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Original Article

Malfunction of Sertoli cell secretion leads to testicular damage in diabetic rats

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Abstract: In this work, we investigate changes in the secretory function of Sertoli cells in diabetic rats and its correlation with testicular damage. Diabetes was induced by intraperitoneal injection of streptozotocin (55 mg/kg/i.p.) in adult rats. Eight weeks later, testicular tissue was harvested. Serum inhibin B (INHB) and testosterone (T) levels were measured by ELISA. The levels of secretory proteins, such as androgen-binding protein (ABP) and transferrin (TF), were measured by western blot analysis. Testicular damage was evaluated by hematoxylin and eosin staining, and apoptosis was identified by terminal-deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL). Seminiferous tubular sperm formation was evaluated by the Johnsen's score. In diabetic rats, ABP protein levels (P = 0.038), serum INHB (P = 0.031), and T levels (P = 0.039) decreased greatly, whereas the decrease in TF protein levels (P = 0.247) was not significant. Additionally, TUNEL-positive cells (P = 0.000) increased and the Johnsen's score (P = 0.000) decreased in diabetic testes. These results suggest that the secretory function of Sertoli cells in diabetic rats was damaged. The altered secretory function of Sertoli cells may be responsible for disturbed spermatogenesis.

Keywords: Diabetes mellitus, sertoli cells, secretory function

Introduction

Diabetes mellitus (DM) is an increasingly prevalent public health threat. Recent studies indicated that male infertility is a major complication of DM [1, 2]. The adverse effects of DM on male semen parameters may be mediated through multiple mechanisms including increased oxidative stress, altered spermatogenesis, degenerative and apoptotic changes in the testes, altered glucose metabolism in the Sertoli cell blood-testis barrier, and reduced serum testosterone levels [3-8].

Sertoli cells, regarded as the nurse cells for germ cells, are somatic cells in the seminiferous epithelium that provide nutritional and physical support for spermatogenesis [9]. Secretory proteins, such as inhibin B (INHB), androgen-binding protein (ABP), and transferrin (TF), play an important role in spermatogenesis [10, 11]. Previous studies indicate that Sertoli cells are also affected in a rat model of DM induced by injecting streptozotocin (STZ) intraperitoneally [7, 12]. However, little is known about the effects of DM-associated factors on the secretory function of Sertoli cells in rats. In this study, we investigated the changes in the secretory function of Sertoli cells in diabetic rats over 8 weeks, a period that represents one spermatogenic cycle.

Materials and methods

Adult male Sprague-Dawley rats, aged 8-9 weeks and weighing 215-235 g, were obtained from Vital River Experimental Animal Center, Beijing, China (Certificate No. SCXK (Beijing) 2012-0001). Rats were housed in individual cages with free access to water and laboratory chow. They were maintained under a 12 h light/dark cycle at constant room temperature (22 ± 1°C) and humidity (60%). All experimental procedures were performed as approved by the local Animal Use Committees of the China-Japan Friendship Hospital.

Rats were divided into the control group (n = 6) and the diabetic group (n = 6). Diabetes was

Table 1. Blood glucose levels, body weight, testis weight, serum INHB, and testosterone ($\bar{x}$ ± s)

Parameter	Control	DM	*p* value
Blood glucose (mmol/L)	6.39 ± 0.77	32.14 ± 1.71*	0.001
Initial body weight (g)	224.9 ± 6.57	225.5 ± 4.22	0.431
Final body weight (g)	551.25 ± 56.52	340.25 ± 44.72*	0.000
Testis weight (g)	3.48 ± 0.28	2.45 ± 0.24*	0.000
Serum INHB (pg/mL)	13.42 ± 2.27	10.82 ± 1.14*	0.031
Serum testosterone (ng/mL)	0.60 ± 0.11	0.41 ± 0.16*	0.039

*$P < 0.05$ compared with control group.

induced by a single intraperitoneal injection of STZ (55 mg/kg, Sigma Aldrich, St. Louis, MO, USA). The animals were considered diabetic if blood glucose values were over 16.7 mmol/L at 72 h after STZ treatment, using a drop of blood obtained by a tail vein puncture. The control animals were sham injected with an equivalent dose of drug vehicle (0.1 M citrate buffer, pH 4.5). Eight weeks after STZ injection, the serum from sacrificed rats was obtained to measure INHB and T concentrations. The testes tissues were harvested for western blot and histological examination.

