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The effect of the metabolic syndrome on the histological structure of the testes tissue and the sperm morphology in the rats

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Abstract: In this study, we aimed to investigate the effect of metabolic syndrome on spermatozoon morphology and histological, histochemical, histomorphometric changes in the testes. Twenty-four male Sprague Dawley rats (60 day-olds) were used for this purpose. The rats in the control group were fed only water and rat food, while the rats in the experimental group were fed with water containing 20% D-fructose and rats food for 16 weeks with ad libitum. At the end of the experimental period, the testes and epididymis of the rats were removed. The testes were embedded in paraffin by performing the necessary histological procedure. In the rat testes with metabolic syndrome, subbasal vacuolization, epithelial vacuolization, desquamation of germinal cells in the epithelium, the presence of germ cells across to tubular lumen for increased. Seminiferous tubule diameter was increased but the epithelial height of the tubules was decreased in the experimental group significantly. It was noted that the basement membrane surrounding the tubules and the capillary wall was thickened in rats with metabolic syndrome. In addition to these findings, apoptotic cells were increased in the experimental group compared to the the control group. It was observed that the rate of abnormal and dead spermatozoa increased. In this study, we determined the negative effects on the testes of the incidence is increasing day by day with metabolic syndrome. Due to its connection with male infertility, it is thought that more comprehensive studies will contribute to the development of scientific data.

Key words: Apoptotic cells, histology, metabolic syndrome, rat, testes

1. Introduction

Metabolic syndrome (metS) is one of the major health problems, which is increasing nowadays and involves a complex series of inflammations that are based on genetic and environmental factors, together with insulin resistance, obesity, dyslipidemia, endothelial dysfunction, hypercoagulation, and hypertension [1]. The main causes of obesity and metabolic disorders that have been observed widely in recent years are low exercise and improper diet with high fat and high fructose content [2]. It was demonstrated that the rats were fed with a high fructose diet had problems with insulin functions in the liver and peripheral tissues due to glucose tolerance. Insulin resistance also causes hyperinsulinemia, hypertension, hyperglycemia, and hypertriglyceridemia [3]. Fructose consumption has negative effects on weight, lipid distribution, and glucose metabolism, and it has been stated that a diet rich in fructose causes obesity, metabolic syndrome, and diabetes mellitus [4]. Fructose is cheaper and sweeter than sucrose, does not create a feeling of satiety, accelerates the second feeling of hunger, the consumption amount creates an advantage in the markets; therefore, its use in foods has increased in recent years [5]. Fructose should not be thought of as a simple energy source that only plays a role in obesity. This monosaccharide is also a foodstuff that has direct metabolic effects such as insulin resistance and anointment as well as excess energy intake [6]. In addition, it causes various metabolic diseases such as nonalcoholic fatty liver, dyslipidemia, and diabetes mellitus [7]. With metabolic syndrome, obesity has many negative effects on health. One of these effects is the decrease in the number of spermatozoa. This decrease in the number of sperm was found in the body parts with high rates of obesity [8]. It has been observed that the semen parameters of obese individuals were dissimilar in terms of motility and volume in sperm count compared to normal individuals [9]. It was also observed that male individuals with higher BMI [body mass index] have a higher risk of infertility [10]. Diabetes has been reported to cause testicular dysfunction, low testosterone levels, and insufficient spermatogenesis [11,12].

In this study, it was aimed to investigate the effects of metabolic sydrome on spermatozoon morphology and histological, histochemical, and histomorphometric changes in the testicular tissues of rats.

