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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 μm^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.

1. Introduction

Hyperglycemia, altered metabolism of lipids, carbohydrates and proteins are characterized of diabetes mellitus as the most common serious metabolic disorders.[1,2]

Type I or insulin dependent, type II or insulin independent and Gestational diabetes are three general classifications of diabetes mellitus.[3]

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.[1,4,5]

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.[3]

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

On the other hand, several studies reported that the induced diabetes in adult male animals causes adverse effect on seminiferous tubules,[12–15] 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 autobioreport 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 μm^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 μm^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±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 ± SEM of blood glucose concentrations before mating and 72 h after STZ injection were 99.60 ± 6.2 and 211.60 ± 6.30 mg/dl in diabetic dams, respectively. In control dams the mean ± SEM of blood glucose concentrations before mating and 72 h after STZ injection were 99.60 ± 6.2 and 92.53 ± 5.3 mg/dl, respectively.

3.2. Body and testis weight

The mean ± 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.

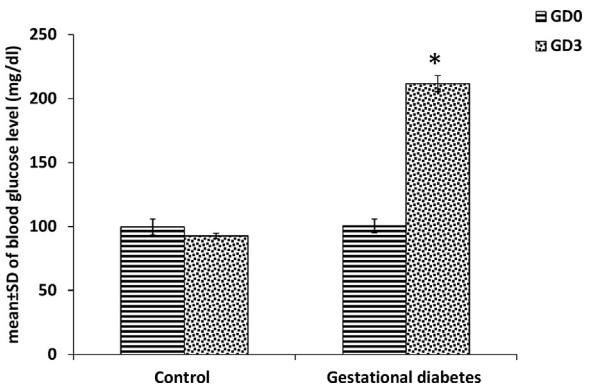

Fig. 1. The mean ± SEM of the blood glucose of dams before and 72 h after induction of diabetes (* *P*-value <0.05).

Table 1
The mean±SEM of body and testicular weight in 12 weeks old offspring (mg) in control and gestational diabetes groups.

weight	Control (n = 6)	Gestational diabetes (n = 6)	p-value
Body	217.81 ± 4.4	214.00 ± 4.0	NS
Testis	1.97 ± 0.07	2.13 ± 0.08	NS

NS: Non significant.

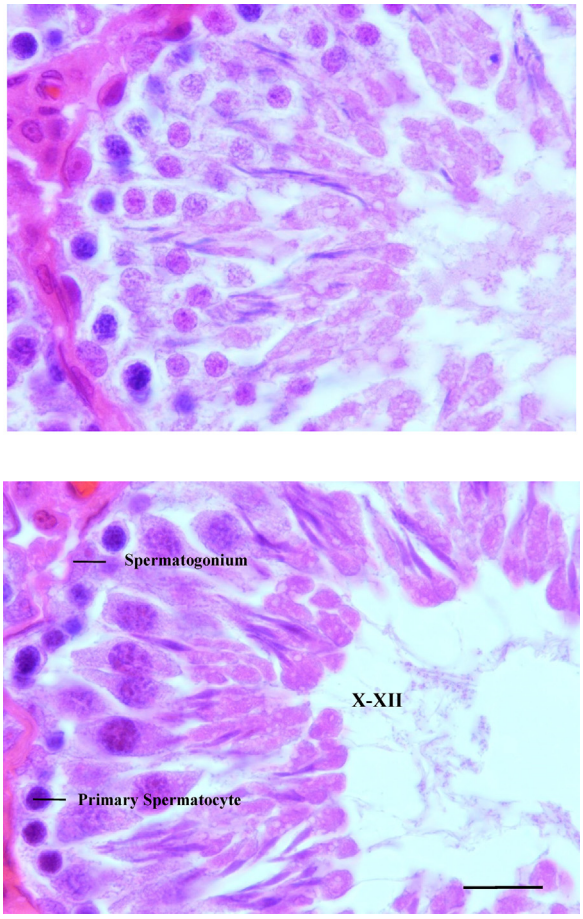

Fig. 2. Photomicrographs histology of testicular tissue from gestational diabetic offsprings did not show pathological findings. Qualitative analysis of histology section of testicular tissue for spermatogenesis cells (spermatogonia, spermatocyte and round spermatid), sertoli and leydig cells non-significantly reduced in gestational diabetic offspring as compared to control. The tissue sections were taken at 5 μm and stained with hematoxylin and eosin. (1000X, Scale bar: 25 μm).

