



## Original Article

## Gestational diabetes reduced sertoli cells in 12 weeks age rat offsprings testis



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## ABSTRACT

**Introduction:** Previous study has shown the adverse effects of type 1 and 2 diabetes mellitus on male reproductive system. This study was done to evaluate the effect of induced gestational diabetes on seminiferous tubule of 12 weeks age offspring rats.

**Methods:** 10 Wistar rats' dams were randomly allocated to control and diabetic groups. Streptozotocin was used to induce diabetes in female rats. Dams in diabetic group received 40 mg/kg/BW of streptozotocin at the first day of gestation and control group animals received an equivalent volume of normal saline by intraperitoneally. Six offspring of each group were randomly selected on day 84 postnatal. Five micrometer sections were taken from testes, stained with hematoxylin and eosin. Photographs of sections were taken using Olympus BX51 microscope and a digital camera DP12. Density and number of spermatogenesis cells, leydig cells, sertoli cells, seminiferous tubule diameter and Seminiferous epithelial height and dUTP end-labeling positive cells were evaluated in 50,000  $\mu\text{m}^2$  area of seminiferous tubules by Olysia Autobioreport software.

**Results:** Spermatogenesis and leydig cells in gestational diabetic offsprings non-significantly reduced in compare to controls. Sertoli cells significantly reduced in gestational diabetic offspring compared to controls. Seminiferous tubular diameter and seminiferous epithelial height non-significantly reduced in gestational diabetic offspring compared to controls. The apoptotic cells in diabetic group non-significantly increased in comparison with controls. The histopathological alterations were not seen in experimental group.

**Discussion:** Uncontrolled gestational diabetes significantly reduces the sertoli cells but non-significantly reduces the spermatogenic cells in the rat offsprings.

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## 1. Introduction

Hyperglycemia, altered metabolism of lipids, carbohydrates and proteins are characterized of diabetes mellitus as the most common serious metabolic disorders.<sup>1,2</sup>

Type I or insulin dependent, type II or insulin independent and Gestational diabetes are three general classifications of diabetes mellitus.<sup>3</sup>

Vascular disorder, retinopathy, cardiomyopathy, altered immune functions, changes in the intestinal function, peripheral neuropathy, and dysfunctions of the central nervous system in both human and animal models of the disease are related to Diabetes mellitus.<sup>1,4,5</sup>

Gestational diabetes mellitus (GDM) defined as impaired glucose tolerance affects approximately 4% of all pregnant women who have never before had diabetes, but who do have high blood glucose levels during pregnancy.<sup>3</sup>

Our previous studies have shown that Gestational diabetes mellitus causes neural alteration in brain cortex, hippocampus, dentate gyrus, cerebellum and retina.<sup>6–11</sup>

On the other hand, several studies reported that the induced diabetes in adult male animals causes adverse effect on seminiferous tubules,<sup>12–15</sup> but there is no study about the effect

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of gestational diabetes on seminiferous tube of offspring. Therefore, this study was done to evaluate the effect of induced gestational diabetes on seminiferous tubule of 12 weeks age offspring rats.

## 2. Materials and methods

This experimental study was performed at the Gorgan faculty of Medicine, Golestan University of medical sciences, Gorgan, Iran. Guidelines on the care and use of laboratory animals and approval of the ethic committee of Golestan University of medical sciences were obtained before study.

### 2.1. Experimental animals

Wistar rats, weighting 180–220 grams (12 weeks old) were used in this study. The animals were maintained in a climate-controlled room under a 12-hour alternating light/dark cycle, 20 °C–25 °C temperature, and 50%–55% relative humidity. Dry food pellets and water were provided *ad libitum*.

### 2.2. Drug

Streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in sterile saline solution (0.85%) to give 40 mg/kg dose intraperitoneally inject to female rats.

### 2.3. Animal groups and treatment

After 2 weeks of acclimation to the diet and the environment, female Wistar rats were placed with a proven breeder male overnight for breeding. Vaginal smears were done the next morning to check for the presence of sperm. Once sperm was detected that day was assigned as gestational day 0 (GD0). On day 1 of gestation, pregnant females randomly divided into two control and diabetic groups.