Body and testes weights

Body weights of all animals were weighed at the beginning and end of the study. Immediately after sacrifice, testes were excised and their weights were recorded.

Serum INHB and T

Concentrations of INHB and T in serum samples were measured with an ELISA kit (CUSABIO, China) according to the manufacturer's protocol. Absorbances were measured with an ELISA plate reader at a wavelength of 450 nm.

Western blot

Testis cell lysates containing 20 μg protein were run on a 10% SDS-polyacrylamide gel and then transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat-milk in TBST for 1 h at room temperature and then incubated with the primary antibodies at a dilution of 1:1000 each for mouse anti-transferrin (Abcam, Cambridge, MA, USA), rabbit anti-androgen binding protein (Abcam), mouse anti-beta actin (Zhongshan, Beijing, China) at 4°C overnight. The membranes were washed and incubated with the secondary anti-rabbit IgG-HRP antibody or antimouse IgG-HRP (Jackson, PA, USA, 1:10,000) at room temperature for 40 min. The membranes were washed three times with TBST, and protein was visualized by enhanced chemiluminescence according to the manufacturer's instructions.

TUNEL assay

Apoptosis was evaluated by a TUNEL assay using an In Situ Cell Death Detection Kit (Roche, Germany) according to the manufacturer's protocol. The sections were deparaffinized, hydrated in a series of alcohol, and deproteinized by proteinase K (20 μg/mL) for 15 min at 37°C. The sections were rinsed and incubated in the TUNEL reaction mixture. The sections were rinsed, visualized with a converter POD with 0.02% 3,3'-diaminobenzidine, and counterstained with hematoxylin. The apoptosis index was defined as the number of apoptotic TUNEL-positive cells per 50 tubules. Two observers blind to the source of testicular tissue performed all measurements. Computerized histomorphometric analysis was performed using Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD, USA).

Examination of spermatogenesis

The testis specimens were fixed in 4% paraformaldehyde and embedded in paraffin blocks. Sections (5 μm) were deparaffinized and stained with HE. The histopathological changes in testicular tissue were evaluated by the Johnsen's testicular score system. Thirty cross-sectioned tubules in each group were evaluated systematically, and the Johnsen's score was used to categorize the spermatogenesis [13].

Statistical analysis

Results are presented as mean ± standard deviation. Statistical analysis was performed by using SPSS 15.0. The significance of differences of body weight, testis weight, serum INHB and serum testosterone between DM and control groups were determined by Independent-Samples T test. The significance of differ-