Seminiferous tubular diameter (242.05 + 2.5 vs 249.44 + 3.8 micrometer) and seminiferous epithelial height (76.71 + 2.1 vs 80.84 + 1.7) non-significantly reduced in gestational diabetic offspring compared to controls.

The number of spermatogenesis cells including spermatogonia, spermatocyte and spermatid non-significantly reduced in gestational diabetic offspring compared to controls (Table 2).

The number of leydig cells was 21.66 + 3.3 and 27.33 + 1.9 in gestational diabetic and control offspring, respectively. This reduction was not significant.

The number of sertoli cells was significantly reduced in gestational diabetic offspring (9.83 + 1.1) compared to controls (17.44 + 1.2) (*P*-value <0.001).

The number of apoptotic cells was 12.83 + 1.3 and 9.83 + 1.1 in diabetic and control groups; respectively. This increase of apoptotic cells in diabetic group was not significant in comparison with controls.

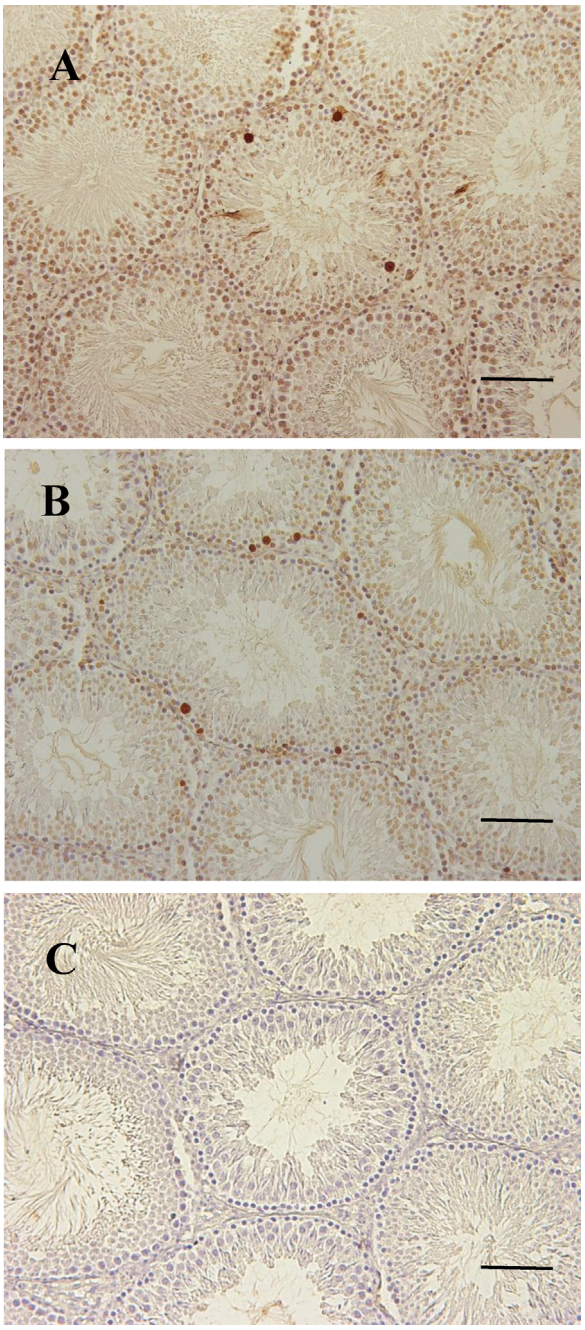

Fig. 3. TUNEL-positive cells in testicular sections of gestational diabetic rat offsprings. Photomicrograph in A is from a 84 days old control and B gestational diabetic offsprings. TUNEL-positive cells non-significantly increased in gestational diabetic offspring as compared to control. C the negative control (200X, Scale bar: 25 μm).

