Commun.Fac.Sci.Univ.Ank.Series C
Volume 28, Number 1, Pages 101-113 (2019)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/tr/pub/communc/issue/45050/587206

HISTOMORPHOLOGICAL CHANGES ON THE TESTICULAR TISSUE IN DIABETIC RATS INDUCED WITH STREPTOZOTOCIN

NIHAL SAÇMAOZU, HATICE MUTLU-EYISON, SUNA CEBESOY

ABSTRACT. Diabetes Mellitus (DM) is a chronic metabolic disease characterized with various complications due to its damage on many organs. One of the most common complications of DM is testicular damage. Therefore, it is necessary to start a treatment agent promptly during this process which may lead to infertility. There are treatment steps that seriously prolong the process of complications of this dangerous disease. However, there are some contradictions in numerous studies conducted regarding which agent the treatment should continue with according to the stage of the diabetes in the patient. In this context, examining the functions of male reproductive system may prevent these contradictions in the future. In this study, 2 groups were used; one of them consisted of the healthy rats and the other consisted of the ones that became diabetes by induction of streptozotocin. The animals of both groups were fed under the same sheltering conditions and testicle tissues of both groups were evaluated under light microscope. As a result of histomorphological examination of testicular tissues, the pathologies such as impaired integrity of the seminiferous tubules especially in the testicles of diabetic rats, the separation of germinal epithelium cells from connective tissue and from each other and the presence of prominent necrosis focuses in spermatogenic cells were found to be noteworthy. In this study, it has been evaluated that cellular pathological changes are clearly revealed under the microscope. It is believed that the results of the study will contribute to the clinical studies regarding the treatment and may shed light on research at molecular level.

1. INTRODUCTION

DM is a serious health problem causing severe complications in cardiovascular system, kidney, eye, nervous system and male reproductive system. In numerous studies conducted on both men with diabetes and animal models, it has been reported that DM causes infertility in males through various mechanisms such as spermatogenesis disorder, degenerative and apoptotic changes in testicle, damage to glucose metabolism in the Sertoli/blood testicle barrier, decreased testosterone synthesis and secretion, ejaculation dysfunction and low libido [2].

Regulation of blood glucose in the body is provided as a result of the complex interaction of many chemicals and hormones. The most important hormone playing a role in the regulation of diabetes is insulin hormone secreted from beta cells of the pancreas [3]. Regarding insulin therapy in diabetic rats, there are various expressions

Received by the editors: April 20, 2019; Accepted: June 18, 2019.
Key word and phrases: Diabetes Mellitus, Testis tissue, Histopathological evaluation.

stating that it partially or completely improves LH and FSH levels [2]. DM is divided into 2 categories: Type 1 and Type 2. Type 1 DM is the type where Leydig cell function and testosterone synthesis significantly decrease due to the loss of regulatory effect of insulin [2, 4]. Here, FSH and LH levels also decrease and this results in a negative effect on sperm formation. Type 2 DM constitutes 90% of the diabetic population, which is the most common hyperglycemic condition in the world. Despite the fact that it is generally seen after the age of 30, it can develop at any age. It is not clearly understood compared to Pathogenesis Type 1 DM [5].

