EXPERIMENTAL STUDY

Mitochondrial transplantation attenuates apoptosis and improves spermatogenic function following testicular torsion-detorsion injury in rats

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ABSTRACT

BACKGROUND: Testicular torsion is a urological emergency that may result in irreversible ischemia-reperfusion injury and long-term impairment of spermatogenic function despite timely surgical detorsion. Mitochondrial dysfunction plays a central role in oxidative stress-induced germ cell damage following torsion. This study aimed to investigate the protective effects of isolated intratesticular mitochondrial transplantation in a rat model of testicular torsion-detorsion injury.

METHODS: Thirty-six adult male Sprague-Dawley rats were randomly allocated into four groups: sham (n=4), torsion (n=10), torsion+vehicle (n=10), and torsion+mitochondria (n=10). Testicular torsion was induced by a 720° rotation for 2 h, followed by surgical detorsion. Isolated mitochondria were administered through intratesticular injection before torsion induction. Histopathological evaluation was performed using hematoxylin–eosin staining and quantified by the Johnsen scoring system. Apoptotic activity was assessed by Caspase-3 immunohistochemistry. Functional outcomes were evaluated through total sperm count, motile sperm count, and detailed sperm motility subtype analysis.

RESULTS: The torsion and vehicle groups demonstrated marked histological damage, significantly reduced Johnsen scores, and increased apoptotic activity compared with the sham group. Mitochondrial transplantation significantly preserved seminiferous tubular architecture and resulted in higher Johnsen scores compared with untreated torsion (9.7±0.4 vs. 6.4±1.0, $p<0.001$). Caspase-3 expression was significantly reduced in the mitochondria-treated group, approaching levels observed in sham animals. Functionally, total and motile sperm counts were significantly higher in mitochondria-treated rats than in torsion and vehicle groups. Detailed motility analysis revealed restoration of progressive sperm motility patterns, whereas progressive motility was completely absent in untreated ischemic groups.

CONCLUSION: Intratesticular mitochondrial transplantation provides significant structural and functional protection against ischemia-reperfusion injury in experimental testicular torsion. By preserving spermatogenic architecture, reducing apoptosis, and restoring progressive sperm motility, mitochondrial therapy represents a promising experimental strategy for fertility preservation in ischemic testicular disorders.

Keywords: Apoptosis; ischemia-reperfusion injury; mitochondrial transplantation; spermatogenesis; testicular torsion.

Cite this article as: Özcan C, Sancı A, Mendeş UD, Sözer M, Ülger Ö, Şimşek G, et al. Mitochondrial transplantation attenuates apoptosis and improves spermatogenic function following testicular torsion-detorsion injury in rats. Ulus Travma Acil Cerrahi Derg 2026;32:1171-1179.

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Ulus Travma Acil Cerrahi Derg 2026;32(10):1171-1179 DOI: 10.14744/tjtes.2026.02503
Submitted: 14.12.2025 Revised: 05.08.2026 Accepted: 18.08.2026 Published: 06.10.2026
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INTRODUCTION

Ischemia-reperfusion (I/R) injury remains one of the leading causes of tissue damage in surgical and urological emergencies, such as testicular torsion, renal transplantation, and major vascular procedures.[1] In particular, testicular torsion-detorsion (TTD) represents a time-critical urological emergency that compromises microcirculatory perfusion, leading to germ cell apoptosis, oxidative stress, and impaired spermatogenesis.[2] Even when timely surgical detorsion is achieved, the restoration of blood flow can paradoxically aggravate tissue damage through the excessive generation of reactive oxygen species (ROS), mitochondrial dysfunction, and disruption of the blood–testis barrier.[3] Current therapeutic strategies, including antioxidant and anti-inflammatory agents, have demonstrated only partial success, and no pharmacological approach has yet been proven to fully restore testicular structure and function following I/R injury.[4-6]