Table 2
The mean±SEM of spermatogenic cell line, leydig and Sertoli cells in 12 weeks old offspring (5000 μm^2) in control and gestational diabetes groups.

Cell	Control (n = 6)	Gestational diabetes (n = 6)	p-value
Spermatogonia	26.05 ± 3.1	19.66 ± 1.6	NS
Primary spermatocyte	58.83 ± 5.1	55.72 ± 4.5	NS
Spermatid	81.22 ± 14.1	69.50 ± 14.4	NS
Sertoli	17.44 ± 1.2	9.83 ± 1.1	0.001
Leyding	27.33 ± 1.9	21.66 ± 3.3	NS

NS: Non significant.
"*P-value <0.05."

The histopathological alteration was not seen in diabetic group in comparison with controls.

4. Discussion

This study showed that gestational diabetes produces a significant reduction in the density of the Sertoli cells and non-significant reduction of the spermatogenic and leydig cells in the 12 weeks of Wistar rat offspring.

Several animal model studies have shown the adverse effect of diabetes mellitus on male reproductive system including disruption of spermatogenesis and morphometric alterations of seminiferous tubules.[12–15]

In our study seminiferous tubular diameter and seminiferous epithelial height non-significantly reduced in diabetic offspring testis in compared to controls. Several animal model studies have shown offsprings born to dams with induced pre-pregnancy diabetes mellitus have low seminiferous tubular diameter and seminiferous epithelial height.[15,16] Also, several animal model studies have reported that diabetes mellitus reduces seminiferous tubular diameter and seminiferous epithelial height in male adult rats.[17–19]

In this study, we observed non-significant reduction of the spermatogenic cells. Animal model studies have shown reduction of spermatogenic cells due to induced diabetes mellitus in male adult rats.[12,17,20] Furthermore, several animal model studies have shown that induced pre-pregnancy diabetes mellitus reduces spermatogenic cells of offsprings.[15,16] In our study, leydig cells in the 12 weeks of Wistar rat offspring non-significantly reduced in diabetic offspring testis in compared to controls. Several animal model studies have shown reduction of leydig cells due to induced diabetes mellitus in male adult rats,[12,17,20] and in offspring born from dams with induced pre-pregnancy diabetes mellitus.[15,16]

In this study, the number of Sertoli cells was significantly reduced in gestational diabetic offspring compared to controls. Our result in this regard is similar to previous studies.[12,14–18] Several animal model studies have shown that dams with induced pre-pregnancy diabetes mellitus born offspring with low Sertoli cells.[15,16] Also, several animal model studies have reported that diabetes mellitus reduces the sertoli cells in male adult rats.[12,14,17,18]

In our study, apoptotic cells in the 12 weeks of Wistar rat offspring non-significantly increased in diabetic offspring testis in compared to controls. Also, other studies have reported increase of apoptotic cells in testis due to diabetes mellitus.[19]

The adverse effect of diabetes mellitus on spermatogenic cell line can be due to dysfunction of leydig cells and reduction of testosterone hormone.[12] Also, hyperglycemia reduces the vital ability of Sertoli cells which can damage to spermatogenic cell line and spermatogenesis.[21]

Indeed, hyperglycemia reduces the blood vessels and blood stream of testis which can damage to sertoli and leydig cells.[22]

Furthermore, hyperglycemia induces ultrastructure of sertoli cells including reduces of reticulum endoplasmic, increase of lipid vacuole, abnormal shape of mitochondria and alteration of cellular junctions of the cells.[14]

Regardless of diabetes mellitus type, it is associated with hyperglycemia. Several possible mechanisms are explained about cellular and hormonal alterations of testis tissue.

