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# BENEFICIAL EFFECTS OF COENZYME Q10 IN REDUCTION OF TESTICULAR TISSUE ALTERATION FOLLOWING INDUCTION OF DIABETES IN ADULT RATS

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## Abstract

***Background and Aims:*** *Various types of infertility are associated with uncontrolled hyperglycemia and diabetes. Development of oxidative stress is one the most important factors in the alteration of spermatogenesis in diabetic conditions. Consequently, the reduction of oxidative stress with antioxidant compounds can be effective in the reduction of tissue alterations. The aim of this study was to evaluate the efficacy of coenzyme Q10 in improvement of spermatogenesis in adult diabetic rats.* ***Material and Methods:*** *32 adult rats were divided into four groups of control and treatment. Coenzyme Q10 (10 mg/kg body weight - b.w.) was administrated to one control and one diabetic (intraperitoneal injection of 45 mg/kg b.w. of Streptozotocin) groups. Blood concentrations of FSH, LH and Testosterone were measured. Histology of testicular tissue and sperm analysis were considered for evaluation of spermatogenesis.* ***Results:*** *Administration of Coenzyme Q10 led to increase of pituitary gonadotropins levels in diabetic rats. Testosterone levels were not changed significantly. Testicular morphology, spermatogenic indices and sperm analysis were improved in treated diabetic rats.* ***Conclusions:*** *The results of this study suggest that the use of Coenzyme Q10 has positive effects in reduction of spermatogenic alterations following induction of experimental diabetes in rats.*

**key words**: *Coenzyme Q10, Diabetes, Rats, Testicular tissue*

## Background and Aims

The acute and chronic complications of diabetes are the major problems that occur in diabetic patients [1]. Increase of blood glucose levels leads to structural and functional changes in various target tissues and organs [2]. Experimentally induced diabetes in male rats is associated with alteration in functions of reproductive system [3]. It was previously reported that diabetes is one of the most important risk factors for male infertility. In this regard, cellular alterations in testicular microenvironment are associated to diabetes mellitus [4]. Diabetes is a condition of increased oxidative stress and impaired energy metabolism. Recent experimental and clinical studies suggest that oxidative stress and

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production of reactive oxygen species (ROS) play a key role in the pathogenesis of both types of diabetes mellitus and subsequently development of diabetes complications [5,6]. The spermatogenic activities of testicular seminiferous tubules are carried out by Sertoli cell mediated glucose uptake. The metabolism of glucose and production of lactate (the preferred energy source of germ cells) occur in the cytoplasm of Sertoli cells. In this regard, the microvascular damages of testicular tissue following diabetic hyperglycemia can alter the transport of glucose and subsequently lead to structural and functional changes of spermatogenesis due to derangement of cellular nutrition [7,8]. Decrease of oxidative stress and ROS production is one of the most important factors in reducing tissue damages related to diabetes mellitus. Coenzyme Q 10 ($CoQ_{10}$) is an endogenous, lipid soluble, benzo-quinone compound that functions as a diffusible electron carrier in the mitochondrial respiratory chain [9]. $CoQ_{10}$ acts as a powerful antioxidant which scavenges free radicals, prevents the initiation and propagation of lipid peroxidation in cellular biomembranes and helps regeneration of tocopherol [10,11]. On the basis of antioxidant activities of Coenzyme Q10, the aim of the present study was to investigate the possible protective role of Coenzyme Q10 on the structural and functional alterations of spermatogenesis after induction of diabetes in rats.

## Material and Methods

### *Treatments and chemicals*

In this study, streptozotocin, STZ, (Sigma, ST. Louis, MO, USA) was used for induction of diabetes in rats. The STZ was dissolved in 0.1 M citrate sodium buffer (*p*H 4.5) and was injected intraperitoneally in overnight fasting animals. Diabetes was confirmed 48 hours after injection of STZ. For this aim, the blood glucose levels of fasting animals was collected from the tail vein and determined with an automated glucose analyzer device (Glucometer, On Call EZ, SD, USA). The animals with blood glucose levels above 200 mg/dl were considered diabetic and were used in this study [12]. Coenzyme Q10 (Holland & Barrett CoEnzyme Q-10) was presented as 30 mg tablets. Daily oral dose for each animal was 10 mg/kg-body weight [13].