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2. Materials and method

In this study, 60 days old 24 male Sprague Dawley rats were used. The rats fed ad libitum with standard rat food and water were kept in a medium of 12 h light/12 h dark and at 23-25°C. Rats were divided into two groups a the control group (n = 10) and the experimental group (n = 14). The whole procedure was carried out by ethical rules (Ethics Committee Approval Decision No: 64583101/2016/016). A group of rats not fed with fructose was called the control group, and rats were fed ad libitum for 16 weeks with just tap water and rat food. A group of rats, which is called the experimental group was fed ad libitum continuously for 16 weeks with 20% D-fructose solution as drinking water (Merck D(-)-Fructose for Biochemistry 1.04007.0250) and rat food. The body weights of both control and the experimental groups were measured and noted regularly each week since the beginning of the experiment time. The abdominal circumference of the animals was measured to see regional lipidosis, and the data of the initial and final days of the experiment were compared. At the end of 16 weeks of feeding, the rats under the effect of ether anesthesia were euthanized by cervical dislocation, and the testes and epididymis of the rats were taken. For each rat, both testes were weighed separately, right and left. The right testicles of the rats were taken into the Bouin fixation solution. The histological procedure was applied to the testicles and embedded in paraffin. Semen samples obtained from epididymis were divided into two groups for semen examination. A part of the semen sample obtained was marked with eosin nigrosin staining, and dead-alive examination and counting of spermatozoa were done using a Leica DMLB research microscope. The other half of the semen sample was fixed with Hancock's solution, and smear was prepared. Spermatozoon anomalies and anomaly zones were determined with an Olympus CX41 Phase contrast microscope. The data were recorded by counting.

2.1. Histological methods

Serial sections of 6 μm thickness and 200 μm spacing were taken from the paraffin-embedded tissues. Crossmon's triple staining and PAS reaction were applied for general histological examination and determination of histometric changes in 6 sections taken serially from paraffin-embedded tissues [13].

2.2. Determining histometric changes

For the groups, during VII and VII. stages tubules [14] density, the diameter of tubules and epithelium height were determined. For this purpose, the diameter of the VII and VII. stages tubules and the height of the seminiferous epithelium were measured, and the measurements were made interactively with the help of Olympus BX43F research microscope and Olympus cellSens Entry image analysis program.

2.3. Determining histological changes

The sections were examined in terms of germ cell shedding into the tubular lumen, subbasal vacuolization, epithelial vacuolization, and epithelial shedding. However, the number of atrophic tubules were determined in 100 tubules from each section, and atrophic tubule density was calculated. These changes were evaluated semi-quantitatively, and subjective scoring (0: Negative, 1: Low, 2: Medium, 3: High) was made according to the images.

2.4. Histochemical method

TUNEL method was applied to determine apoptosis in two serial sections of 6 μm thickness taken from each rat at 200 μm spacing [15,16]. In this method, available as a commercial kit (ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit, Chemicon International S7101) was used.

2.5. Determination of apoptosis density

The number of apoptotic cells corresponding to one tubule for each group, and % TUNEL positive rate was determined. Brown precipitation reaction observed in the preparations examined under the light microscope (Leica DMLB) in the X40 objective was evaluated as positive. A total of 200 apoptotic cells in all tubules were counted for an animal, with 100 tubules for each of the two sections of each rat and the number of apoptotic cells per tubule was calculated. In addition, the number of tubules containing at least one apoptotic cell among 200 tubules counted for each rat was determined, and the rate of TUNEL positive tubules % was calculated [16].

2.6. Cytological method

200 μL PBS was prepared, one corresponding to the cauda epididymis of each rat and taken semen from cauda epididymis was incubated in drops of PBS for 30 min at 35.5°C. In this way, semen was diffused in PBS, and tissue fragments of the cauda epididymis were separated from the medium at the end of the time. Thus, the sperm suspension was obtained and when the solution was ready, the semen and eosin-nigrosine (1:1) were taken with a micropipette, dropped onto a slide and followed by preparing smear to count the dead/alive spermatozoa under a light microscope with a x100 objective [17].

2.7. Hancock method [Liquid Fixation Method]

1 mL of Hancock solution prepared with 62.5 mL formalin (37%), 150 mL saline solution, 150 mL Buffer solution and 500 mL ultrapure water was taken, and 10 μL of fresh semen was added into this solution and mixed with the tip of the micropipette [18]. Then, 5 μL of this mixture was taken with a micropipette and dropped on the slide, and after it was covered with a coverslip, normal-abnormal spermatozoon count was made under a phase-contrast microscope. At least 200 spermatozoa from each sperm sample were examined and normal-abnormal structures were determined. Abnormal spermatozoon was categorized

as head, midpiece, 'S' shaped, and tail anomalies.