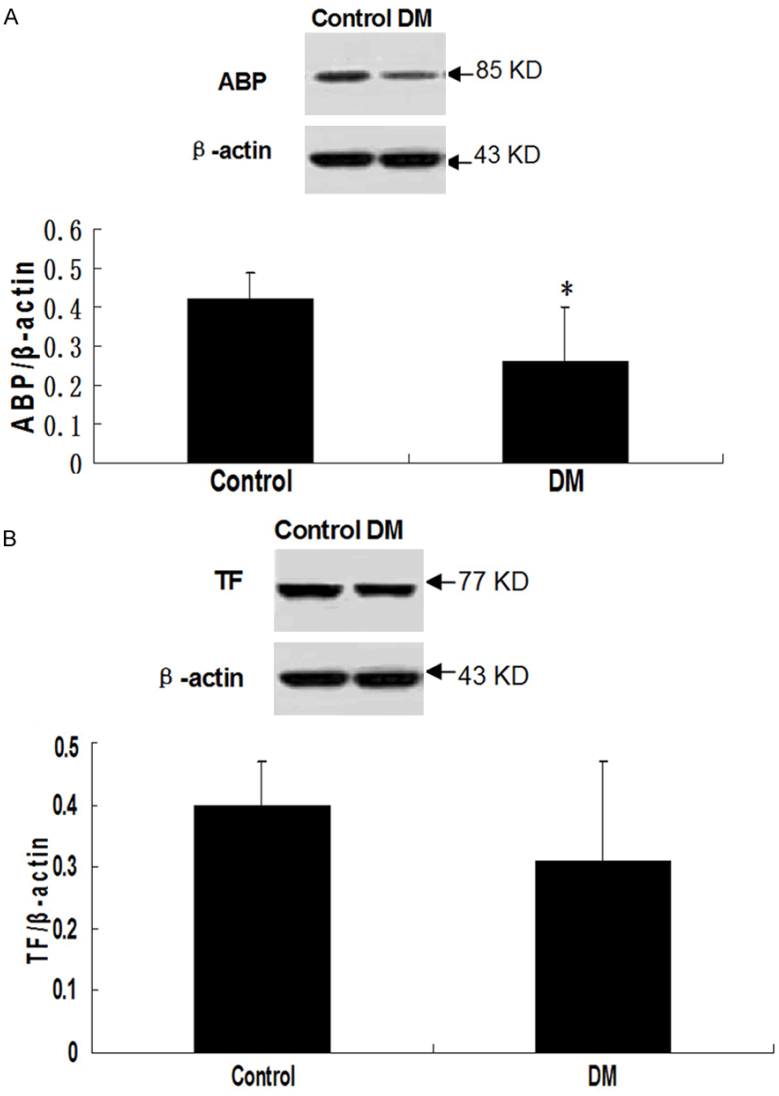

Figure 1. Evaluation of protein expression. A. Top: expression of ABP; bottom: quantitative analysis of ABP protein expression. B. Top: expression of TF; bottom: quantitative analysis of TF protein expression. Data are shown as mean ± SD. *$P < 0.05$ versus the control group.

ences of blood glucose was determined by Nonparametric Tests of 2 Independent Samples. A value of $P < 0.05$ was considered as significant.

Results

Blood glucose level, body weight, and testis weight

The blood glucose level, body weight, and testis weight of the rats at week 8 are presented in **Table 1**. Blood glucose levels in the DM rats were significantly higher than those in the control group ($P = 0.001$). In addition, the final body weight and testis weight of the rats in the STZ group were significantly lower than those in the control group ($P = 0.000$).

Serum concentrations of INHB and T

Rats in the DM group exhibited decreased serum INHB ($P = 0.031$) and T ($P = 0.039$) concentrations compared with those in the control group (**Table 1**).

ABP and TF protein expression in rat testis

Decreased ABP protein levels were observed in the DM group compared with the control group ($P = 0.038$, **Figure 1A**). However, the TF protein levels were not decreased significantly compared with the control group ($P = 0.247$, **Figure 1B**).

Evaluation of TUNEL staining

The number of TUNEL-positive cells in the DM group ($P = 0.000$) was significantly increased compared with the control group. Most of the apoptotic cells observed were spermatogenic cells (**Figure 2**).

Testicular histopathology

Testicular tissues in the control group showed a normal arrangement of germinal and Sertoli cells with no histopathological lesions (**Figure 3A**). The DM group showed moderate to severe testicular atrophy with severe cellular disorganization and degeneration in seminiferous tubules (**Figure 3B**). The Johnsen's score was lower in the DM group compared with the control group ($P = 0.000$).

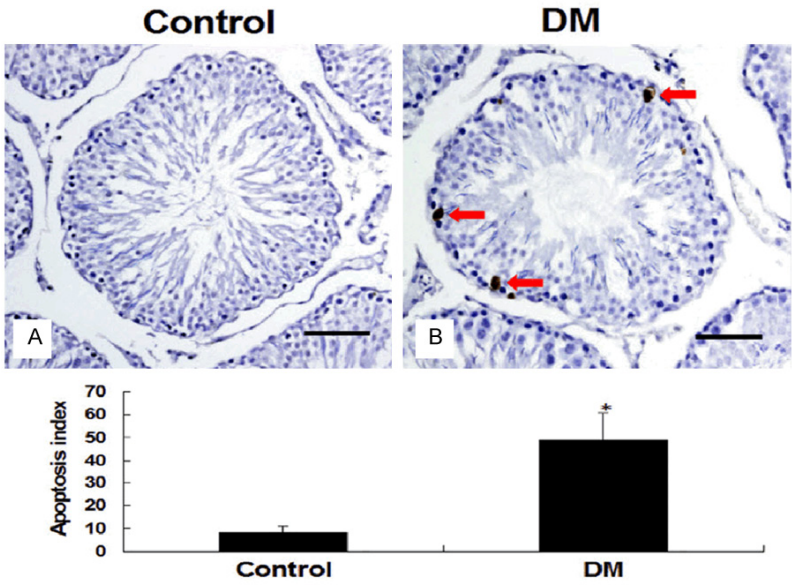

Figure 2. Evaluation of TUNEL. Top: Evaluation of TUNEL staining. Arrows indicate TUNEL-positive apoptosis cells (×200). DM: diabetes mellitus. Bottom: quantitative analysis of apoptosis index in testes. Scale bar, 50 μm. Data are shown as mean ± SD. *P < 0.05 compared with the control group.

