Diabetes mellitus induced impairment of sperm parameters in mice: A stereological method

Sara Dadras[1,2], Mohammad Bayat[2], Marefat G. Novin[1,2], Hamid Nazarian[1], Amir Raoofi[3,4], Shabnam Abdi[5], Sina Sadeghzadeh[6], Faezeh Tajari[7], Mohammad-Amin Abdollahifar[1,2]

[1] Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Department of Anatomical Sciences, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran

[4] Leishmaniasis Research Center, Department of Anatomy, Sabzevar University of Medical Sciences, Sabzevar, Iran

[5] Department of Anatomical Sciences & Cognitive Neuroscience, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

[6] Bachelor's degree, Cognitive Neuroscience and Evolutionary Psychology, Harvard University, Cambridge, Massachusetts, USA

[7] Student Research Committee, School of Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

SUMMARY

In this study, the effects of diabetes mellitus on stereological changes of sperm parameters in mice were investigated. Mice under standard housing conditions were assigned into two experimental groups: (I) control (II) diabetic (N = 6 mice per group). The sperm samples were collected from the right cauda epididymis of the mice for measuring the sperm and sperm count. The volume of the sperms' heads was estimated using the nucleator method. The length of the sperms flagellum and mid-piece was estimated by counting the number of intersections of the tails and Merz grid test line in an unbiased counting frame, superimposed on live images of sperms. The results showed that the total sperm count and sperm motility in Streptozotocin (STZ)-induced animals was decreased, in comparison with the control groups. Our results showed a significant difference in the volume and surface area of the head and the length of the flagellum between the sperms in the control and diabetic groups. In conclusion, our results indicated that type 1 diabetes induced impairment in sperm parameters.

Key words: Diabetes mellitus – Mice – Morphology – Sperm – Stereology

INTRODUCTION

A chronic illness, diabetes mellitus is caused by chronic elevations in blood sugar levels due to the inability of the pancreas for producing insulin as a result of the destruction of the pancreatic beta cells (Scarano et al., 2006). Diabetes mellitus is associated with abnormalities in carbohydrate, fat, and protein metabolism in target tissues. The

Corresponding author:
Mohammad-Amin Abdollahifar. Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: m_amin58@yahoo.com / abdollahima@sbmu.ac.ir

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chronic hyperglycemia of diabetes also causes such complications and other dysfunctions in various organs, particularly the eyes, kidneys, heart, blood vessels and reproductive organs (Ning et al., 2009; Ghanbari et al., 2012). Previous studies show that diabetes can affect the neuroendocrine reproductive tract axis and cause sexual dysfunctions. It has been also reported that hyperglycemia can negatively affect sperm quantity and quality in rats (Arikawe et al., 2006; Navarro-Casado et al., 2010). Previous studies reported the adverse effects of diabetes mellitus on the functions of reproductive organs, particularly ejaculation and spermatogenesis; moreover, by dropping testosterone levels, the disease can cause harm to the tissues and the reduction the capacity of testis (Mosher, 1988).

Morphology sperm plays a most important role to determine sperm quality and successful fertilization (Rønn et al., 2000; Noorafshan and Karbalay-Doust, 2010; Kuster et al., 2004; Panahi et al., 2017). Although the World Health Organization (WHO) recommends the investigation of different parts of sperm morphology, little research has been conducted on the volume of the sperms' heads and the mid-piece and tail sperm length, despite the essential role they play in sperm motility (Mossman et al., 2012; de Paz et al., 2011). In other words, most studies have focused on a narrow range of parameters such as analyzing the sperm length and head (Maroto-Morales et al., 2010; Noorafshan and Karbalay-Doust, 2010). This study was conducted through the unbiased application of stereological tools. Regarding the subject mentioned above, this study was conducted in order to explore the effects of diabetes on the sperm head volume of and length of their mid-piece by using stereological methods.

MATERIALS AND METHODS

Animals

This study was conducted on 12 adult mice (28-30 g) that were purchased from the animal center laboratory of the Pasteur Institute in Tehran, Iran. This study was conducted according to the standard directive recommended and approved by the research authorities of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR. SBMU. RETECH.REC.1395.440). The mice were divided into two groups: (I) Control; (II) Diabetic (40mg/kg STZ). Each group included 6 mice that were housed in standard rat cages and room temperature 22-24°C, 12:12 h light-dark schedule, and were provided with water ad libitum.