2.8.Statistical analysis

SPSS (Statistical for Social Sciences) for Windows 22 (SPSS Inc., Chicago, IL, USA) package program was used for statistical analysis of the data obtained. The compliance of the data to normal distribution was evaluated using the Shapiro–Wilk test. Differences between groups that did not show normal distribution were evaluated using the Mann–Whitney U test. Difference between groups showing normal distribution was made with student's t. Values of $p < 0.05$ from the results obtained from the statistical analysis performed were considered significant. All data were given as mean and ± standard error. Histological changes determined semi-quantitatively [germ cell shedding into the tubules lumen, subbasal vacuolization and epithelial vacuolization, interstitial area] were evaluated by Kruskal–Wallis variance analysis. The post hoc 97 multiple comparison test [19] was conducted to determine which group caused the difference.

3. Results

3.1.Body weight, percent body weight change

Body weight and body weight change percentages of control and experimental groups are given in Table 1. When the body weight was examined, it was seen that the average body weight increased in the experimental group rats. However, this difference was not statisticaly significant. When the first and last weight change percenteges of the animals were examined, a statistically significant difference was found ($p < 0.001$).

3.2.The abdominal circumference

It was observed that the abdominal circumference measurement of the rats in the experimental group were increased compared to the control group ($p < 0.001$). The data are given Table 1.

3.3.Testicular weight and testicular weight index (TWI)

The right and left testicular weight and testicular weight index of the control and experimental groups are given in Table 1. While there was a small increase in the right testicle in the experimental group compared to the control group, although a decrease was observed in the left testicle, no statistically significant difference was observed between the two groups in terms of weight difference. According to the data obtained, it was determined that there was a statistically significant decrease in the experimental group compared to the control group as a result of the testicular weight index calculation ($p < 0.001$).

3.4.Histological findings

It was observed that tubules seminiferous contorts were normal and the spermatogenetic epithelial layer formed a thick layer in the control group sections. It was determined that the Sertoli cells located on basement membrane, and Leydig cells surrounding capillary vessels in the interstitial area. Any of anomaly was not observed in evaluated tubules. It was observed that the testicles had a normal testicular appearance (Figure 1A) and tubular (Figure 1B). In the experimental group in which metabolic syndrome was formed by giving fructose, tubules seminiferous contorts were observed to be irregular. When the testicular tissue taken from the rats in the experimental group was examined, it was seen that the diameter of the tubules increased compared to the control group [tubular dilatation], but the seminiferous tubule epithelial layer became thinner and the density of germ cells decreased. Gaps and splitted areas were noted, indicating the presence of edema in the connective tissue between the seminiferous tubules (Figure 1C). Tubules with irregular contours and tubular atrophy were observed (Figure 1D). It was noted that the basal membrane surrounding the tubules in the experimental group was thickened compared to the control group. Splits between Sertoli cells and deformity of the Sertoli cell structure starts from the basal membrane to the lumen were observed (Figure 1E). Again, in the seminiferous tubules in the experimental group, multinucleated giant cells (Figure 1F) and mitotic figures were observed (Figure 1G). It was observed that

Table 1. Body weight and percent body weight change, abdominal circumference measurements, and testicular weight index (TWI) of in control and experimental groups.

Group	n	Body weight (g)	Percent body weight change (g)	Abdominal circumference (cm)	Testicular (g)		TWI
					Right	Left	
Control	10	387.40 ± 10.17	36.84 ± 0.83	18.59 ± 0.45	1.95 ± 0.02	1.96 ± 0.03	1.01 ± 0.01
Experimental	14	407.14 ± 8.91	41.06 ± 0.37	21.27 ± 0.34	1.95 ± 0.07	1.94 ± 0.07	0.95 ± 0.02
p		0.161	0.001	0.001	0.538	0.681	0.007

Control: No fructose in diet.
Experimental: 20% D fructose in diet.

