HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net

Original research http://dx.doi.org/10.1016/j.apjr.2015.06.008

Diet-induced obesity alters kinematics of rat spermatozoa

I.P. Oyeyipo[1,2], P.J. Maartens[1], S.S. du Plessis[1*]

[1]*Division of Medical Physiology, Department of Biomedical Sciences, Stellenbosch University, South Africa*

[2]*Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Osun State, Nigeria*

ARTICLE INFO

Article history:
Received 14 Apr 2015
Received in revised form 16 May 2015
Accepted 20 May 2015
Available online 24 July 2015

Keywords:
Spermatozoa
Obesity
Viability
Motility

ABSTRACT

Objective: To investigate the effect of DIO on the kinematics and viability of spermatozoa in an albino rat model.

Methods: Sperm suspensions from normal (Control) and diet-induced obese (DIO) Wistar rats were collected and incubated for various times (30, 60, 120 or 180 min at 37 °C). Motility parameters were analyzed with computer-aided sperm analysis (CASA), while viability was assessed by means of a dye exclusion staining technique (eosin/nigrosin).

Results: Results reveal that there was a significant time dependent decrease ($P < 0.05$) in progressive motility, curvilinear velocity and beat cross-frequency after 60 min, while amplitude of lateral head displacement and sperm viability was significantly reduced ($P < 0.05$) after 120 min in the DIO group compared to control spermatozoa.

Conclusions: These results provided evidence that obesity is detrimental to sperm parameter in rats possibly through increased testicular temperature as a result of a rise in fat deposition.

1. Introduction

Infertility has become very common among couples of childbearing age with about 15% of the general population affected in industrially developed countries [1] while approximately 50% of known causes of primary infertility can be attributed to male factor [2].

The etiology of male factor infertility is still poorly understood due to idiopathic infertility. Apart from the fact that certain individuals may be genetically predisposed to be sub-fertile, epigenetic factors are also implicated as potential causes of male infertility [3]. The much speculated decline in male reproductive potential and semen quality over the past 50 years is currently gaining considerable attention. Several studies revealed that sperm parameters have deteriorated by 50% since the 1940s in some parts of the world [4,5]. These observations on semen quality impact negatively on male fertility and thus contribute to the overall decline in male reproductive potential [4,6].

Obesity is a public health issue that affects both children and adults and is currently taking on pandemic proportions. It is associated with a combination of an increasingly sedentary lifestyle and unhealthy diet [7]. According to statistics, approximately 400 million adults were classified as obese with another 1.6 billion adults classified as overweight in 2005. It is predicted that currently in 2015, 700 million adults are obese and 2.3 billion overweight [8]. Obesity has been documented as a risk factor for non-insulin-dependent diabetes, osteoarthritis, cardiovascular disease, particular types of cancer, and certain metabolic and reproductive disorders [9]. Obesity has also been shown to be associated with disturbance in the hormonal milieu which can affect the reproductive system, as observed in obese women [10]. However, in men this relationship is not well understood, due to too few and inconclusive studies available in the literature [11,12]. However, there is a strong belief that the decrease in fertility can be directly related to the paralleled increase in obesity. In support of this theory, Swan *et al.* revealed that sperm counts have continued to decrease between 1934 and 1996 by as much as 1.5% annually in the USA as well as other parts of the Western world with such decrease not observed in regions where obesity is less prevalent [13].

From the available literature it is clear that the relationship between excessive adiposity and specific sperm parameters is

*Corresponding author: S.S. du Plessis, Division of Medical Physiology, Stellenbosch University, P.O. Box 241, Cape Town 8000, South Africa.
Tel: +27 21 938 9388;
Fax: +27 21 938 9476
E-mail: ssdp@sun.ac.za
Peer review under responsibility of Hainan Medical College.

not very well established with various contradictory findings being reported. In an epidemiological study by Chavarro and co-workers it was reported that body mass index (BMI) is unrelated to sperm concentration, motility or morphology. However, the authors observed a steady decrease in ejaculate volume with increasing BMI levels [12]. Studies have also shown considerably more DNA damage in sperm from obese men than in normal-weight men [12,14]. On the other hand it has been documented that BMI correlated positively with abnormal sperm morphology and negatively with sperm concentration and motility [15]. Jensen *et al.* [16] reported decreased sperm counts in obese normozoospermic men compared to non-obese fertile subjects. Interestingly, Rybar *et al.* [17] found no significant relationship between mean BMI and standard semen parameters.