Induction of diabetes mellitus

A single dose (55 mg/kg body weight) of intraperitoneal streptozotocin (STZ) injection can cause type 1 diabetes in mice (Zanosar Pharmacia and Upjohn Co, Kalamazoo, MI, USA). One week following the STZ injection, blood samples were taken from the veins of mice's tail in order to analyze the blood glucose levels (Biomine, Rightesttm GM300, Biomine Corporation, Switzerland). Mice with blood glucose level greater than 250 mg/dL were considered to be type 1 diabetic. In our study, the mice's body weight and blood glucose levels were recorded every 2 weeks until the end of the research. Sperm morphology examinations were conducted 30 days after the STZ injection.

Sperm Collection

Sperm samples were collected from the right cauda epididymis of the mice. The sperm sample incubation at 37 °C for 20 minutes, 10 µl of the sample were placed on a slide and sperm motility was observed. The sperm count was measured by counting chamber. Then the sperm smear was prepared for analysis, placed on a slide, air-dried at room temperature, and fixed in methyl alcohol. Then, the sample was stained with Diff-Quik (Seed et al., 1996).

Stereological study

Length of sperms' mid-piece and flagellum estimation

The length of sperms' mid-piece and flagella estimation under the microscope is an application of length estimation in two-dimensional planes (Panahi et al., 2017). The mean length of the sperms' mid-piece and flagella was estimated using the following formulae (Fig. 1):

$$\sum L = \left(\frac{\pi}{2}\right) \cdot \left(\frac{a}{l}\right) \cdot \left(\frac{1}{asf}\right) \cdot \sum I, \quad L = \frac{\sum L}{\sum N}$$

In this formula a/l is the Merz grid constant which was obtained as follows: the area of each basic tile of the grid was X multiplied by Y. Within this tile, there were two semicircles of length of π.d (perimeter of a circle), In this formula d is the diameter of the semicircle. Thus the Merz grid constant a/l was (X_Y) π.d. In this formula asf is the area of the basic tile divided by the area of the counting frame. In this formula ΣI is the summation of the intersection of the tails with the semicircles. In this formula ΣN is the total number of the counted sperms in the unbiased counting frame.

Mean volume estimation of sperm heads

The sperms' volume of head was estimated using the nucleator method (Karlsson and Cruz-Orive, 1997). In this method for each sampled nucleus, two horizontal directions (intercept, Ln) were considered from the central point within the nucleus to the cell borders (Figure 1). The sperm head volume in the number weighted distribution was estimated using the following formula:

$$V_N = \frac{4\pi}{3} \times \overline{l_n^3}$$

Statistical analysis

The data were analyzed using the non-parametric tests (Kruskal-Wallis). Differences were regarded as statistically significant if $P \leq 0.05$.

RESULTS

Total sperm count, motility and sperm mid-piece and tail defect

Our data showed that the total sperm count in STZ-induced animals was decreased, in comparison with the control groups ($P < 0.05$). the