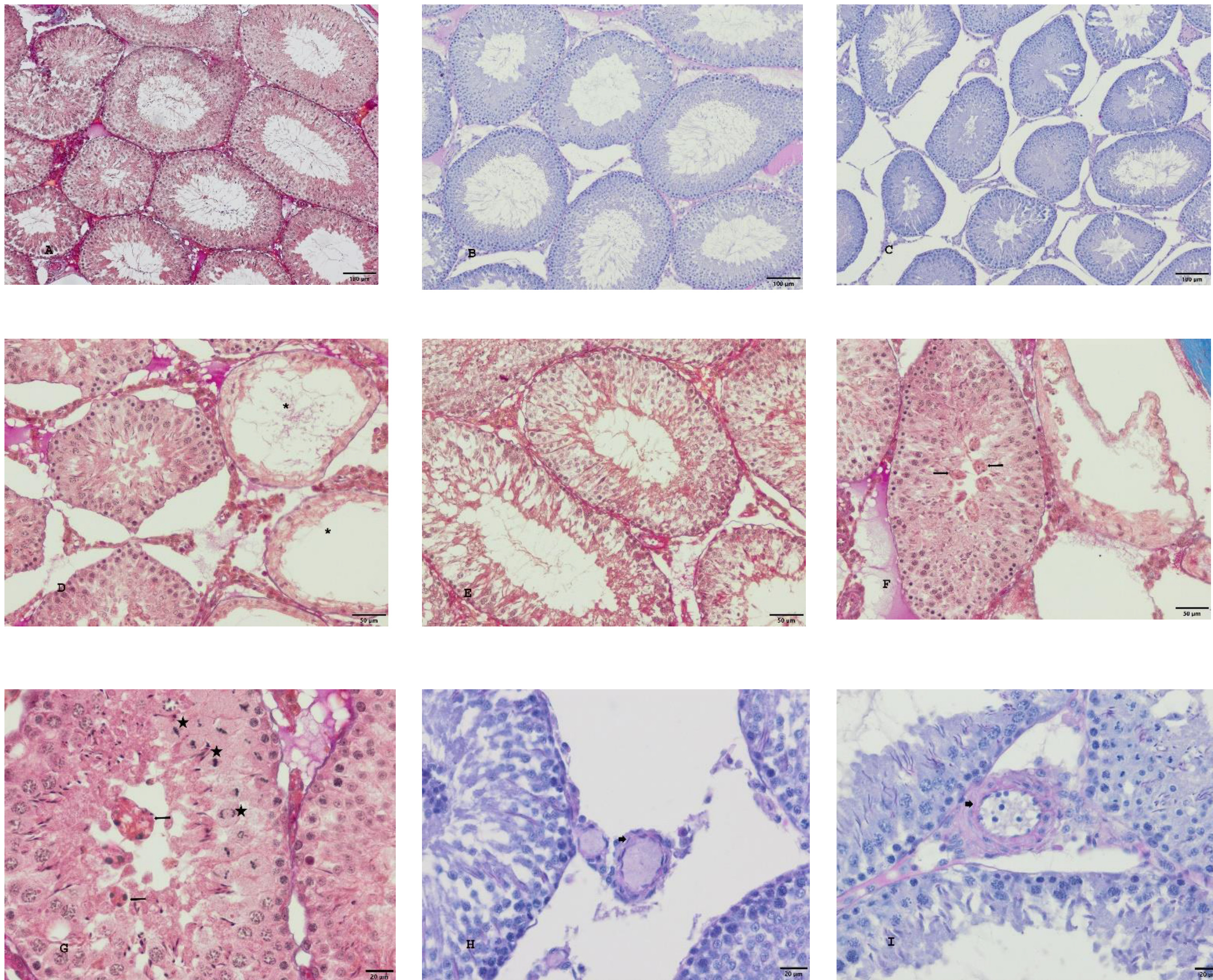

Figure 1. A. General image of tubules in control groups. Triple stain. Bar.100 µm. **B.**General image of tubules in control groups. PAS. Bar.100 µm. **C.**The image of gaps and splitted area indicating between the seminiferous tubules of experimental group. PAS. Bar.100 µm. **D.**Tubules with irregular contours and tubular atrophy of experimental group (*). Triple stain. Bar.50 µm. **E.**Deformity of the Sertoli cell structure in experimental groups. Triple stain. Bar.50 µm. **F.**The image of multinucleated giant cells (arrow) in experimental groups. Triple stain. Bar. 50 µm. **G.**The image of multinucleated giant cells and the mitotic figures (stars) in experimental groups. Triple stain. Bar. 20 µm. **H.**The thickness of the wall of the vessels (arrow) in control group. PAS. Bar. 20 µm. **I.**The thickness of the wall of the vessels (arrow) in experimental group. PAS.Bar. 20 µm.

thickened in the wall of the structure of the vessels in the experimental group (Figure 1I) compared to the control group (Figure 1H). Tunica albuginea was found to be thickened in experimental group sections compared to control group sections.