90% of the testicles consist of tubular structures called seminiferous tubules where sperm is formed. The cells found in the testicles called the Leydig cell produce testosterone. Hypothalamus in the brain inform pituitary about the amount that testosterone will be produced. For this purpose, gonadotropin releasing hormone (GnRH) is secreted from the hypothalamus and the hormone that reaches the pituitary allows the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary [6]. While LH, secreted from the pituitary gland, increase testosterone secretion from the Leydig cell; FSH, secreted by the pituitary gland, produces sperm in seminiferous tubules. Secreted testosterone reduces LH secretion from the pituitary gland. Under the influence of FSH hormone, hormones named inhibin and activin are secreted from the Sertoli cells in the testicles. While inhibin reduces FSH secretion from the pituitary, it increases activin hormone [2, 6, 7]. Activin is produced both in pituitary gland and testicles. Testicular tissue consists of the parts of seminiferous tubule, where sperm is produced, and interstitium, where hormone is produced. The Leydig cells in the interstitium produce the male hormone, called testosterone. While testosterone hormone affects sperm production in the testicle, it also shows its effect in distant tissues by being carried in the blood (for example: sexual desire in the brain, having deep voice, growing beard and mustache, male type development in muscles and bones etc.) [8]. Sperm is produced in seminiferous tubules. The seminiferous tubules are composed of germ cell layers and canaliculus in the center that is called lumen. Germ cells lay these canaliculi, while round immature sperm cells are located externally, mature spermatozoa are found in the lumen. Lumens combine to provide sperm flow to the epididymis. The effect of FSH hormone secreted by the pituitary gland in males is to provide sperm production in seminiferous tubules. The LH hormone controls the production of testosterone from the Leydig cells, having indirect effect on sperm production and its maturation. Germ cells show development of certain parts and complete maturation phases from spermatogonia to the phase of spermatozoa [9].

Diabetes mellitus is an important metabolic disease and seen in the majority of the society. It is a disease which has great importance and needs to be examined due to causing complications such as infections, urogenital system disorders, vascular

disorders, digestive system disorders, retinopathy, nephropathy, neuropathy and diabetic ketoacidosis [10].

DM is one of the important diseases of our time and is a serious health problem that should not be neglected due to the fact that it affects all the systems. In this study, the pathological changes caused by Type 2 DM in testicular tissue will be evaluated. As a result of the histological examination of the testicles of diabetic rats, there are studies available regarding loss of spermatogenic series cells in seminiferous tubules, atrophic tubules, capillaries in interstitial tissue and mural thickening in arterioles. Also, the development of multicore giant cells in tubule, vacuoles in Sertoli cells, inflammatory cell infiltration and atrophy in interstitial connective tissue has been observed [11-13].

The aim of the study is to examine the pathological changes that will occur as a result of the experimental diabetes generated in rats induced with STZ (streptozotocin). It is believed that the findings of the study will contribute to the clinical studies regarding treatment and shed light on researches conducted at the molecular level.

2. Materials and methods

The animal experiment part of the study was conducted within the scope of permission obtained from Gazi University Animal Experiments Local Ethics Committee. At this stage, 19 Wistar albino male rats weighing 200-220 g were used. All subjects were fed in standard cages, under similar environmental and nutritional conditions at room temperature 23 ± 2 ºC with 40-50% humidity, and standard pellet feed and water. The duration of the experiment was set at 21 days, and the rats were divided into two groups, as control and diabetic groups. Intragastric lavage with tap water was applied to the healthy control group (G1, n = 9), for 21 days. Streptozotocin solution (STZ, Serva 35503, Heidelberg, Germany), diabetogenic agent, was applied to the diabetic group (G2, n = 10), as 45 mg / kg single dose and intraperitoneally (i.p.) in 0.1 M sodium citrate in buffer solution (pH = 4.5). Blood was taken from the tail veins of the subjects 3 days after STZ injection and blood glucose levels were measured with manual glucometer (Glucotrend; Accu-Chek, UK). As a result of the blood glucose measurement, the rats with 240 mg / dl and above were evaluated as diabetic and included in the study. On day 22, both groups of rats were sacrificed under anesthesia (sodium thiopental;50 mg/kg). Testicular tissue samples were fixed with 10% formaldehyde. Fixed tissues were embedded in paraffin. In the research, microtome sections were taken from these blocks and Masson's trichrome, Silver staining, PAS (Periodic acid shiff) and H&E (Hematoxylin-eosin) staining techniques were used for the testicles. These stained sections were examined under a light microscope and photographed. The photos of

all preparations stained were taken by Leica DFC320 camera connected to Leica DM LS2 model microscope.