Mitochondria play a central role in energy metabolism, redox balance, and cell survival.[7] They are particularly abundant in testicular and sperm cells, where they supply adenosine triphosphate (ATP) required for spermatogenesis and sperm motility.[8] Increasing evidence indicates that mitochondrial injury during reperfusion is a key event driving germ cell apoptosis and testicular atrophy.[9] Therefore, strategies aimed at preserving or restoring mitochondrial function have gained growing attention. Mitochondrial transplantation has recently emerged as a promising therapeutic strategy for ischemic tissue injury.[10] Studies in cardiac, hepatic, and renal models have demonstrated that the delivery of isolated functional mitochondria into damaged tissues can restore bioenergetic capacity, reduce oxidative stress, and improve histological recovery.[11-13] Experimental findings further suggest that transplanted mitochondria are capable of integrating into host cells, maintaining membrane potential, and activating prosurvival signaling pathways.[14] Although mitochondrial transplantation has been successfully applied in several organ systems, its potential role in testicular I/R injury remains largely unexplored.[15] Previous research has mainly focused on mesenchymal stem cell-based or paracrine antioxidant mechanisms, while the direct restoration of mitochondrial integrity in reproductive tissues has not been systematically evaluated.[16] Moreover, the mechanisms by which exogenous mitochondria modulate germ cell apoptosis and spermatogenic recovery following torsion-detorsion injury are not yet fully understood.

In this study, we aimed to investigate the protective and regenerative effects of isolated mitochondrial transplantation in a rat model of TTD injury, focusing on its impact on spermatogenesis, histopathological alterations, and mitochondrial integrity markers.

MATERIALS AND METHODS

All experimental procedures were approved by the Saki Yenilli Experimental Animals Production and Research Laboratory Local Ethics Committee (Approval No: 14, Date: 02.12.2022) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals

A total of 36 adult male Sprague-Dawley rats (12–14 weeks old, 250–350 g) were used. Animals were maintained under standard laboratory conditions (temperature 22–24°C, 45–55% humidity, 12-h light/dark cycle) with free access to food and water. Before all procedures, animals were fasted overnight but allowed water ad libitum. Two additional rats were used exclusively for mitochondrial isolation and verification procedures.

Experimental Design

Rats were randomly assigned to four groups: Sham, torsion, torsion+vehicle, and torsion+mitochondria. The sham group underwent scrotal incision and immediate replacement of the testis without torsion. In the torsion group, the left testis was twisted 720° clockwise and fixed for 2 h to induce ischemia, followed by detorsion.[17,18] The vehicle group received intratesticular injection of respiration buffer before torsion, while the mitochondria group received an equal volume containing isolated mitochondria.[12,19] The 2-h torsion period was selected based on prior studies demonstrating reproducible ischemic damage without total necrosis, allowing subsequent evaluation of reperfusion-related recovery.[17,18]

Anesthesia and Surgical Procedure

Anesthesia was induced by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Through a midline scrotal incision, the left testis was gently exteriorized and rotated 720° clockwise, then fixed to the scrotal wall with a 4-0 atraumatic silk suture to maintain ischemia. After 2 h, detorsion was achieved by returning the testis to its natural position, and the incision was closed with interrupted 4-0 silk sutures. In the sham group, only scrotal incision and immediate repositioning were performed without torsion. Postoperative analgesia was ensured with meloxicam (1 mg/kg, s.c.), and all animals were monitored for recovery.

Mitochondrial Isolation

Allogeneic biceps femoris muscle was used as the mitochondrial source. Isolation was carried out at +4°C under hypothermic conditions using a commercial kit (Mitochondria Isolation Kit for Tissue and Cultured Cells, ab288084; Abcam, UK) according to the manufacturer's instructions.[12,19]

Briefly, 200 mg of freshly harvested skeletal muscle was washed twice in isolation buffer, minced into small fragments, and homogenized with a Dounce homogenizer (10 strokes). The homogenate was centrifuged at 600 × g for 10 min to remove debris, followed by centrifugation of the supernatant at 7000 × g for 10 min to obtain the mitochondrial pellet. The pellet was washed with isolation buffer, resuspended in storage buffer, and kept on ice until transplantation.

Mitochondrial protein concentration was quantified using a BCA Protein Assay Kit (Thermo Fisher Scientific, 23225), and

the target transplantation dose was calculated

Mitochondrial Transplantation Procedure

Under sterile conditions, isolated mitochondria (150 µg protein/100 µL respiration buffer) were injected into the left testis using a 30G insulin syringe.[10,12,19] The needle was inserted at a 45° angle from the lower pole toward the upper pole; injection was performed slowly while withdrawing the needle to ensure homogeneous distribution. In the vehicle group, an equivalent volume of respiration buffer was administered. All injections were performed immediately before torsion induction.