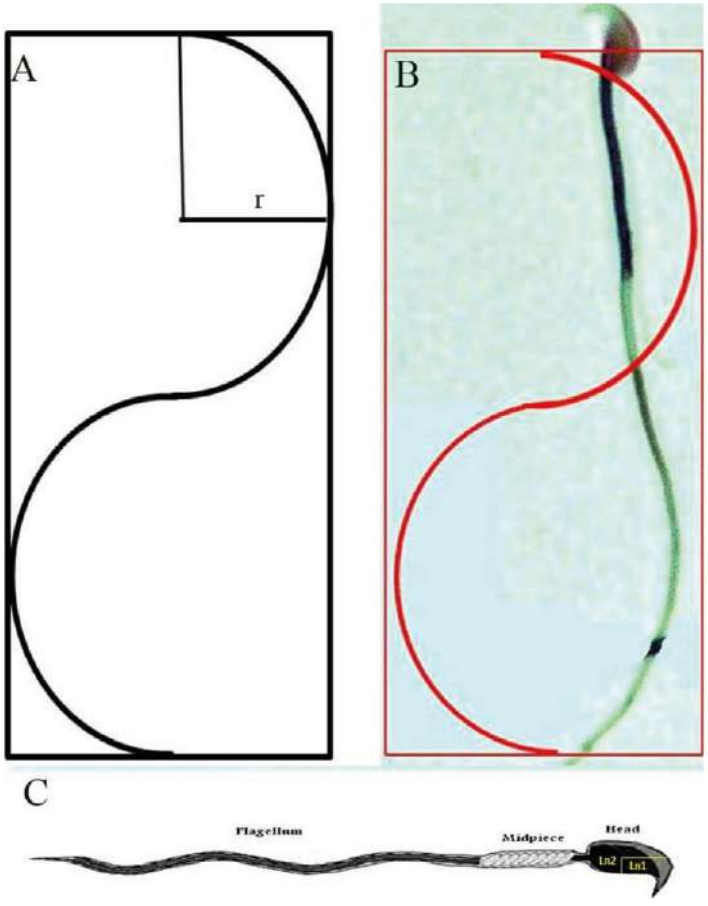

Fig. 1.- Estimation of the sperms mid-piece and flagellum length. (**A**) Two semicircles were located at a rectangle. The length of each semicircle was equal to twice the length of its minor axis (r). The area associated with the semicircle was calculated by multiplying the X by Y and divided by the length of the two semicircles to achieve the area per length. (**B**) The total number of the intersections between the sperms mid-piece and flagellum axes and the cycloid were counted. The semicircle was positioned parallel to the vertical axis. (**C**) For nucleator methods, two horizontal directions (intercept, Ln1 and Ln2) were considered from the central point within the sperm head to the plasma membrane.

results also showed that the sperm motility also decreased in the diabetic groups in comparison to the control groups (P<0.05) (Fig. 2).

Sperm mid-piece length

The results for length of the sperms' mid-piece revealed no significant differences between the control and diabetic groups. Therefore, sperm mid-piece length was not correlated with abnormal sperm morphology in diabetic groups (Fig. 3).

Sperm flagellum length

Our result indicated that the length of the flagellum was decreased in the diabetic groups in comparison to the control rats ($p < 0.05$) (Fig. 3).

Sperm head Volume

The results of stereological analyses are shown in table 1. The results showed that there was a significant difference in the volume of the sperm heads between the control and diabetic groups ($p<0.05$). Therefore, sperm head volume was associated with abnormal sperm morphology (Fig. 3).

DISCUSSION

In this study investigated the morphological changes of the sperms in STZ-induced diabetic mice using stereological techniques. Our finding showed that the reduction in the sperm count and sperm motility in the diabetic groups in comparison with the control groups. Our results also showed that the reduction in the sperm heads volume and the length of sperm flagellum in the diabetic groups in comparison with the control groups. These sperm structure changes have proven to be detrimental to the chance of success pregnancy (Guneli et al., 2008). Diabetes increased blood glucose levels and created histological changes in mice's testis by reducing spermatogenic cells, cell apoptosis, and other histological changes in the seminiferous tubules. These changes were associated with morphological abnormalities in