3. Results

The healthy control group (G1) rats were wrapped with tunica albuginea on the external connective tissue. Seminiferous tubules were seen in different diameters. There were interstitial connective tissues between seminiferous tubules (Figure 1).

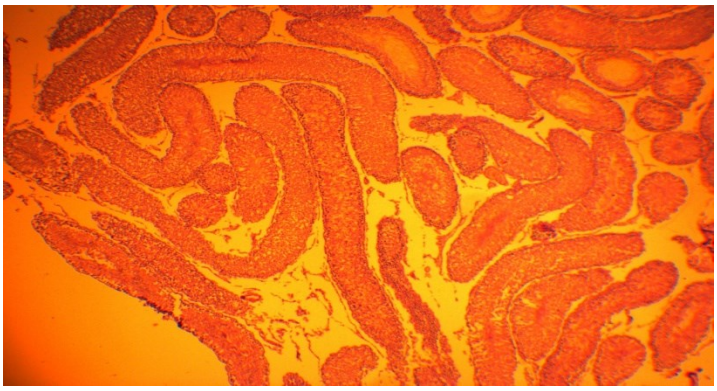

Figure 1. The regular seminiferous tubules and interstitial connective tissues in the healthy control group (G1) rats. PAS. X100.

On the basal membrane of seminiferous tubules, spermatogenic series cells with Sertoli cells were monitored. There were capillary vessels and Leydig cells with eosinophilic cytoplasm in interstitial tissue (Figure 2).

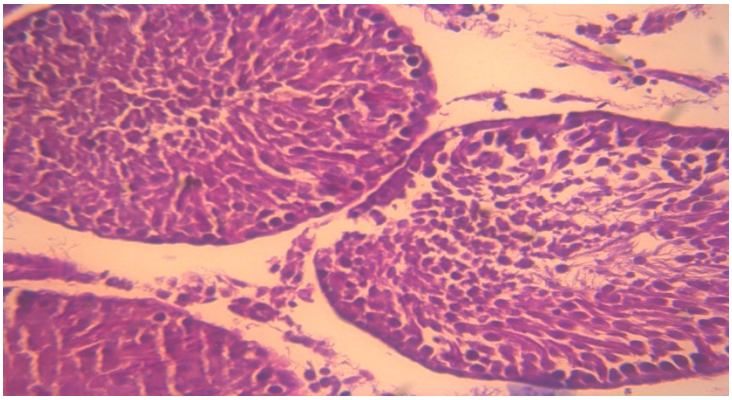

Figure 2. Spermatogenic series cells (spermatogonium, primary spermatocytes, spermatitis) with sertoli cells on seminiferous tubules and capillary vessels with leydig cells

in interstitial connective tissue between tubules in the healthy control group (G1) rats. PAS. X400.

In testicle sections of the Diabetic group (G2) irregularity in the germinal epithelium cell line and degeneration of some cells, edema in interstitial tissue and vein wall thickening attracted attention (Figure 3 and Figure 4).

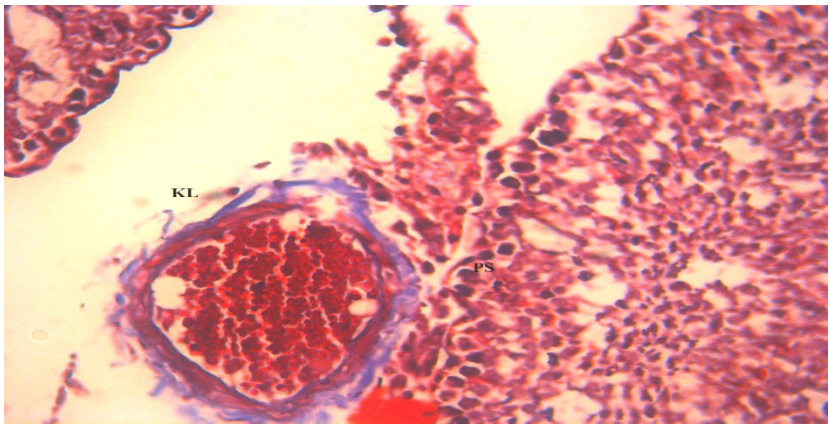

FIGURE 3. Thickening of the vessel wall and fragmented collagen fibers (KL) around of the vessel and some cells degeneration in the diabetic group (G2) rats. Masson's trichrome. X400.