Diabetes mellitus is a chronic endogenous stressor that is associated with increased oxidative stress. Male reproductive system complications of diabetes mellitus could be mediated through excessive free radicals generation, these radicals contribute to increase cell death by oxidizing proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes.[23,24] Oxidative stress by free radicals can cause testicular degeneration, testicular weight loss, decrease of motility of sperm and fertility rate.[23,25–29]

Other mechanism may be due to insufficient of insulin hormone. Insulin hormone regulates Leydig and sertoli cells activities, therefore decrease of insulin hormone

Causes adverse effect on spermatogenic cell line through decrease of FSH and LH.[14,16,21]

Also, other possible mechanism in cause of program cell deaths in diabetes mellitus can be due to decrease insulin or insulin-like growth factor signaling, or an increase in cytokines such as TNFa.[28,30–32] Hyperglycemia could induce cellular death by enhancing tissue acidosis.[33]

5. Conclusion

In conclusion, This study revealed that uncontrolled gestational diabetes significantly reduces the sertoli cells and also it non-significantly reduces the spermatogenic cells in the rat offsprings. Further studies is require to determine the exact mechanism and alterations of gene expression of spermatogenic cell line and Sertoli cell of gestational diabetes offsprings.

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References

1. Gispen WH, Biessels GJ. Cognition and synaptic plasticity in diabetes mellitus. *Trends Neurosci*. 2000;23:542–549.
2. Yurii V, Lebeda MA, Orlovskya AG, et al. Early reaction of astroglial cells in rat hippocampus to streptozotocin-induced diabetes. *Neurosci Lett*. 2008;444:181–185.
3. Persaud ODD. Maternal diabetes and the consequences for her offspring. *J Dev Disab*. 2007;13(1):101–104.
4. McCall AL. The impact of diabetes on the CNS. *Diabetes*. 1992;41:557–570.
5. Biessels GJ, Heide LPV, Kamal A, et al. Ageing and diabetes: implications for brain function. *Eur J Pharmacol*. 2002;441:1–14.
6. Ghafari S, Golalipour E, Golalipour MJ. Alterations of the giant pyramidal neurons (Betz cells) in brain cortex of rat offspring born from gestational diabetic dams: a morphometric study. *Int J Morphol*. 2015;33:1120–1125.
7. Najafdari S, Rezaei N, Malekzadeh Shafaroodi M, et al. Ganglionic cells apoptosis in retinal layer of rat offspring due to gestational diabetes. *Int J Morphol*. 2014;32:1131–1135.
8. Razi EM, Ghafari S, Golalipour MJ. Effect of gestational diabetes on Purkinje and granule cells distribution of the rat cerebellum in 21 and 28 days of postnatal life. *Basic Clin Neurosci*. 2015;6:6–13.
9. Razi EM, Ghafari S, Hojati V, et al. Effect of gestational diabetes on neuronal cells in rat cerebellum in early postnatal life. *Int J Morphol*. 2014;32:420–425.
10. Kafshgiri SK, Ghafari S, Golalipour MJ. Gestational diabetes induces neuronal loss in dentate gyrus in rat offspring. *J Neurol Sci*. 2014;31:316–324.
11. Golalipour MJ, Kafshgiri SK, Ghafari S. Gestational diabetes induced neuronal loss in CA1 and CA3 subfields of rat hippocampus in early postnatal life. *Folia Morphol (Warsz)*. 2012;71:71–77.
12. Ricci G, Catizone A, Esposito R, et al. Diabetic rat testes: morphological and functional alterations. *Andrologia*. 2009;41:361–368.
13. Shalaby M, Mouneir SM. Effect of Zingiber officinale roots and cinnamon zeylanicum bark on fertility of male diabetic rats. *Glob Vet*. 2010;5:341–347.
14. Kianifard D, Sadrkhanlou RA, Hasanzadeh Sh. The histological, histomorphometrical and histochemical changes of testicular tissue in the metformin treated and untreated streptozotocin-induced adult diabetic rats. *Vet Res Forum*. 2011;2:13–24.
15. Elbastawisy YM, Elsaed WM, Algaidi SA. Hormonal and testicular ultrastructural changes at puberty in rat offspring from diabetic mothers. *J Am Sci*. 2012;8:437–442.
16. Jelodar G, Khaksar Z, Pourahmadi M. Endocrine profile and testicular histomorphometry at puberty in rat offspring from diabetic mothers. *Comp Clin Pathol*. 2010;19:135–139.
17. Altay B, Cetinkalp S, Do-anavsargil B, et al. Streptozotocin-induced diabetic effects on spermatogenesis with proliferative cell nuclear antigen immunostaining of adult rat testis. *Fertil Steril*. 2003;80:828–831.