Animal studies on diet-induced obesity (DIO) in male mice have been associated with decreases in total sperm motility [18,29] number of post copulatory plugs and pregnancy rate [18] fertilization rate as well as increased sperm intracellular reactive oxygen species (ROS) and DNA damage [19]. Other studies revealed that obesity led to a lesser number of ejaculates per day [20] and reduced sperm quality by lowering sperm motility without affecting other sperm parameters [21].

In spite of the growing body of knowledge of the effect of obesity on male reproduction, there are contradicting findings with regards to sperm motility and it is currently still unclear if male obesity has any impact on sperm motility parameters. The disparities observed in the literature might be as a result of several limitations inherent to human studies. These studies can be affected by confounding factors such as lifestyle and co-pathologies which can also impair sperm function or even self-reporting of these parameters which can lead to under reporting. Secondly, most of the studies originated from fertility clinics, where patient cohorts are usually biased towards, sub-fertile or infertile men, which may also confound findings further. Lastly very few studies employed computer-aided sperm analysis (CASA) as an unbiased means to report sperm motility parameters. It is therefore relatively unknown whether obesity affects sperm motion parameters. The present study was therefore accordingly designed to investigate the effect of DIO on the kinematics and viability of spermatozoa in an albino rat model.

2. Materials and methods

2.1. Animals

Twelve inbred male Wistar rats were used for this study. Obesity was induced in rats by feeding them with a hyperphagia-inducing diet. Animals were randomly and equally divided into control (C) and DIO groups. The C animals were fed normal rat chow while the DIO animals' food was supplemented with sucrose and condensed milk for a period of 16 weeks [22]. All animals had free access to food and fresh water and were kept separately on a 12 h day/night cycle in an AAALAC (Association for the assessment and accreditation of laboratory animal care international) accredited animal facility. This study was ethically approved by the institutional review board.

2.2. Sperm sample preparation

Animals were humanely killed by euthanasia (intraperitoneal injection of 160 mg/kg pentobarbital) and exsanguination. Blood glucose concentrations for each group were measured immediately using a blood glucose monitor (Glucoplus Inc. Canada). Visceral fat mass, Testes and epidydimides were also excised and also weighed immediately. Both epididymides were removed and the cauda epididymis carefully isolated through dissection. Sperm from the cauda epididymis of the left side was isolated, by placing the structure into 2 mL of HAMS (Sigma Chemical Co.) medium supplemented with 3% bovine serum and cutting it into 1 mm lengths. It was subsequently incubated for 30 min at 37 °C, thereby allowing for release of the spermatozoa into the medium. The sperm suspension was diluted with fresh HAMS-BSA medium to give an approximate concentration of 1×10^6 sperm/mL.

2.3. Assessment of sperm motility

Motility parameters of spermatozoa from C and DIO rats were measured at various points in time (30, 60, 120 or 180 min) post collection by means of CASA. The settings for the Sperm Class Analyzer (SCA, Microptic, Barcelona, Spain) were Pseudo Negative phase, Ph2/3 condenser, 4× objective lens, no filter, Brightness ± 450, Contrast ± 100. kinematic parameters such as total motility, progressive motility (percentage of A + B level of spermatozoa) curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), amplitude of lateral head displacement (ALH), linearity (LIN), straightness (STR) and beat cross-frequency (BCF) were measured by filling a 8 chamber leja slide with 2 μL of sperm suspension.

2.4. Assessment of sperm viability

The number of viable spermatozoa was assessed by means of a dye exclusion staining technique (Eosin/Nigrosin). In brief, a modified technique of Eliasson was used where sperm, eosin and nigrosin were mixed in a 1:2:3 ratio. A smear was subsequently made for light microscopy analysis (×100 magnification). Unstained spermatozoa were identified as viable, while stained spermatozoa (pink) were identified as non-viable [23]. A total number of 200 spermatozoa were counted in duplicate and the results were expressed as percentage viability.

2.5. Statistical analysis

All data are expressed as mean ± SEM. A Student's *t*-test was performed to compare the various parameters from the C and DIO animals at each observation time point. All statistical comparisons and test were performed using the Statistical Package for Social Sciences (SPSS Inc, Chicago, IL, USA). Difference between groups were considered statistically significant when $P < 0.05$.

3. Results

3.1. Anthropometry parameters

A significant change ($P < 0.001$) was observed in final body weight, visceral fat mass when compared to controls. However, no significant change was observed in the testicular and epididymal weight and plasma glucose level as shown in Table 1.