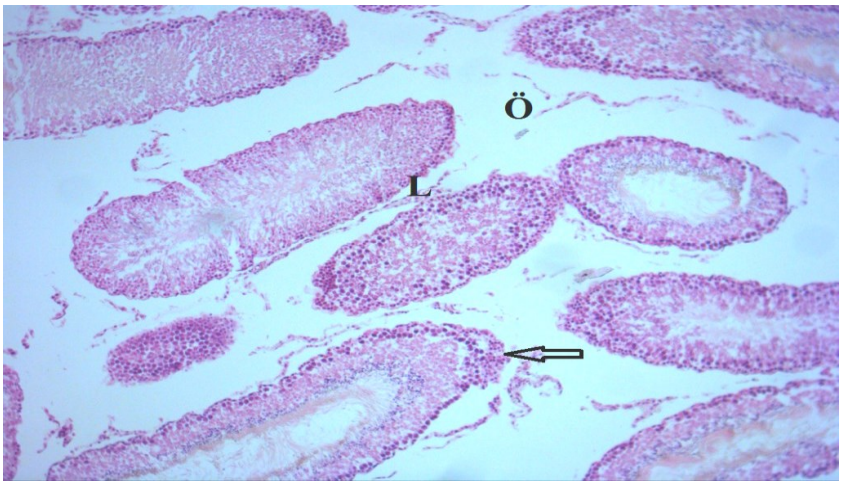

FIGURE 4. Edema in interstitial tissue (Ö), Leydig cell (L) and proliferating primary spermatogonium around the seminiferous tubule (→) in the diabetic group (G2) rats. H &E. X100.

Furthermore, it was observed that the connective tissue was interrupted, the integrity of the seminiferous tubule was disrupted (Figure 5) and germinal epithelial cells were separated from the connective tissue and from each other in the testicles of the rats in the G2 group in which experimental diabetes was generated by inducing STZ.

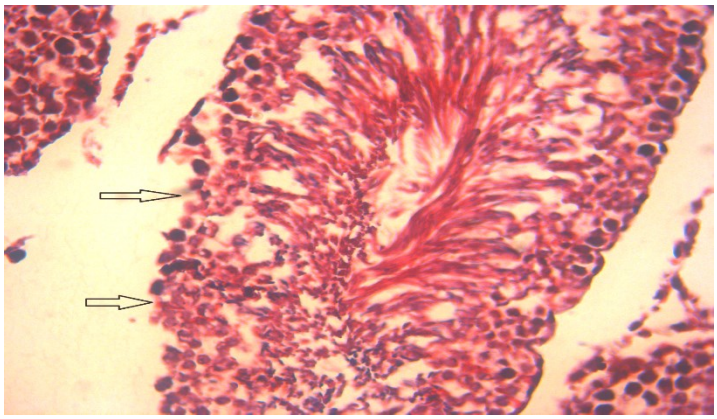

FIGURE 5. Degenerative basal membrane of the seminifer tubule (→) and edema in interstitial tissue in the diabetic group (G2) rats. Masson's trichrome. X400.

However, invagination in tubules was quite remarkable (Figure 6) Gaps, which were seen among the cells starting from primary spermatocytes, were among important findings (Figure 7).