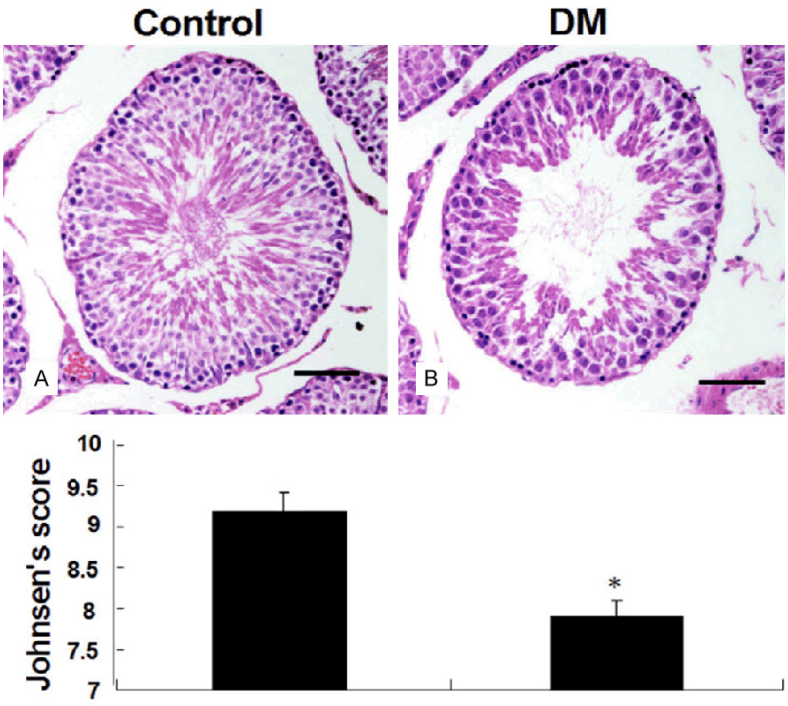

Figure 3. Evaluation of histologic changes. Top: Comparison of the histological changes between the control and DM groups. Seminiferous tubules and interstitium in testicular tissue were normal in the control animals. There were fewer germinal cells, and they were degraded and disorganized in diabetic rats (×200). Bottom: quantitative analysis of the Johnsen's score in testes. Scale bar, 100 μm. Data are shown as mean ± SD. *P < 0.05 compared with the control group.

Discussion

Sertoli cells are a target of DM induced by STZ. In diabetic rats, an ultrastructural study showed mitochondrial changes and reduction in the smooth endoplasmic reticulum in Sertoli cells [12]. Moreover, the vimentin apical extension of Sertoli cells decreased significantly in the STZ group [7].

ABP, INHB, and TF are biological markers of the secretory functions of Sertoli cells. Owing to the importance of Sertoli cells in the testis, any agent that impairs the secretory function of Sertoli cells may have adverse effects on spermatogenesis [14]. ABP binds and transports androgens, protects them from degradation, and controls their bioavailability in the testis [15, 16], which facilitates the development and maturation of spermatogenic cells [17]. T is the main in vivo regulator of ABP synthesis in diabetic rats. The marked increase in T downregulates ABP expression in rat testis [18, 19]. ABP and androgens may be factors that regulate germ cell apoptotic waves in mice [20]. However, there are contradictory results about ABP expression in diabetic rats. Premalatha et al. [21] showed that the concentration of ABP decreased in diabetic rats compared with normal rats, whereas Velten et al. [22] showed that ABP concentration was increased but ABP content was not changed in testicular tissue. This discrepancy may

arise from differences in STZ dose and duration. In our study, ABP protein levels decreased in the DM group, and the total serum testosterone level in our study was significantly lower in the DM group, which was consistent with most previous studies [7]. However, we did not observe the related changes in the T-ABP levels in the rat testis.