3.5. Histological changes

In the evaluation procedure for subbasal vacuolization (Figure 2A), epithelial vacuolization (Figure 2B), epithelial shedding (Figure 2C), and germ cells shedding into the tubular lumen (Figure 2D), according to the comparison of the control and experimental groups, the increases in the experimental group were found to be statistically significant ($p < 0.002$). The data obtained are given in Table 2. When the epithelial vacuolization was examined, there was again a statistically significant increase in the experimental group ($p < 0.001$). When the epithelial shedding was examined in the control and experimental groups, it was found that epithelial shedding was very intense in the experimental group and was statistically significant ($p < 0.001$). In the evaluation performed to determine the atrophic tubule density, when the control and experimental groups were compared, the increase in the experimental group was found to be statistically significant ($p < 0.001$). The data is given in Table 3.

3.6. Histometric changes

During VII and VII. stages seminiferous tubule diameter and seminiferous tubular epithelial height of the control and experimental groups are given in Table 3.

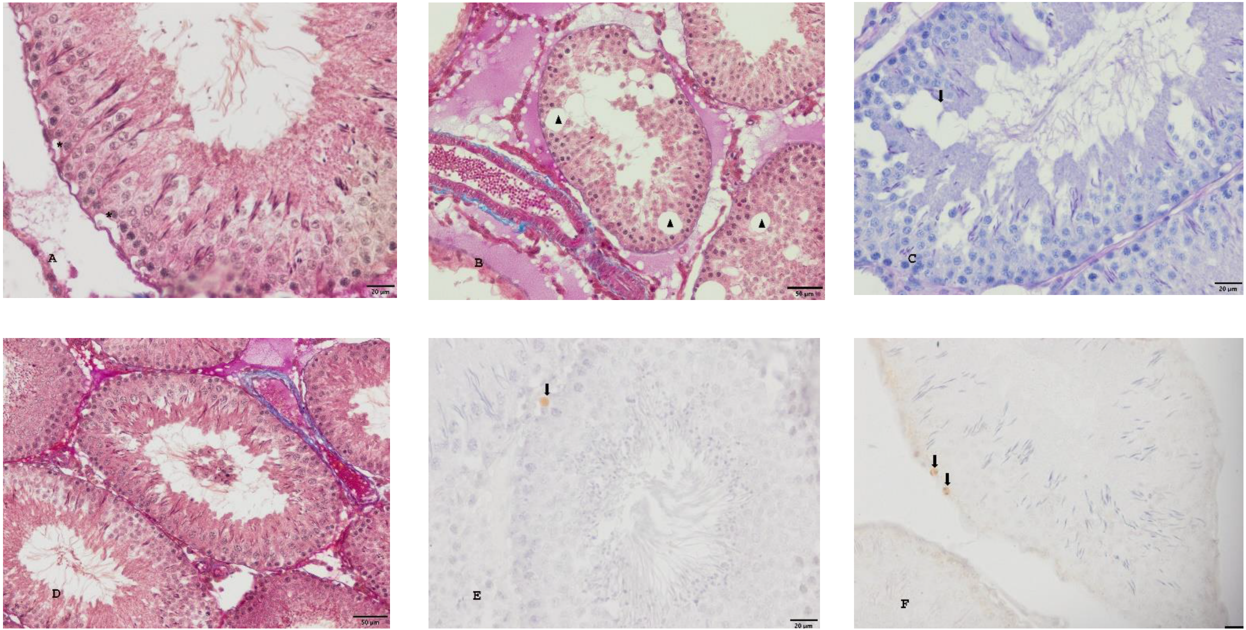

Figure 2. A. Subbasal vacuolization (*) in experimental group. Triple stain. Bar 20 µm. **B.**Epithelial vacuolization (triangles) in experimental group. Triple stain. Bar. 50 µm. **C.**Epithelial shedding (arrows) in experimental group. PAS. Bar. 20 µm. **D.**Germ cells shedding into the tubular lumen of experimental group. Triple stain. Bar. 50 µm. **E.**Apoptotic cells (arrow) of control group. TUNEL method. Bar. 20 µm. **F.**Apoptotic cells (arrows) of experimental group. TUNEL method. Bar. 20 µm.