### *Animal procedures*

A total of 32 adult male Wistar rats with a body weight 180±20 g were used in this study. The baby rats were placed in standard cages (four animals per each) under light for 12-hour: dark cycle with room temperature of 23 -25°C until they reached the desired body weight for the study to begin. All animals received standard laboratory animal's chow and water *ad labitum* during the whole period of experiment. All animal procedures used in this study, were approved by the University of Tabriz standards for human care and use of laboratory animals, in accordance with the Research Ethical Committee of the Ministry of Health and Medical Education of Iran (adopted in April 17, 2006) based on the Helsinki Protocol (Helsinki, Finland, 1975).

### *Experimental design*

The animals were divided into four groups, each holding eight rats: ***Control:*** normal and apparently healthy rats that did not receive any type of treatment; ***Control+$CoQ_{10}$:*** the animals of this group were treated with $CoQ_{10}$ (10 mg/kg body weight - b.w.) orally for a period of four weeks and then euthanized at the end of study; ***Diabetic:*** in the animals of this group experimental diabetes was induced by a single intraperitoneal injection of STZ (45 mg/kg b.w.). The animals were euthanized four weeks after induction of diabetes; ***Diabetic+ $CoQ_{10}$:*** this group consists of diabetic animals were treated with $CoQ_{10}$ (10 mg/kg b.w.) orally for a period of four weeks.

### *Analytic procedures in plasma samples*

At the end of study, the blood glucose levels were determined by spectrophotometery according to the glucose oxidase method (Unico 1200, Japan), blood plasma testosterone levels were measured by an enzyme-linked immunosorbent assay (ELISA) method using a commercial kit (Diaplus Inc. USA), blood plasma FSH and LH levels were determined by ELISA assays using specific commercial kits (DRG Instruments GmbH, Germany).

### *Tissue preparation and histological techniques*

The testicular tissues were immediately fixed in 10% formaldehyde in buffered solution containing 54 mM $NaH_2PO_4$ and 28 mM $Na_2HPO_4$ (*p*H 7.4) and kept at 4°C. After 48 hours, the transverse section was made on the middle part of each testis and kept immersed in the fixative solution for the completion of tissue fixation. Then, formaldehyde-fixed samples were embedded in paraffin and sliced with thickness of 6-7 micrometer and were mounted onto albumin-pre-coated glass slides. The mounted tissue samples were deparaffined with xylol and stained by the Hematoxylin and Eosin method for histological observations by light microscopy.

### *Morphometeric analysis*

For morphometeric assessment of seminiferous tubules, the slides were studied at 200× magnification. To get extra precise results, only the seminiferous tubules that sectioned transversely were studied and the shortest diameter of seminiferous tubules was considered for measurement. The investigated parameters for morphometeric analysis were the height of germinal epithelium (GEH), seminiferous tubules diameter (STD) and the diameter of lumen of seminiferous tubules (STLD). The analyses were performed from images obtained and digitalized using an Olympus DP70 digital camera (Olympus Europe, Hamburg, Germany). Then, the images were processed by the computerized image analysis system software cell* (Olympus Soft Imaging Solutions GmbH, Münster, Germany). The scale bar was 200 μm and twenty tubules from each specimen were measured in different fields of tissue.

### *Evaluation of spermatogenesis in testicular tissue*

For estimation of spermatogenesis in testicular tissue, three different indices were used. Tubular differentiation index (TDI), repopulation index (RI) and spermiogenesis index (SPI). To determine the tubular differentiation index, the number of seminiferous tubules with more than three layers of germinal cells derived from type A of spermatogonia was calculated. To find out the repopulation and spermiogenesis indices, the ratio of active spermatogonia to inactive cells and, respectively the ratio of the number of seminiferous tubules with spermatozoids to the empty tubules, were calculated [14].