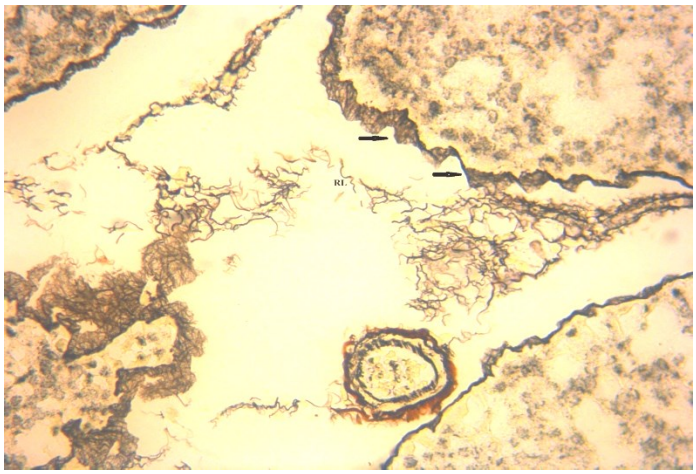

FIGURE 6. View of the invagination in tubules (→) in testicle with silver staining techniques and fragmented connective tissue fibers in the diabetic group (G2) rats. X400.

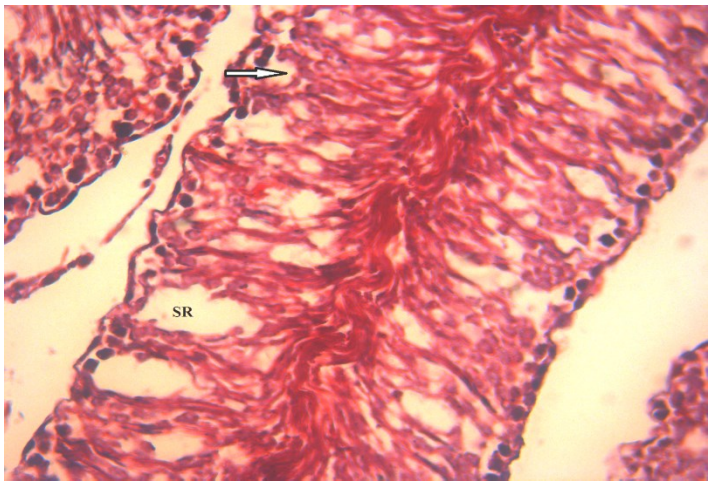

FIGURE 7. Gaps between cells starting from primary spermatocytes (→) and degenerated sertoli cell (SR), Masson's trichrome. X400.

In H&E stained diabetic rat testicles, germinal epithelium separated from basal lamina was observed. (Figure 8). Furthermore, gap, which were among the cells starting from necrosis and primary spermatocytes, were found in diabetic rat testicles that were stained with H & E in spermatogenic cells which lay seminiferous tubule's lumen (Figure 9).

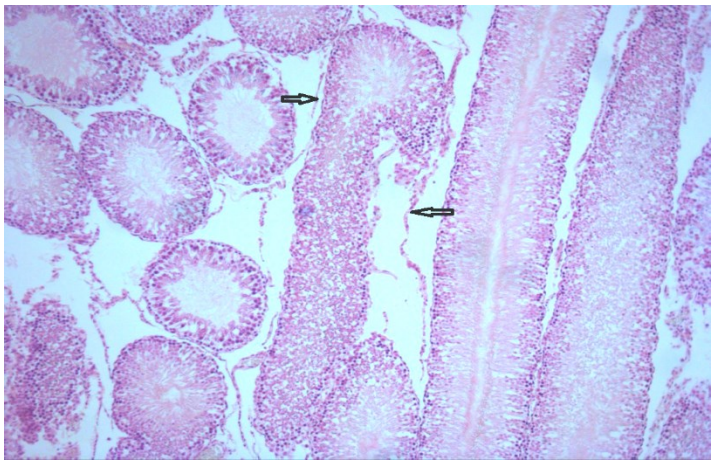

FIGURE 8. The view of the germinal epithelium (→) separated from basal lamina in seminiferous tubules in testicle with H&E staining techniques in the diabetic group (G2) rats. X100.