Table 2. Histological changes in control and experimental groups.

Group	n	Subbasal vacuolization	Epithelial vacuolization	Epithelial shedding	Germ cells shedding into the tubular lumen
Control	10	0.03 ± 0.03	0.03 ± 0.09	0.23 ± 0.08	0.26 ± 0.09
Experimental	14	1.71 ± 0.11	2.09 ± 0.14	2.38 ± 0.12	1.02 ± 0.16
p		0.001	0.001	0.001	0.002

Control: No fructose in diet.
Experimental: 20% D fructose in diet.

Table 3. Density of atrophic tubules and diameter of seminiferous tubulus and epithelial height of seminiferous tubulus.

Group	n	Normal tubules	Atrophic tubules	Diameter of seminiferous tubulus (µm)	Epithelial height of seminiferous tubulus (µm)
Control	10	99.90 ± 0.06	0.10 ± 0.06	287.45 ± 2.20	85.52 ± 1.63
Experimental	14	95.03 ± 0.63	4.96 ± .63	299.57 ± 0.20	55.59 ± 0.25
p		0.001	0.001	0.001	0.001

Control: No fructose in diet.
Experimental: 20% D fructose in diet.

3.7. Histochemical findings

The number of apoptotic cells per tubule was recorded by observing apoptotic cells giving TUNEL positive reaction in the control and experimental groups. % TUNEL positive tubules values calculated by determining tubules containing at least one apoptotic cell are given in Table 4. It was observed that the number of apoptotic cells per seminiferous tubules in the experimental group (Figure 2F) increased compared to the control group (Figure 2 E), ($p < 0.001$).

Table 4. Number of apoptotic cells per seminiferous tubules and tubules containing at least one apoptotic cell, normal – abnormal spermatozoon count results and the numbers of dead – alive spermatozoon.

Group	n	Number of apoptotic cells per seminiferous tubules	Number of tubules containing at least one apoptotic cell	Normal spermatozoon	Abnormal spermatozoon	Number of dead spermatozoon	Number of alive spermatozoon
Control	10	4.90 ± 0.48	4.00 ± 0.34	166.60 ± 5.32	53.30 ± 4.50	4.90 ± 1.39	186.80 ± 4.13
Experimental	14	17.42 ± 0.98	12.89 ± 0.79	171.14 ± 10.01	73.28 ± 8.85	16.57 ± 1.84	182.92 ± 7.25
p		0.001	0.001	0.725	0,088	0.001	0.682

Control: No fructose in diet.
Experimental: 20% D fructose in diet.

3.8.Spermatozoon morphology

Normal-abnormal spermatozoon count results of the control group and experimental group are given in Table 4. Compared to the control group, it was observed that the abnormal spermatozoon rate increased in number in the experimental group, but this increase was not statiscally significant.

3.9. Dead-alive spermatozoon

The numbers of dead-alive spermatozoon belonging to the control and experimental groups are given in Table 4. It was noted that there was a statistically significant increase in the amount of dead spermatozoon in the experimental group compared to the control group ($p < 0.001$).