### *Sperm analyses*

For analyses of sperm, the cauda epididymis was separated from testis and cut into small pieces in one milliliter of Ham's F10 culture medium. The epididymal sperm count was evaluated by hemocytometer with light microscope at 400× magnification. Sperm motility was assessed with phase contrast microscope at 400× magnification. In average 10 microscopic fields were observed and the mean of counted sperms was considered as sperm motility for each rat. To estimate the percentage viability, a volume of 20 μl of sperm suspension was mixed with an equal volume of 0.05 percent eosin-Y. The prepared slides were viewed by bright-field microscope at 400× magnification. Two hundred sperms were considered for calculating the indices for the experimental groups [15].

### *Statistical analyses*

The obtained results were analyzed using the GraphPad PRISM® software version 5.04 (GraphPad Software, Inc. USA). All data were reported as mean ± SEM. The comparison of means between experimental groups was evaluated by using the one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Differences were considered to be statistically significant if $P < 0.05$.

## Results

### *Blood plasma samples analysis*

As expected, the mean blood glucose levels in the two diabetic groups were significantly higher than in the control groups (Table 1). The blood concentration of testosterone was non-significantly reduced in the diabetic groups in comparison to controls. Moreover, the diabetic animals treated with $CoQ_{10}$ had non-significantly higher serum testosterone levels in comparison to the untreated diabetic group. In the diabetic groups, the mean blood level of FSH and LH was lower than in the control groups. The reduction of FSH was significant between control groups and diabetic groups. The reduction of LH was only significant between untreated diabetic group and control groups. As shown in Table 1, the administration of $CoQ_{10}$, led to a non-significant elevation of FSH and LH levels in diabetic rats.

**Table 1.** Hormonal analysis in experimental groups (mean ± SEM, n=8).

| | **Control** | **Control+$CoQ_{10}$** | **Diabetic** | **Diabetic+$CoQ_{10}$** |
|---|---|---|---|---|
| **FSH (mIU/ml)** | 0.28±0.06$^{*£}$ | 0.28±0.09$^{*£}$ | 0.19±0.10 | 0.21±0.07 |
| **LH (mIU/ml)** | 0.25±0.04$^{*}$ | 0.26±0.06$^{*}$ | 0.18±0.06 | 0.22±0.08 |
| **Testosterone (ng/ml)** | 5.37±1.05 | 5.41±2.34 | 5.16±4.23 | 5.22±3.37 |
| **Glucose (mg/dl)** | 149.61±7.87$^{*£}$ | 152.72±7.69$^{*£}$ | 258.06±8.91 | 245.27±9.34 |

Significantly different compared to $^{*}$ Diabetic group, $^{£}$ Dibetic+$CoQ_{10}$ group ($P < 0.05$ for all groups); FSH - follicle-stimulating hormone; LH - luteinizing hormone

**Table 2.** Morphometeric values of testicular seminiferous tubules (mean ± SEM, n=8).

| | **Control** | **Control+$CoQ_{10}$** | **Diabetic** | **Diabetic+$CoQ_{10}$** |
|---|---|---|---|---|
| **STD (µm)** | 458.63±6.57$^{*£}$ | 449.97±5.25$^{*£}$ | 379.43±6.31 | 402.12±6.92$^{*}$ |
| **GEH (µm)** | 162.28±4.39$^{*£}$ | 164.29±5.78$^{*£}$ | 118.37±4.61 | 134.71±3.76$^{*}$ |
| **STLD (µm)** | 181.61±6.44$^{*£}$ | 186.16±7.21$^{*£}$ | 205.09±5.43 | 201.29±4.88 |

Significantly different compared to $^{*}$ Diabetic group, $^{£}$ Dibetic+$CoQ_{10}$ group ($P < 0.05$ for both); STD - seminiferous tubule diameter; GEH - germinal epithelium height; STLD - seminiferous tubule lumen diameter

### *Morphometric analysis of testicular tissue*

Table 2 summarizes the results of testicular morphometry. The diameter of seminiferous tubules (STD) was reduced significantly in the two diabetic groups. This decrement in tubular diameter was seen in more intensity in the untreated diabetic group. The alterations of germinal epithelium height (GEH) were similar to the results of STD. As shown in Table 2, there was a significant difference in ST lumen diameter (STLD) between control and diabetic groups.