INHB, which is mainly secreted by the Sertoli cells in the testis, is a glycoprotein that modulates follicle-stimulating hormone secretion via a negative feedback loop [23]. INHB is also a marker of Sertoli cell damage and spermatogenic disturbance. Markedly decreased INHB levels have been associated with severe damage to the testes [24, 25]. In rats, INHB levels reflect major spermatogenic alterations, and greatly decreased INHB levels may indicate irreversible alterations and even infertility [26]. Meanwhile, sperm count and testicular volume in patients were significantly and positively correlated with INHB [27]. Similarly, we showed that the total serum INHB level was significantly lower in the DM group.

TF, which is also secreted by Sertoli cells, plays a critical role in the delivery of iron from the somatic compartment to the germ cells sequestered by the blood-testis barrier [28]. This observation suggests a relationship between germ cell and Sertoli cell transferrin synthesis. In addition, a mutant mouse that can not synthesize normal amounts of transferrin has a reduced ability to produce functional sperm [29]. Loss of germ cells induced by mild hyperthermia is associated with reduced transferrin expression [30]. Although the decreases in TF levels in the testis were not significant, our results showed that TF secretion was impaired. In summary, the decreased concentrations of ABP, INHB, and TF in DM rats could reflect impaired Sertoli cell secretory function. Significant increases in the numbers of apoptotic cells in testes and decreases in the number of germinal cells and mean testicular biopsy score (MTBS) values have been observed in STZ-induced diabetic rats [31]. Our data showed that the apoptosis index was significantly increased in the DM group. In addition, we observed a much lower Johnsen's score in the DM group compared with the control group. These data further support the view that DM impairs spermatogenesis [7].

We showed that ABP, INHB, and TF in Sertoli cells were involved in the regulation of spermatogenesis and germ cell apoptosis in diabetic rats, and our results indicated that the secretory function of Sertoli cells was damaged. Therefore, the altered secretory function of Sertoli cells may contribute to disruption of spermatogenesis.

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Disclosure of conflict of interest

None.

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References

[1] La VS, Condorelli R, Vicari E, D'Agata R and Calogero AE. Diabetes mellitus and sperm parameters. J Androl 2012; 33: 145-153.

[2] Bhattacharya SM, Ghosh M and Nandi N. Diabetes mellitus and abnormalities in semen analysis. J Obstet Gynaecol Res 2014; 40: 167-171.

[3] Zhao Y, Tan Y, Dai J, Li B, Guo L, Cui J, Wang G, Shi X, Zhang X, Mellen N, Li W and Cai L. Exacerbation of diabetes-induced testicular apoptosis by zinc deficiency is most likely associated with oxidative stress, p38 MAPK activation, and p53 activation in mice. Toxicol Lett 2011; 200: 100-106.

[4] Sisman AR, Kiray M, Camsari UM, Evren M, Ates M, Baykara B, Aksu I, Guvendi G and Uysal N. Potential novel biomarkers for diabetic testicular damage in streptozotocin-induced diabetic rats: Nerve growth factor beta and vascular endothelial growth factor. Dis Markers 2014; 2014: 129-138.

[5] Jiang X, Bai Y, Zhang Z, Xin Y and Cai L. Protection by sulforaphane from type 1 diabetes-induced testicular apoptosis is associated with the up-regulation of Nrf2 expression and function. Toxicol Appl Pharmacol 2014; 279: 198-210.

[6] Alves MG, Martins AD, Rato L, Moreira PI, Socorro, S and Oliveira PF. Molecular mechanisms beyond glucose transport in diabetes-