4. Discussion

Recently, it has been reported that fructose taken with diet causes many health problems along with obesity [20]. MetS model can be created with high fructose consumption. This model can induce hypertension, hypertriglyceridemia, hyperinsulinemia, and insulin resistance in mice [21]. In this study, the body weights of the experimental group rats, in which metabolic syndrome was formed by fedon 20% D-fructose solution as drinking water for eight weeks, increased by 13 grams compared to the control, but this increase was not statistically significant [22]. In the presented study, it was determined that the body weights of the experimental group rats increased compared to the control group. However, it was seen that the difference between them was not statistically significant. These results are consistent with Bircan's [22] study. When the percentages of body weight change calculated based on the first and last weight measurements of the groups were examined, the difference was found to be statistically significant. In a study that caused diabetes by applying streptozotocin, when the TWI (testicular weight index) results of the experimental and control groups were examined, a statistically significant increase was observed between the control-diabetes groups [23]. In the study, a small increase in the right testicle was observed in the experimental group compared to the control group. Although there was a decrease in left testicular weight, there was no statistically significant difference between the two groups. According to the data obtained from the TWI calculated using body weight and the sum of right and left testicular weight for each animal, a statistically significant decrease was found in the testicular weight index of the experimental group compared to the control group. It has been reported that serum testosterone levels decrease significantly in men with MetS compared to healthy individuals [24]. It has been demonstrated that low testosterone level is an important marker for type II diabetes and MetS [25]. However, as a result of low testosterone level, decrease in muscle mass, increase in insulin resistance, dyslipidemia and related oxidative stress, it causes fat accumulation in the abdominal area with an increase in lipoprotein lipase activity, which is the main enzyme that regulates triglyceride uptake in fat cells [26]. In the present study, an increase in abdominal circumference was found due to fat accumulation in the abdominal area of the experimental group animals. The difference between the abdominal circumference measurements of the experimental and control groups was found to be statistically significant ($p = 0.001$, Table 2).

It was observed that the seminiferous tubules were irregularly shaped and germ cells decreased in the seminiferous tubule epithelium in rats with experimental diabetes. Edema and residual bodies were observed in the interstitial area [23]. In the study, testicular atrophy was observed in the experimental group, unlike the control group. It was observed that the tubulus seminiferous contorts were irregular, the splits between the tubules and a significant part of the spermatogenic cells in some of the tubules have degenerated. Splits between Sertoli cells, disruption of the Sertoli cell structure from basal to lumen and vacuoles between germ cells and interstitial space attracted attention. Thickening was detected in the tunica albuginea of the experimental group.

Kaya [27], associated the spermatogenic serial cell degeneration with the death of Sertoli cells in his study. Fooding of Sertoli cells and spermatogenic serial cells located in the seminiferous tubules is by diffusion from the blood vessels in the interstitial connective tissue. These blood vessels are important not only for the nourishment of Leydig cells but also for the nourishment of the tubule. It has been reported that thickening of the interstitial blood vessels may cause tubular atrophy by reducing tubular blood supply [28]. It is demonstrated that the tubule fooding, which is already impaired by the thickening of the blood vessel walls, will further deteriorate the spermatogenesis by further deterioration with tubule basement membrane thickening [28]. Thickening of the capillary vessel wall was observed. In experimental diabetes studies, thickening of the interstitial vessel walls is likely to cause chronic ischemia in seminiferous tubules [27]. It is seen that the negativity in tubule nutrition also negatively affects spermatogenesis. It is thought that Sertoli cells and spermatogenic cells may be more sensitive to acute arterial malnutrition and to testosterone deficiency, respectively [29]. It can be said that testosterone deficiency and arterial malnutrition have an important effect on testicular atrophy observed in diabetes. Similar findings were found in this study as in arterial malnutrition observed in diabetes. In the presented study, when subbasal vacuolization, epithelial vacuolization, epithelial shedding, and germ cells pouring into the tubular lumen were evaluated, it was observed that the values in the experimental group increased statistically significantly compared to the control group ($p < 0.001$).

Öztürk et al. [29] demonstrated in their study that they observed multinucleus giant cells in some seminiferous tubules. Multinucleus giant cells were shown with systemic, toxic, infectious agents that cause tubular atrophy, ischemia, and cryptorchidism [30]. Although it is not known exactly how they were formed, it is believed that giant cells with multiple nuclei were formed as a result of the combination of spermatids [27]. It is possible to observe the nucleus chromatin of these giant cells as a crescent-like appearance on the inner surface of the nucleus membrane. This appearance conforms to the chromatin structure observed in apoptotic cells [31]. In the presented study, giant multinucleus cells poured into the lumen were observed in the experimental group.