4. Discussion

Diabetes is a chronic disease where glucose levels in the blood are at very high levels. It has been monitored in the numerous studies performed that DM has negative effects on male reproductive system on a large-scale. In this context, Bondarenko et al. found that the testicles of the rats in which they created diabetes using STZ were relatively lighter and degenerative changes in the testicles such as the separation of the epithelium layer from the tubular basal membrane and the presence of thrombosis in the veins were seen [14]. In their histomorphological studies performed with STZ in diabetic rat testicles, Kianifard et al. having a more detailed study on the same subject, determined shrinkage in the germinal epithelium width and seminiferous tubule diameter, edema in interstitial tissue, disruption of spermatogenesis along with loss of germ cells [15].

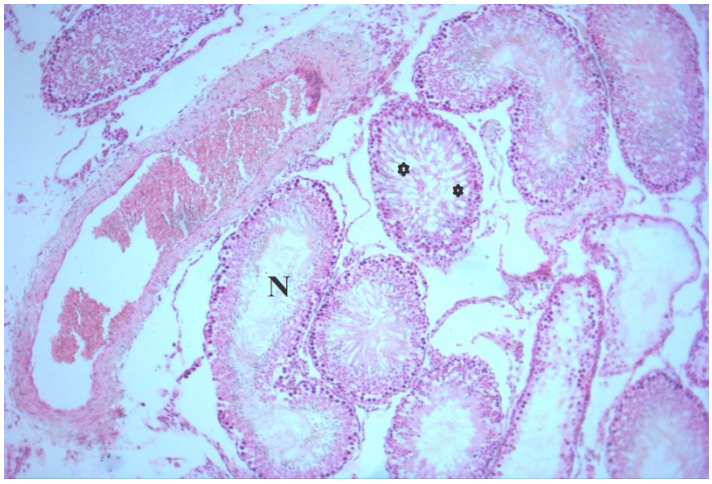

Figure 9. The view of the necrosis (N) of spermatogenic cells laying the lumen of the seminiferous tubule and gaps (*) between cells starting from primary spermatocytes in testicle with H&E staining techniques in the diabetic group (G2) rats. X100.

Similarly, degeneration in cells and edema in interstitial tissue were seen in histological cross-sections performed with light microscobe in the study. In one of his studies, Koh reported a significant decrease in the weight of the epididymis with increased apoptotic cell death and degenerative germ cells [16]. Furthermore, the tubular atrophy seen in histological cross-sections were the first remarkable finding in diabetic rats in the study. In a large number of experimental diabetes studies with STZ, including the research of Mohasseb et al., Wright et al., Anderson and Thlivers,

Altay et al. and Cai et al., tubular atrophy was observed in the testicles of the subjects [17-21]. Feeding of sertoli cells and spermatogenic series cells settled into seminiferous tubules are carried out through diffusion from the interstitial connective tissue in the blood vessels. These blood vessels play a significant role not only for feeding of leydig cells, but also for feeding of the tubules. It has been reported that thickening of interstitial blood vessels may lead to tubular atrophy by reducing tubular feeding [22]. Another result is the thickening seen in tubular basal membrane. Similarly, in the study of Cameron et al. on individuals with diabetes, thickening of the basal membrane of seminiferous tubules due to diabetes has been identified [22]. They stated that the tubule feeding, which was already impaired by thickening of blood vessel walls, would further deteriorate due to the thickening of the basal membrane of the tubule and it would negatively affect spermatogenesis. Similarly, Öztürk et al. observed tubular atrophy as a result of histological examination of the testicles of the same type of diabetic rats [23].