### *Indices of spermatogenesis*

All indices of spermatogenesis were reduced in the diabetic groups in comparison to control groups (Table 3). In this regard, all indices were increased in treated diabetic group but, this improvement was not significant between the two diabetic groups. As shown in Table 3, significant decrement of spermatogenic indices was seen between control groups and diabetic

groups for repopulation index and between control groups and untreated diabetic groups for tubular differentiation index. No significant differences were recorded for the spermiogenesis index (SPI) between the four groups.

**Table 3.** Spermatogenesis indices of testicular tissue (mean ± SEM, n=8).

| | **Control** | **Control+$CoQ_{10}$** | **Diabetic** | **Diabetic+$CoQ_{10}$** |
|---|---|---|---|---|
| **TDI (%)** | 90.57±3.34$^{*}$ | 91.88±4.36$^{*}$ | 73.44±5.66 | 82.18±6.42 |
| **SPI (%)** | 89.97±4.21 | 89.52±6.32 | 81.66±7.19 | 83.31±8.16 |
| **RI (%)** | 83.28±3.12$^{*£}$ | 84.19±5.88$^{*£}$ | 65.97±6.51 | 69.13±5.47 |

Significantly different compared to $^{*}$ Diabetic group, $^{£}$ Dibetic+$CoQ_{10}$ group ($P < 0.05$ for both); TDI - tubular differentiation index; SPI - spermiogenesis index; RI - repopulation index.

**Table 4.** Epididymal sperm analysis (mean ± SEM, n=8).

| | Control | Control+$CoQ_{10}$ | Diabetic | Diabetic+$CoQ_{10}$ |
|---|---|---|---|---|
| Sperm count ($10^{6}$/ml) | 76.34±4.94 | 78.19±8.31 | 68.67±5.12 | 71.09±6.39 |
| Sperm Motility (%) | 87.36±6.21 | 86.42±7.39 | 77.26±7.92 | 79.19±6.97 |
| Sperm Viability (%) | 88.22±7.11 | 87.98±9.38 | 79.46±8.82 | 81.89±7.93 |

No significant differences were seen between groups.

### *Sperm analysis*

As shown in Table 4, all indices of epididymal sperm analysis were reduced in the diabetic groups in comparison to control groups. Moreover, the results from sperm analysis showed that, administration of $CoQ_{10}$ led to improvement of sperm analysis indices in treated diabetic group in comparison to untreated diabetic animals. However, all differences in sperm analysis between study groups were statistically non-significant.

## Discussions

Following induction of diabetes, the microscopic changes of testicular tissue and the alteration in normal spermatogenesis have been observed to increase [4,5]. In this regard, the excessive production of reactive oxygen species and microvascular damage are reported to substantially contribute to the occurrence of structural and functional changes [16-18]. Our previous studies showed that induction of diabetes leads to both steroidogenic and spermatogenic dysfunctions and impairment of testicular function [4,19]. It seems that, prevention of oxidative damage with natural antioxidants can be considered as one of the most effective strategies in control and reduction of diabetic complications in laboratory animals. The results obtained in this study, are valid for experimental diabetes in rats and cannot be extrapolated automatically to human diabetes. CoQ10 acts as a powerful antioxidant which scavenges free radicals and prevents the initiation and propagation of lipid peroxidation in cellular biomembranes [10,11].

