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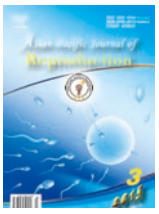

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Diet-induced obesity alters kinematics of rat spermatozoa

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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 ℃). 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 child-bearing 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 aetiology 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 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

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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 1mm lengths. It was subsequently incubated for 30 minutes at 37 ℃, 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 uL 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 with 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. Anthropometric 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.

3.2. Sperm motility parameters

Kinematic parameters of spermatozoa from C and DIO rats measured at various time points (30, 60, 120 and 180 min) are shown in Figure 1(a-i). No significant differences in any of the parameters were observed after 30 minutes, (however a trend of decrease was observed in the majority of DIO samples). The percentage of viable spermatozoa was significantly reduced (P<0.05) at both 120 min and 180 min in the DIO animals. This observation was similarly reflected in the decrease in motility at these two time points as well. Interestingly specific parameters such as progressive motility, BCF and VCL appeared to be more sensitive and already showed signs of decrease at 60 minutes of incubation. Sperm motility vigor was significantly reduced, as evident by decreases in VCL, ALH and BCF with increasing duration of incubation as shown in Figure 1(c, d and e). The significant decreases in VCL and BCF were observed after 60 min of incubation while ALH was significantly decreased (P<0.05) after 120 min of incubation when values were compared with control.

There was a significant decrease in VSL after 180 min of incubation when compared with the control as shown as Figure 1(f). VAP, STR and LIN appeared not to be affected between groups at any time point as shown in figure 1(g-i).

3.3. Sperm viability

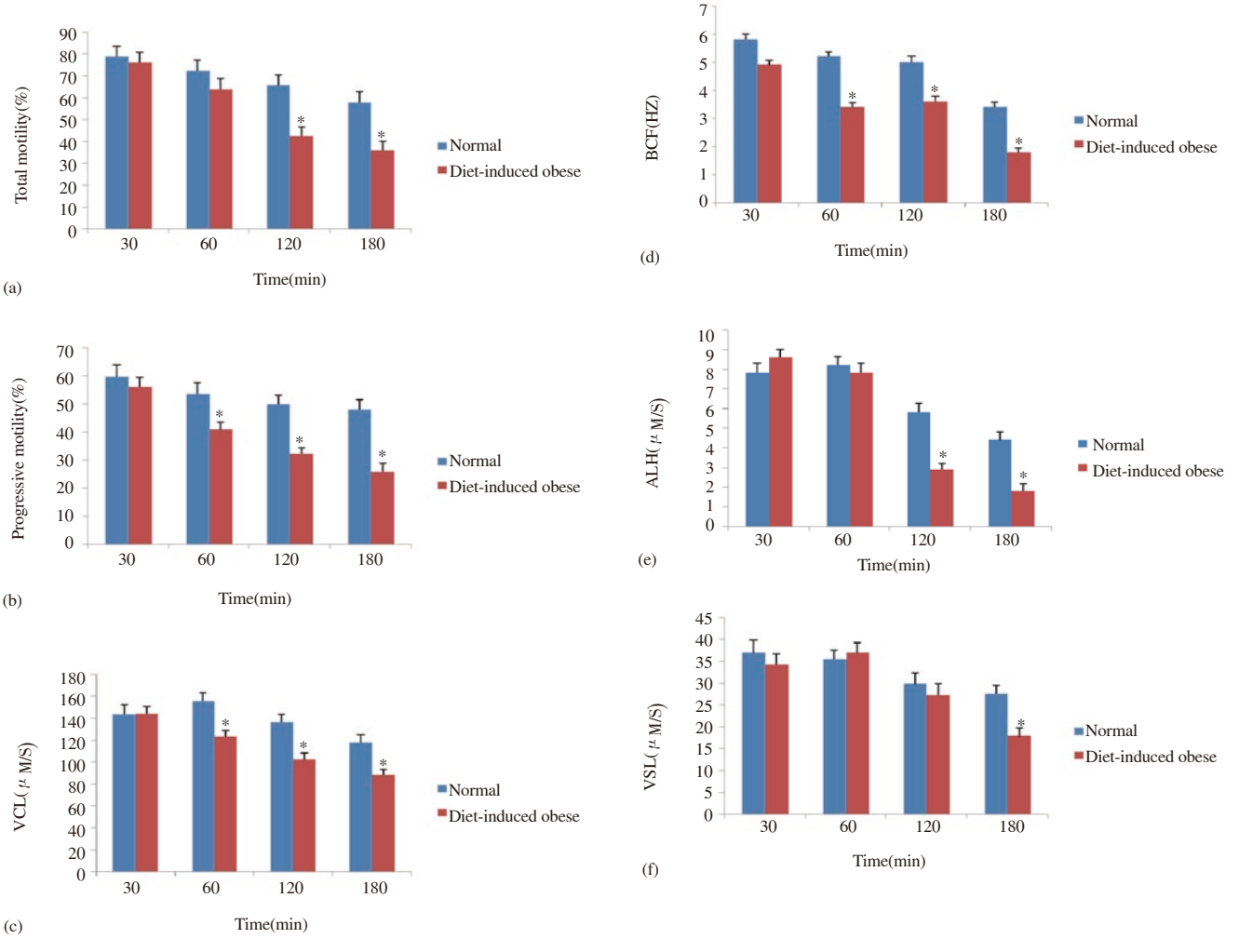
The viability of spermatozoa was significantly decreased (P<0.05) after 120 min of incubation in the DIO group when compared with the control as shown in Figure 1(j).