related male infertility. Biochim Biophys Acta 2013; 1832: 626-635.
[7] Xu Y, Lei H, Guan R, Gao Z, Li H, Wang L, Song W, Gao B and Xin Z. Studies on the mechanism of testicular dysfunction in the early stage of a streptozotocin induced diabetic rat model. Biochem Biophys Res Commun 2014; 450: 87-92.
[8] El BK and Azar ST. Low testosterone and diabetes. Curr Diabetes Rev 2013; 9: 418-421.
[9] Reis MM, Moreira AC, Sousa M, Mathur PP, Oliveira PF and Alves MG. Sertoli cell as a model in male reproductive toxicology: Advantages and disadvantages. J Appl Toxicol 2015; 35: 870-883.
[10] Liu X, Nie S, Huang D and Xie M. Effects of nonylphenol exposure on expression of cell receptors and secretory function in mouse Sertoli TM4 cells. Environ Toxicol Pharmacol 2014; 37: 608-616.
[11] Feng Y, Fang X, Shi Z, Xu M and Dai J. Effects of PFNA exposure on expression of junction-associated molecules and secretory function in rat Sertoli cells. Reprod Toxicol 2010; 30: 429-437.
[12] Kianifard D, Sadrkhanlou RA and Hasanzadeh S. The ultrastructural changes of the sertoli and leydig cells following streptozotocin-induced diabetes. Iran J Basic Med Sci 2012; 15: 623-635.
[13] Johnsen SG. Testicular biopsy score count-a method for registration of spermatogenesis in human testes: normal values and results of 335 hypogonadal males. Hormones 1970; 1: 2-25.
[14] Xiong X, Wang A, Liu G, Liu H, Wang C, Xia T, Chen X and Yang K. Effects of p,p'-dichlorodiphenyldichloroethylene on the expressions of transferrin and androgen-binding protein in rat Sertoli cells. Environ Res 2006; 101: 334-339.
[15] Bardin CW, Musto N, Gunsalus G, Kotite N, Cheng SL and Larrea F. Extracellular androgen binding proteins. Annu Rev Physiol 1981; 43: 189-198.
[16] Joseph DR. Structure, function, and regulation of androgen-binding protein/sex hormone-binding globulin. Vitam Horm 1994; 49: 197-280.
[17] Pogach L, Giglio W, Nathan E and Huang HF. Maintenance of spermiogenesis by exogenous testosterone in rats treated with a GnRH antagonist: relationship with androgen-binding protein status. J Reprod Fertil 1993; 98: 415-422.
[18] Tindall DJ and Means AR. Concerning the hormonal regulation of androgen binding protein in rat testis. Endocrinology 1976; 99: 809-818.
[19] Danzo BJ, Pavlou SN and Anthony HL. Hormonal regulation of androgen binding protein in the rat. Endocrinology 1990; 127: 2829-2838.
[20] Jeyaraj DA, Grossman G and Petrusz P. Dynamics of testicular germ cell apoptosis in normal mice and transgenic mice overexpressing rat androgen-binding protein. Reprod Biol Endocrinol 2003; 1: 1-11.
[21] Premalatha R, Jubendradass R, Rani SJ, Srikumar K and Mathur PP. A phytooxysterol, 28-homobrassinolide modulates rat testicular steroidogenesis in normal and diabetic rats. Reprod Sci 2013; 20: 589-596.
[22] Kuhn-Velten N, Schermer R and Staib W. Effect of streptozotocin-induced hyperglycaemia on androgen-binding protein in rat testis and epididymis. Diabetologia 1984; 26: 300-303.
[23] Hayes FJ, Pitteloud N, DeCruz S, Crowley WJ and Boepple PA. Importance of inhibin B in the regulation of FSH secretion in the human male. J Clin Endocrinol Metab 2001; 86: 5541-5546.
[24] Petersen PM, Andersson AM, Rth M, Daugaard G and Skakkebaek NE. Undetectable inhibin B serum levels in men after testicular irradiation. J Clin Endocrinol Metab 1999; 84: 213-215.
[25] Wallace EM, Groome NP, Riley SC, Parker AC and Wu FC. Effects of chemotherapy-induced testicular damage on inhibin, gonadotropin, and testosterone secretion: a prospective longitudinal study. J Clin Endocrinol Metab 1997; 82: 3111-3115.
[26] Pfaff T, Rhodes J, Bergmann M and Weinbauer GF. Inhibin B as a marker of sertoli cell damage and spermatogenic disturbance in the rat. Birth Defects Res B Dev Reprod Toxicol 2013; 98: 91-103.
[27] Kumanov P, Nandipati K, Tomova A and Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. Fertil Steril 2006; 86: 332-338.
[28] Sylvester SR and Griswold MD. The testicular iron shuttle: a "nurse" function of the Sertoli cells. J Androl 1994; 15: 381-385.
[29] Bernstein S. Hereditary hypotransferrinemia with hemosiderosis, a mutine disorder resembling human atransferrinemia. J Lab Clin Med 1987; 110: 690-695.
[30] Vallés AS, Aveldaño MI and Furland NE. Altered Lipid Homeostasis in Sertoli Cells Stressed by Mild Hyperthermia. PLoS One 2014; 9: e91127.
[31] Aktas C, Kanter M, Erboga M and Timurkan H. Effects of experimental diabetes on testis proliferations and apoptosis in rats. J Exp Clin Med 2011; 28: 94-98.