Five female rats in diabetic group receiving 40 mg/kg/body weight of streptozotocin (STZ) and control groups (five rats) receiving an equivalent volume normal saline injection intraperitoneally (IP). Blood was sampled from the tail at 1 week after STZ injection. The dams with blood glucose level 120–250 mg/dl were labeled as having Gestational Diabetes Mellitus (GDM). The pregnancy of dams was terminated physiologically.

Six offspring of gestational diabetic mothers and control mothers on day 84 postnatal were randomly selected and sacrificed. For light microscope preparations right testis was fixed in 10% neutral-buffered formalin for histological procedure. Five micrometer sections were taken from testis, stained with hematoxylin and eosin.

### 2.4. Blood glucose measurements

Blood glucose level of mothers (before mating and after STZ injection) and offspring was obtained via tail vein and was estimated with a glucometer (ACCU-CHEK® Active Glucometer, Roche Diagnostics, Mann-heim, Germany).

### 2.5. Morphometric techniques

In each sample, ten similar sections of right testis were selected and images of five separate fields were captured by Olympus BX 51 microscope and DP12 digital camera attached to OLYSIA auto-biopsy software (Olympus Optical, Co. LTD, Tokyo, Japan).

Density and number of spermatogenesis cells, leydig cells, sertoli cells, seminiferous tubule diameter (STD) and Seminiferous epithelial height (SEH) and dUTP end-labeling (TUNEL)-positive

cells were evaluated in 50,000  $\mu\text{m}^2$  area of seminiferous tubules by OLYSIA Autobioreport software.

### 2.6. Terminal transferase dUTP nick-end labeling (TUNEL) techniques

The whole-mounted testis stained with the terminal transferase dUTP nick-end labeling (TUNEL) reaction to detect apoptosis (in situ cell death detection kit; fluorescence; Roche, Mannheim, Germany) according to the manufacturer's instructions. Tissue slices were pre-treated with proteinase K (10 mg/mL) in 0.05 M Tris-HCl buffer, pH 7.4, washed in phosphate-buffered saline (PBS), then labeled with TUNEL reaction mixture

Nuclear DNA fragmentation were analyzed under a fluorescence microscope (Olympus BX51, Japan) and camera DP72 using an excitation wavelength in the range of 450–500 nm, and detection was in the range 515–565 nm (green). The number of TUNEL- nuclei was counted in 1000010,000  $\mu\text{m}^2$  area of the seminiferous tubules of testis in 400X magnification using OLYSIA Autobioreport software (Olympus Optical, Co. LTD, Tokyo, Japan).

### 2.7. Statistical analysis

Morphometric data is expressed as the mean  $\pm$  SEM and analyzed by the Student's "t" test using SPSS 16.5 software. *P*-value <0.05 was considered significant.

## 3. Results

### 3.1. Blood glucose concentrations

The blood glucose of dams before and 72 h after induction of diabetes is depicted in Fig. 1.

The mean  $\pm$  SEM of blood glucose concentrations before mating and 72 h after STZ injection were 99.60  $\pm$  6.2 and 211.60  $\pm$  6.30 mg/dl in diabetic dams, respectively. In control dams the mean  $\pm$  SEM of blood glucose concentrations before mating and 72 h after STZ injection were 99.60  $\pm$  6.2 and 92.53  $\pm$  5.3 mg/dl, respectively.

### 3.2. Body and testis weight

The mean  $\pm$  SEM of body and testis weight of 84- day old offspring non-significantly reduced in diabetic in comparison with controls (Table 1).

### 3.3. Morphometric results

The morphometric findings are depicted in Figs. 2 and 3, Table 2.

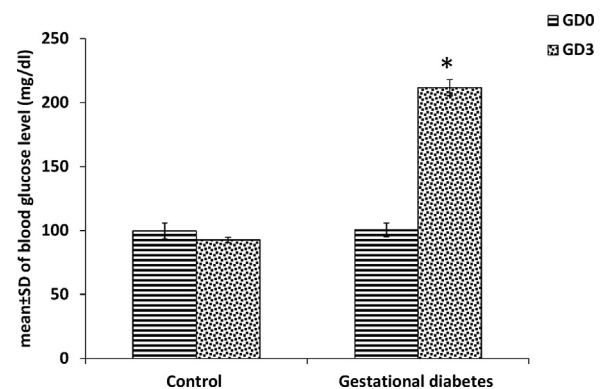


Fig. 1. The mean  $\pm$  SEM of the blood glucose of dams before and 72 h after induction of diabetes (\* *P*-value <0.05).

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