DM causes damages to the reproductive system in males through direct effect of insulin on sperm cells and testicles or makes changes in the hypothalamic-pituitary-gonadal axis. In this context, various studies could be found in the literature. For instance, Murray et al. reported a significant decrease in plasma FSH and testosterone levels [11]. However, in a similar study, Perez Diaz et al. reported that plasma LH and prolactin levels decreased whereas FSH concentration did not show any changes [24]. Hutson et al. reported that serum levels of FSH, LH, PRL and GH decreased in epididymis, testicles and seminal vesicle weights and only FSM levels and testicle weight returned to normal with insulin therapy [7]. In his research, Steger observed that there were disturbances in initiating sexual intercourse, intromission and ejaculation with a significant decrease in plasma testosterone, LH, FSH, PRL and LHRH levels of diabetic rats. Although testosterone replacement had increased plasma testosterone to normal levels, the mating behavior of STZ-induced diabetic rats did not show any improvement [25].

Another result of the study is the decrease in the number of spermatogenetic series cells in seminiferous tubules. Different degrees of loss in spermatogenetic series cells in seminiferous tubules and Sertoli cells were reported in a study conducted by Öztürk et al. Decreased testosterone levels may explain testicular atrophy in diabetes [23]. Another finding is that multi-core giant cells were seen in the seminiferous tubules. Similarly, Kaya et al., Leon MD et al., Cernochova D et al., Torgersen HM et al., Sasagawa I et al. observed multinucleated giant cells in some seminiferous tubules [26-30]. At the same time, these cells were found in systemic, toxic, infectious, ischemic pathologies causing tubular atrophy and in cryptorchidism. In previous experiments, core chromatins of these giant cells were monitored as settled in the inner side of the core membrane to give a crescent-like appearance. This

appearance matches the chromatin structure monitored in apoptotic cells [31]. Another important finding is that there is no histological change except for the number decrease in Leyding cells. This finding has been interpreted by Joan B et al. as diabetes reduces fertility and sexual desire [32]. Decrease in number and loss of function were seen in Leydig cells in testicles. Although serum levels of testosterone, FSH and LH decreased remarkably, there was a significant relationship found between insulin and LH and FSH levels. It is believed that the reason for the decrease in Leydig cell function and testosterone production is the decreased stimulatory effect of insulin on these cells. Decrease in FSH due to decreased insulin adversely affects both sperm production and fertility [32]. Likewise, F. Öztürk et al. reported the changes not seen in Leydig cells as well. As a result of the histological examination of testicles of the same type diabetic rats, F. Öztürk et al. found a loss of spermatogenic series cells in seminiferous tubules, atrophic tubules, capillaries in interstitial tissue and membrane thickening in arterioles [23]. However, they also noted the development of multicore giant cells in tubule, vacuoles in sertoli cells, inflammatory cell infiltration in the interstitial connective tissue and atrophy. In recent years, studies have shown that spermatogenetic serial cell death in rat testicles is apoptosis due to spontaneous and gonadotropic hormone insufficiency and heat increase [6]. There was increase in testosterone levels found when diabetic rats which were induced with STZ were given insulin externally [33].

As is seen, although the same results were reported as the finding of the histomorphological examinations of testicular tissues in the rat models in which diabetes was induced with STZ, numerous studies have included hormonal measurements. As a result of these measurements, they examined whether both hormonal and cellular changes were improved with insulin replacement therapy. The major limit of this study was the inability to determine the hormone levels of diabetic rats. Nevertheless, it is believed that cellular pathological changes have been clearly displayed under a microscope.

5. Conclusion

Diabetes can cause pathological changes in the testis as in many tissues. The data from the other studies that DM alters histomorphological parameters of testicles like testicular atrophy and induces degenerative changes in the testicles and impairment of fertility in male diabetic patients as well as in experimental diabetic animals. Our data confirmed that Streptozotocin induced experimental diabetes lead to testicular damage in rats. Several mechanisms have been suggested to explain these effects.

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