One of the most important functions of insulin is the modulation of blood FSH levels and strong correlation have been found between FSH and insulin levels in blood plasma [20]. The comparison of FSH levels between different groups in our study revealed that, treatment of diabetes with $CoQ_{10}$ leads to an increase of blood FSH levels to some extent. Like FSH, blood LH levels were lower in the diabetic groups. Insulin has a key role in the metabolism of Leydig cells (as main target cells of LH) and maintenance of LH receptors on these cells [3]. Reduction of insulin secretion resulted to LH decrement and subsequently malfunctions of Leydig cells. Our results showed that use of $CoQ_{10}$ has a positive effect, leading to an elevation of blood LH levels. This effect is thought to be achieved through the improvement of insulin function in diabetic rats.

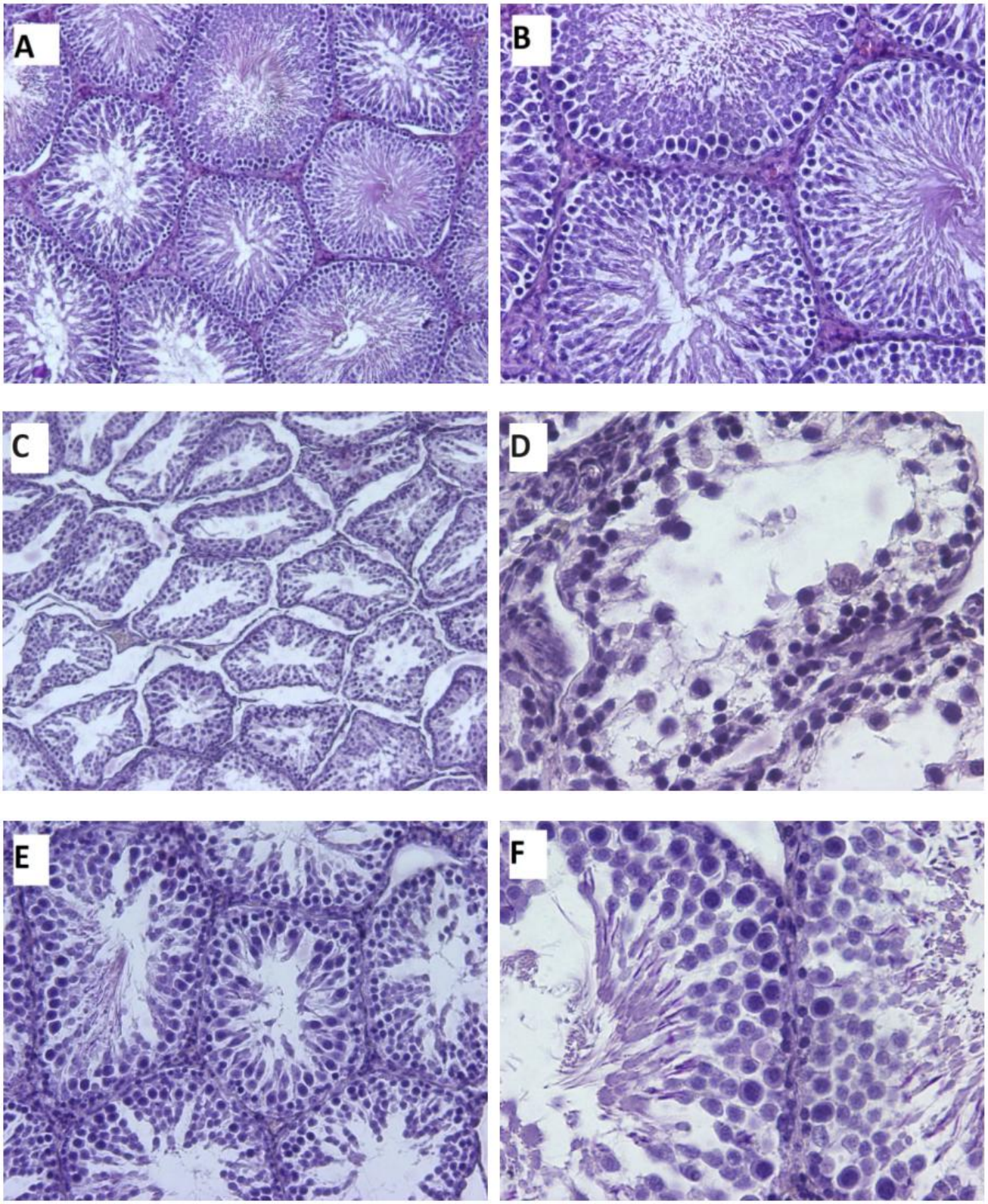


**Figure 1.** *Cross section of testicular tubules in various groups.* A, B: control; C, D: untreated diabetic; E, F: treated diabetic. Seminiferous tubules in control groups have normal structure and germinal epithelium have normal cellular arrangement. Atrophy and malformation of testicular tubules with disarrangement of spermatogenic cells and increase of interstitial connective tissue was seen in sections from diabetic groups. Disarrangement of cells was reduced, spermatogenic cells population and arrangement was improved in treated diabetic groups. Hematoxylin and Eosin staining. Magnification: A, C 100×; B, E 200×; D, F 400×.

The results of blood testosterone measurement in our study showed that, the administration of $CoQ_{10}$ leads to slight elevation of blood testosterone levels in treated diabetic rats. Increase of blood testosterone levels in treated diabetic rats showed that the function of Leydig cells may have a direct relationship with blood glucose levels.

In this study and in our earlier reports [4,19], the reduction in diameter of seminiferous tubules (STs) and depletion in germinal cell population was seen following induction of diabetes (Figure 1).

Decrement in diameter of STs was accompanied with depletion in the height of germinal epithelium which causes the atrophy of seminiferous tubules. These histological observations in STs illustrate the depressed cellular activity of spermatogenic cells in diabetic conditions. Oxidative stress in testicular tissue has a direct relationship with abnormal spermatogenesis due to decrement of glutathione in male germ cells which leads to incomplete functional maturation and capacitation of spermatozoa [21,22].