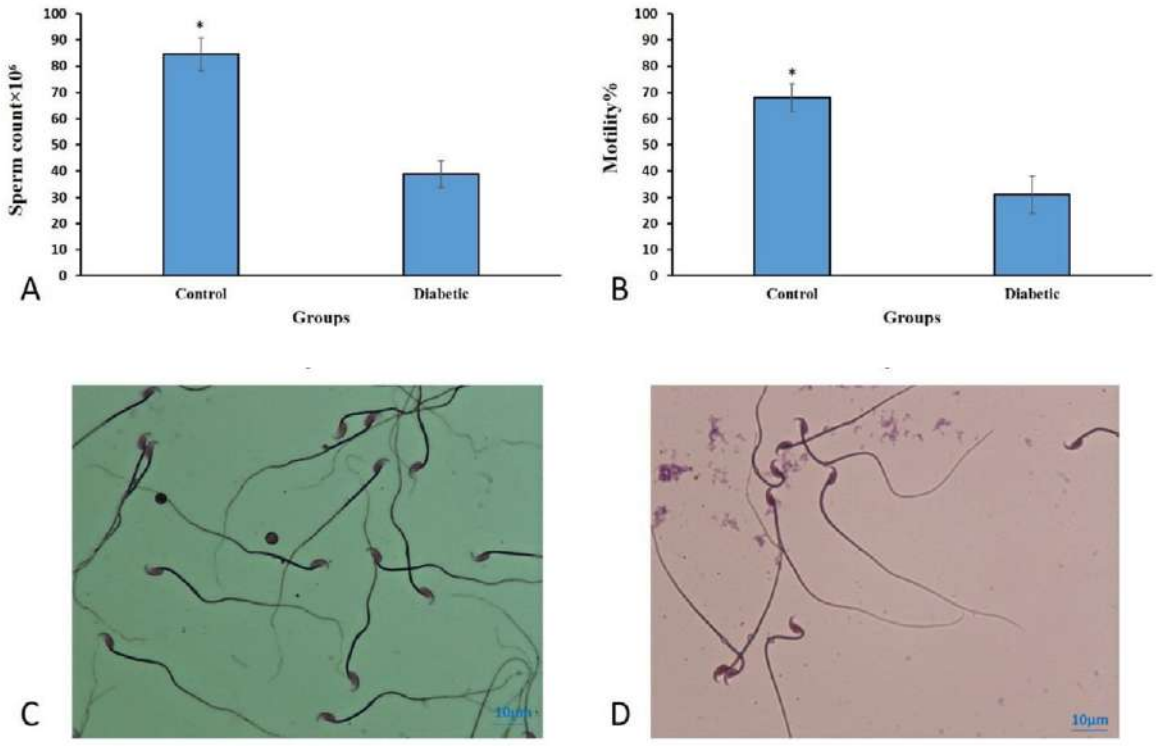

Fig. 2.- Total sperm count and sperm motility in the control and diabetic groups are shown (**A** and **B**). The significant difference between control groups in comparison to the diabetic groups is indicated. *p < 0.05. Diff quick staining of sperms. (**C**) Control group and (**D**) Diabetic group.

spermatogenesis (Guneli et al., 2008). Previous studies showed that diabetes could cause a reduction in testosterone levels in the serum and testes tissues in male mice by changing the function of Sertoli cells and affecting the leydig cells (Kanter et al., 2013). The dysfunction of leydig cells causes oxidative stress and increases free radicals, which could be detrimental to male fertility (Shrilatha, 2007). Moreover, diabetes can affect several sperm-related parameters of male fertility such as erection, semen concentration, semen volume, sperm count, sperm motility and testosterone levels; however, the relationship between standard sperm-related parameters and diabetes was not clear (Ficher et al., 1984; Hassan et al., 1993). It has been shown that diabetes is associated with the reduction of semen volume and the vitality and motility of spermatozoa. Also, spermatozoa of diabetic mice had less chromatin condensation and lower DNA integrity (Arikawe et al., 2006; Ricci et al., 2009).

The motility of sperms is associated with the mid-piece, which contains mitochondria for ATP synthesis which is therefore responsible for human fertility (Lüpold et al., 2009; Hargreave and Elton, 1983). Moreover, sperm viability is related to sperm morphometry, which was confirmed by observing that spermatozoa with a small head, width, area, and perimeter died after freezing and thawing (Marco-Jiménez et al., 2006). There are few studies conducted on the morphometric measurements of sperms (Jeyendran et al., 1986). One study on American soldiers analyzed the relationship between sperm length and fertility impairment (DeStefano et al., 1989; Boyle et al., 1992). The relationship between the dimensions of the sperm head with fertility after human intra uterine in semination (IUI) and intra cytoplasmic sperm injection (ICSI) has also been studied (Soler et al., 2005). Although the WHO recommends the evaluation of sperm morphometry, little attention has been paid to this parameter and its benefit in predicting fertility.

