) was mixed with 0.1 ml liquefied semen and incubated at 37°C for 30 min. Spermatozoa were examined under a phase-contrast microscope, with the swelling of sperm tails counted in duplicate in 100 spermatoza (al-Mogazy et al., 1993).

Seminal ROS level

The ROS levels were estimated by detecting the chemiluminescence activity using luminol (5-amino-2, 3 dihydro-1, 4 phthalazinedione reagent ($C_8H_7N3O_2$) (MP Biomedicals, Santa Ana, CA, USA). Liquefied semen specimens were centrifuged at 300 g for 7 min and the seminal plasma was removed. The pellet was washed twice with phosphate buffered saline (PBS) pH 7.4 by centrifugation at 300 g for 5 min then resuspended in the PBS at a concentration of 20 x 10^6 sperm/ml. A total of 5 mmoles of luminol dissolved in 750 μl dimethyl sulphoxide (Sigma Chemical Co., Sigma-Aldrich., St. Louis, MO, USA) was added to 400 μl of sperm suspension. Each sample was tested in duplicate. Chemiluminiscence activity was measured with an Autolamat Luminometer (Berthold Technologies, Bad Wildbad, Germany) in integrated mode for 15 min to assess ROS levels. The results were expressed as relative light unit/ 20 million spermatoza (Wang et al., 2003).

Sperm DNA fragmentation

Sperm DNA fragmentation was conducted on fresh semen using flowcytometry (Beckman Coulter, Fullerton, CA, USA) based on the fluorescence emission from individual spermatozoon stained with propidium iodide and excitation with a 488-nm argon laser. Semen samples were diluted with PBS (pH 7.4) to 2 × 10^6 sperm/ml. Fifty microliters were incubated with a 100 μl lysing reagent for 15 s and 2 ml of propidium was added. After staining, flowcytometry was used for data acquisition in which the intensity of its emission corresponded to the DNA content. Flowcytometry analysis displays a characteristic bimodal non-artifactual DNA pattern confirming the existence of two distinct populations. The main population was represented by a peak followed by a shoulder, which is the marginal population representing DNA damage, yielding unstable chromatin appearing more stainable. The percentage of sperm cells with DNA damage was automatically calculated by flowcytometry after the acquisition of 5000 spermatoza (Piasecka et al., 2007).

Statistical analysis

Data were analysed and expressed as mean values ± SD; SPSS version 21.0 (IBM Corp., USA) was used for data processing.

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