18. Erfani Majd N, Bbahrami M, Morovati H, et al. The protective effect of aloe vera on histological and histometrical structure of diabetic rat testis. *Sci-Res Iran Vet J*. 2013;9:78–87.
19. Ozdemir O, Akalin PP, Baspinar N, et al. Pathological changes in the acute phase of streptozotocin-induced diabetic rats. *Bull Vet Inst Pulawy*. 2009;53:783–790.
20. Arikawe A, Daramola A, Odofin A, et al. Alloxan-induced and insulin-resistant diabetes mellitus affect semen parameters and impair spermatogenesis in male rats. *Afr J Reprod Health*. 2006;10:106–113.
21. Gondos B, Rivkind Y, Jovanovic L. Effect of increasing glucose concentrations on sertoli cell viability in the nonobese diabetic mouse. *Ann Clin Lab Sci*. 1998;28:236–241.
22. Piryaei A, Najar A, Bayat M. Effects of pentoxifylline administration on histomorphological parameters of streptozotocin-induced diabetic rat testes. *Lab Anim Res*. 2015;31:111–116.
23. Amaral S, Moreno AJ, Santos MS, et al. Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. *Theriogenology*. 2006;66:2056–2067.
24. Mallidis C, Green B, Rogers D, et al. Metabolic profile changes in the testes of mice with streptozotocin-induced type 1 diabetes mellitus. *Int J Androl*. 2009;32:156–165.
25. Raza H, Ahmed I, John A. Tissue specific expression and immunohistochemical localization of glutathione S-transferase in streptozotocin induced diabetic rats: modulation by Momordica charantia (karela) extract. *Life Sci*. 2004;74:1503–1511.
26. Shrilatha B. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences. *Reprod Toxicol*. 2007;23:578–587.
27. Zhao H, Piao C, Gong S. Diabetic damage to male reproduction and its mechanism. *Zhonghua nan ke xue = Nat J Androl*. 2004;10:767–770 [Abstract].
28. Arroba AI, Frago LM, Argente J, et al. Activation of caspase 8 in the pituitaries of streptozotocin-induced diabetic rats: implication in increased apoptosis of lactotrophs. *Endocrinology*. 2005;146:4417–4424.
29. Zakerabasali A, Keshavarz M, Mozafar A, et al. Protective effects of vitamin E and selenium on spermatogenesis in adult male rat insulin-resistant. *J Fasa Uni Med Sci*. 2013;2:3008–3013.
30. Nishikawa T, Araki E. Impact of mitochondrial ROS production in the pathogenesis of diabetes mellitus and its complications. *Antioxid Redox Signal*. 2007;9:343–353.
31. Lechuga-Sancho AM, Arroba AI, Frago LM, et al. Reduction in the number of astrocytes and their projections is associated with increased synaptic protein density in the hypothalamus of poorly controlled diabetic rats. *Endocrinology*. 2006;147:5314–5324.
32. Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science*. 2002;296:1634–1635.
33. DeBoer T, Wewerka S, Bauer PJ, et al. Explicit memory performance in infants of diabetic mothers at 1 year of age. *Dev Med Child Neurol*. 2005;47:525–531.