Table 1

Anthropometry parameters in the experimental groups.

Groups	Initial body weight(g)	Final body weight (g)	Visceral fat (g)	Testicular weight (g)	Epididymal weight (g)	Relative testicular weight (%)	Relative epididymal weight(%)	Plasma glucose (mmol/L)
SD	166.80±5.34	353.80±8.57	9.90 ±0.81	3.32 ±0.16	1.46 ±0.08	0.93±0.02	0.41±0.02	8.27 ±0.81
DIO	169.20±4.57	429.00 ±11.74***	23.50 ±3.53***	3.22 ±0.08	1.34 ±0.11	0.75±0.02	0.31±0.02	8.80 ±0.91

Note. Data are expressed as mean ± SEM (n=6). Control (SD), Diet-induced obese (DIO), ***P<0.001 *vs.* Control.



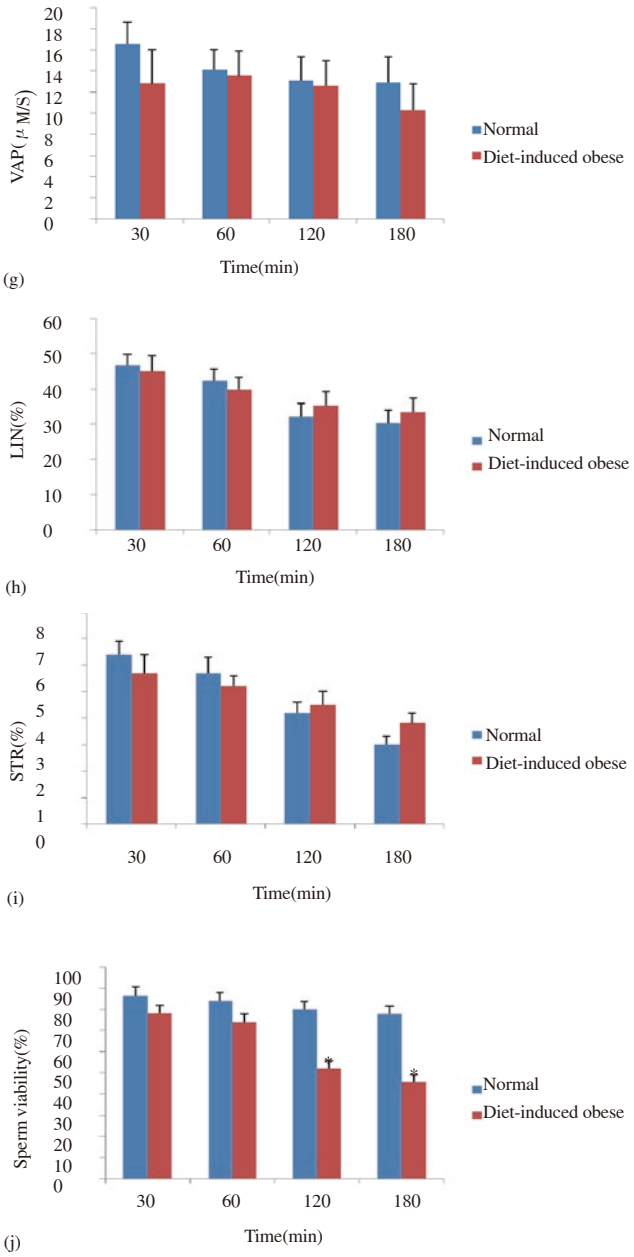


Figure 1. Effected of diet-induced obesity on sperm motion and kinematic parameters (a-i) as well as viability (j) after 30, 60, 120 and 180 min of incubation.