Our study showed that the administration of $CoQ_{10}$, leads to improvement of histomorphometry of testicular tissue (Figure 1). The evaluation of microscopic indices of spermatogenesis confirms these histologic results. Diminished tubular differentiation (TDI) and spermiogenesis (SPI) indices in diabetic rats indicates that, conversion of spermatogonia to primary spermatocytes is reduced. Reduction of repopulation index in diabetic rats demonstrates the number of inactive spermatogonia increased after induction of diabetes. This process causes a decline of the number of primary spermatocytes derived from spermatogonia cells. These alterations in cellular conversion and/or activity lead to reduction in production of spermatozoids. The results from different indices of sperm analysis in this study indicate that the number, ability of movement and the mortality rate of spermatozoids have a relationship with cellular activity of testicular germinal epithelium. The structure of spermatozoids has large quantities of lipids. Oxidative stress can influence the normal structure of developing spermatozoids due to induction of excessive lipid phosphorylation [22]. An excess of ROS weakens sperm cell function and plays a negative role in male fertility. $CoQ_{10}$ may play a positive role in the treatment of asthenozoospermia because of its antioxidant properties. It was shown that $CoQ_{10}$ levels increased in seminal plasma and in sperm cells after treatment [23]. Due to its energy supporting properties, $CoQ_{10}$ is also responsible for sperm production and all energy-dependent processes in the sperm cell. In a research on 17 patients with low fertilization rates were given 60 mg of $CoQ_{10}$ for 103 days, it was shown that sperm's motility rate nearly doubled [24]. Because of the fact that benefits of $CoQ_{10}$ to sperm production and that a related molecule, $CoQ_7$, may increase the production of healthy sperm, CoQ10 was studied in infertile men and it was considered as one part of a pregnancy plan [24,25].

## Conclusions

As the results of this study indicate, administration of $CoQ_{10}$ leads to improvement of cellular activity indices of testicular tissue and sperm analysis. In conclusion, the results of this study suggest that, use of natural antioxidants, can be used as one important strategy for control and reduction of diabetic complications in reproductive system in rats.

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***Conflicts of Interest.*** The authors declare that they have no conflict of interest.

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