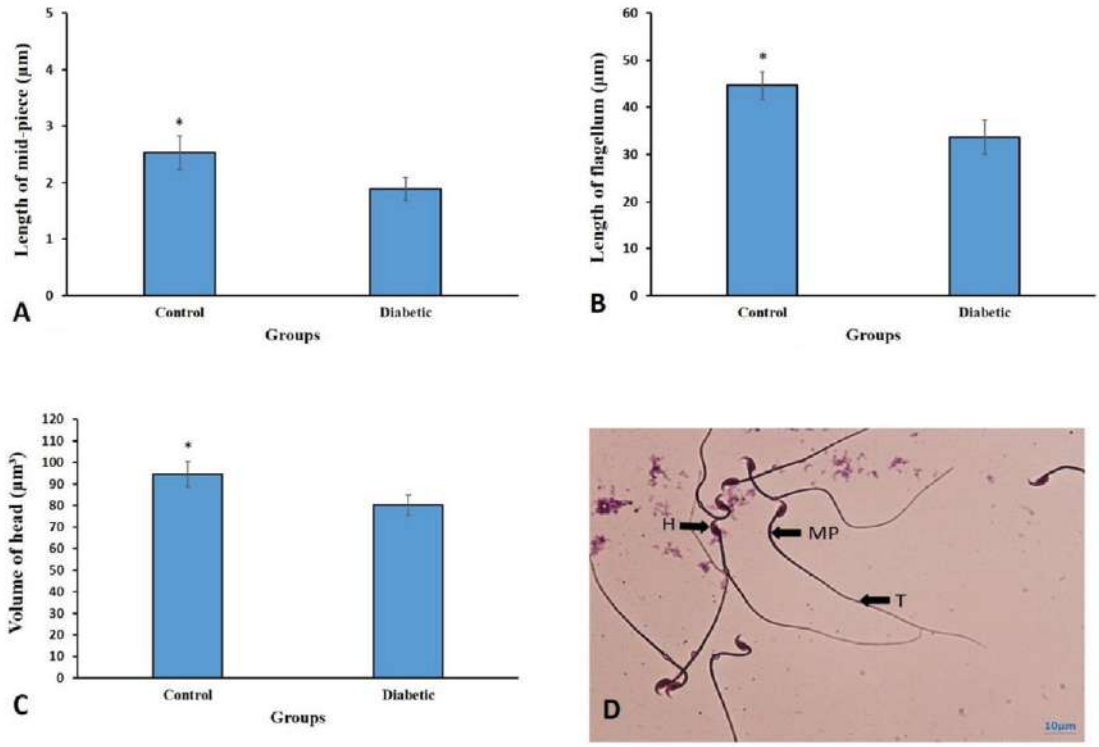

Fig. 3.- The sperms' mid-piece (**A**) and flagellum (**B**) length in the different groups are shown. The significant difference between control groups in comparison to the diabetic groups is indicated. *p < 0.05. The sperms' head volume (**C**) in the different groups are shown. The significant difference between control groups in comparison to the diabetic groups is indicated. *p < 0.05. (D) Diff quick staining of sperms, sperm head (H), sperms mid-piece (MP), sperm tail (T).

In recent research projects, stereological methods are growingly used in order to evaluate various morphometric parameters of three-dimensional objects (Rønn et al., 2000).

As stereological techniques have scientific advantages over quantitative results, this study utilized stereological methods for estimating sperm structure. Design-based stereology can enable invaluable three-dimensional (3D) information and quantitative data regarding the structural changes in sperm morphology such as sperm volume and sperm mid-piece and flagellum length that cannot be obtained using any other physiological, biochemical, or molecular techniques (Mühlfeld et al., 2010). The mentioned methods could not only be used in sperm analysis, but also in quantitative investigations of sperm parameters in various different studies. This study uses design-based stereological methods in order to obtain scientifically reliable estimates of the sperm head volume and sperm flagellum length in the control and diabetic mice. Our data indicated that the reduction of the sperm head volume and sperm flagellum length in diabetic mice in comparison with the control groups. These data support that the changes head volume and sperm flagellum length may related to the sperm fertility potential in diabetic mice.

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