Values are expressed as mean ± SEM of 6 animals *P<0.05 versus control. (VCL, curvilinear velocity; VSL, straight line velocity; ALH, amplitude of lateral head displacement; BCF, beat cross-frequency; VAP, average path velocity; LIN, Linearity; STR, straightness)

4. Discussion

This present study shows that DIO could directly reduce motility, motion parameters and viability of rat spermatozoa when incubated *in vitro*. The increased final body weight and visceral fat mass observed in the DIO group is an indication that obesity was induced by the diet. The adverse effects observed in the DIO group became more pronounced after longer periods of incubation indicating that there is a relationship between length of incubation and potency of sperm parameters. This has a direct implication on fertility potential as it implies that spermatozoa function will deteriorate *in vivo* during transit through the female tract or similarly whilst incubated during in vitro fertilization (IVF) procedures.

Motility is one of the most important features of fertile spermatozoa. It was the first, and remains the most widely used indicator of sperm function[24]. Sperm motility is a vital attribute, because it is readily identifiable and reflects several structural, and functional competencies, as well as important aspects of spermatozoa metabolism[25] CASA has been described as an objective technique providing reproducible data for various motility parameters that cannot be measured manually[26]. The accuracy and precision of CASA systems allow for the detection of subtle changes in sperm motion via examination of sperm motion parameters, which are useful markers for assessing toxicity. Different sperm motility parameters are indicators of specific function; VSL, VAP, STR and LIN have been shown to indicate sperm progression while VCL, ALH and BCF are pointers of sperm vigor. The swimming patterns are described by LIN and STR whereas VCL and BCF are indicators of sperm viability[27, 28].

Results indicated that the percentage total sperm motility decreased in a time dependent manner, but was significantly affected after 120 min of incubation in DIO rat while the percentage progressive sperm motility which is a summation of the fast and slow motile sperm cell was decreased after 60 min of incubation. In this study, progressive motility was decreased in the DIO group. This important sperm attribute is needed to move spermatozoa linearly through the cervix, uterus and fallopian tubes. The observed reduction implies that spermatozoa will not be able to reach site of fertilization. This is in consonance with previous studies that showed that DIO is associated with reduced total sperm motility in rats[21]. Altered motility parameters may also lead to an inability of the sperm to penetrate the cervical mucus[29, 30] thus, preventing the sperm from migrating along the female reproductive tract and reaching the oocyte. In addition, high fertilization index is related to a high percentage of sperm with progressive motility[30].

The values of VCL and BCF of spermatozoa of obese rats were progressively reduced after 60 min of incubation while ALH was reduced after 120 min. This is an indication that DIO has an adverse effect on fertility potentials. Studies have shown that these parameters are necessary for in vitro fertilization in rats[31] and boars[32]. In addition, it is worth noting that these parameters

are markers of sperm strength, cervical mucus penetration and effective rate of fertilization necessary as the sperm transverse the female reproductive tract with a view of fertilizing the oocyte[33, 34]. During the incubation period, there was no observed significant change on LIN and STR, however, there indicators of sperm swimming pattern was increased in the DIO rat after 120 min of incubation when compared with the control. This might point towards reduced fertilizing potential since LIN and STR are lowered in hyperactivated spermatozoa indicating that DIO group might not be able to penetrate the Zona Pellucida in an attempt to fertilize the oocyte even if it reaches it.

The decreases in VSL after 120 min of incubation of spermatozoa DIO rats indicated that sperm progression is altered with diet-induced obesity[27].

This result suggests that diet-induced obesity exerted effects on sperm viability, progression and vigor without affecting sperm swimming pattern in male albino rat. There was a significant decrease in the viability of spermatozoa of diet-induced obese rat after 120 min of incubation. The reduction in viability agrees with the reduction in progressive sperm motility and sperm viability markers (VCL and BCF) that were also significantly decreased after 60 min of incubation. This is in consonance with previous studies in humans[35].

This report suggests that VCL, BCF, VSL and ALH were the most sensitive indicators of altered sperm kinematics in diet-induced obese rat, while LIN and STR appeared less sensitive. We speculate that the declined sperm parameters observed in the DIO group might be as a result of rise in the local testicular temperature resulting from increased fat deposition within the abdominal and reproductive visceral cavity. There are report in literature that increase fat deposition could reduce radiation of heat, compromise the efficacy of thermoregulation, increase generation of ROS and development of oxidative stress in reproductive organs resulting in reduce sperm quality[36–38] However, further studies are recommended to validate and elucidate the mechanism of altered sperm kinematics associated with obesity condition.

Conflict of interest statement

We declare that we have no conflict of interest.

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