It was found that the diameter of the seminiferous tubule in diabetic rats was statistically decreased compared to the control group [23]. Tubular dilatation was found in the presented study; it was observed that the diameter of the seminiferous tubule in the experimental group showed a statistically significant increase compared to the control group. In addition, the experimental group detected a statistically significant decrease in the thickness of the seminiferous tubule epithelium. The decrease in the thickness of the tubule epithelium may be due to the degeneration of the cells in spermatogenesis.

It has been stated that infertility is a common complication in individuals with diabetes, mainly due to apoptotic cell death and loss of spermatogenic cells [32]. It was found that apoptotic spermatogenic cells increased in diabetic male rats at the end of twelve weeks compared to the control group. It has been reported in the study that spermatogenic disorder may be related to the increase in apoptotic cells [33]. Zhao et al. [32] investigated the effects of resveratrol on the testicles in mice with type I diabetes. Testicular apoptosis and oxidative stress levels were significantly higher in mice with diabetes than controls. Resveratrol application has been shown to reduce the number of apoptotic cells. Shi et al. [34] found that apoptotic cells in the testes increased in mice with streptolysin-induced type I diabetes, while Lithium barbarum polysaccharide (LBP), which is considered to have fertility-enhancing properties in traditional medical practices in China, significantly improved sperm parameters and increased antioxidant enzyme activities in mice with diabetes. They have shown that it reduces apoptotic cell death. They stated that the protective effect of LBP on male spermatogenic dysfunction is due to the increase in antioxidant enzyme activity and prevention of cell death. Ding et al. [35] reported that vitamin D supplementation in diabetic rats can protect spermatogenic cells by suppressing inflammation factors and reducing apoptotic cell death, as well as regulating the expression of genes associated with reproduction and testosterone synthesis. In different studies conducted on rats with diabetes [36, 37]. It was stated that Pentoxifylline (PTX) caused a significant decrease in the number of apoptotic cells in the testes and a decrease in blood vessel density. Orman et al. [38] reported that oxidative stress, increased nitric oxide level, and apoptotic cell death played an important role in diabetic rat testicular damage, and that treatment of diabetic rats with aminoguanidine (AG) protected spermatogenic cells against oxidative stress and apoptotic cell death. It is stated that royal jelly use has a protective effect against the number of apoptotic cells that increase with diabetes [39, 40]. Rashid and Sil. [41] reported that curcumin protects against mitochondrial and endoplasmic reticulum-dependent apoptotic death of diabetic testicular cells and that this molecule can be used as a potential therapeutic in the treatment of diabetic testicular dysfunction. Roy et al. [42] determined that ferulic acid prevents testicular damage by decreasing the number of apoptosis and reducing oxidative stress against the increase in apoptotic cells in the testes caused by diabetes. Again, Li et al. [43] found that the NADPH oxidase inhibitor apocynin significantly reduced the production of reactive oxygen species (ROS) and apoptotic cells and increased the total testosterone level.

It is reported that there is an association between metabolic syndrome and testicular dysfunction. It has been found that metabolic syndrome affects testicular function by making a particularly negative effect on spermatogenesis [44, 45, 46]. In our study, it was determined that the number of apoptotic cells per tubules and the number of tubules containing at least one apoptotic cell significantly increased in the experimental group given fructose compared to the control group ($p < 0.001$).

It has been shown that the spermatozoon anomaly in diabetic animals is more than the control group spermatozoon anomaly, and this anomaly generally occurs as a structural disorder accompanied by the corrugation in the tail section [23]. According to the data obtained from this study, a distinct anomaly, especially tail anomaly, was observed in the sperm sample of the experimental group. While the abnormality was not observed in the control group semen, it was observed that the number of abnormal spermatozoon morphology samples increased in the experimental group semen. As a result, there was no statistically significant difference.

As a result, the increasing frequency of metabolic syndrome in the world, the increase in fructose consumption per person to 85–100 g today is of great importance in terms of causing serious health problems as well as testicular dysfunction. Considering that it may cause infertility in metabolic syndrome such as diabetes, attention should be drawn to the subject and new studies should be conducted.

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