Tissue Collection

At 14 days after detorsion, rats were anesthetized and sacrificed by cardiac exsanguination under deep anesthesia (National Research Council, 2011). Bilateral orchiectomy was performed. The left testis and epididymis were dissected, rinsed with isotonic saline, and divided for histopathological, immunohistochemical, and spermatological analyses. Tissues for histology were fixed in 10% neutral-buffered formalin, while samples for molecular assays were snap-frozen in liquid nitrogen and stored at −80°C.

Histopathological Evaluation

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 5 µm and stained with hematoxylin and eosin. Fifty seminiferous tubules per section were examined under light microscopy at 400× magnification. Spermatogenic activity was scored using the Johnsen scoring system (0–10 scale). Mean Johnsen scores were 3.6±0.8 (vehicle), 6.4±1.0 (torsion), 9.2±0.5 (sham), and 9.7±0.4 (mitochondria), indicating partial to complete recovery in the treatment group.[20]

Immunohistochemical Analysis

Apoptotic activity was assessed by Caspase-3 immunohistochemistry.[21] Sections were deparaffinized, rehydrated, and incubated with primary anti-Caspase-3 antibody (1:100, Abcam, UK), followed by a secondary antibody system. Visualization was achieved using 3,3′-diaminobenzidine substrate, and nuclei were counterstained with hematoxylin. The percentage of Caspase-3-positive cells was semi-quantitatively scored based on staining intensity and localization testis.

Spermatological Evaluation

The epididymis was dissected in accordance with its anatomical structure and minced into small pieces in 5 mL of normal saline at 37°C. Samples were incubated for 15 min, and the supernatant was filtered through gauze. The sperm suspension was examined using a Makler counting chamber under 200× magnification.[22] The epididymal sperm preparation protocol was adapted from Makler's method with minor modifications suitable for rodent models.

Motile and immotile spermatozoa were counted in 100 fields, and three independent counts were averaged per animal. The mitochondria-treated and sham groups exhibited significantly higher total and motile sperm counts compared with the torsion and vehicle groups ($p<0.05$). Sperm motility was evaluated according to the World Health Organization criteria and categorized into fast progressive (Type A), slow progressive (Type B), non-progressive (Type C), and immotile (Type D) spermatozoa. Total motility was calculated as the sum of Types A and B. In addition, sperm concentration ($\times 10^6$/mL) and normal morphology percentage were assessed using standard light microscopy.

Statistical and Power Analysis

Statistical analyses were performed using IBM Statistical Package for the Social Sciences Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed for normality using the Shapiro–Wilk test. Continuous variables are presented as mean±standard deviation. For comparisons among more than two independent groups, one-way analysis of variance (ANOVA) was used for normally distributed data, followed by Tukey's post hoc test for multiple comparisons. For non-normally distributed data, the Kruskal-Wallis test was applied, followed by pairwise Mann–Whitney U tests with Bonferroni correction. Categorical variables, including sperm motility subtype distributions, were analyzed using the Chi-square test or Fisher's exact test where appropriate. A two-tailed $p<0.05$ was considered statistically significant. Post hoc power analysis was conducted using G*Power software (version 3.1.9.7; Heinrich Heine University, Düsseldorf, Germany). Based on the observed effect sizes for the primary outcomes, including Johnsen score and motile sperm count, the achieved statistical power exceeded 0.80 for detecting large effect sizes ($f\geq0.45$) at an alpha level of 0.05.

RESULTS

A total of 36 adult male Sprague-Dawley rats were included in the study: sham (n=4), torsion (n=10), torsion+vehicle (n=10), and torsion+mitochondria (n=10) groups. Two additional animals were used exclusively for mitochondrial isolation and verification. All animals survived the procedure, and no perioperative complications or mortality occurred. TTD was successfully completed in all rats, and post-operative recovery was uneventful. Comparative biochemical and histopathological findings are summarized in Table 1, and representative photomicrographs are shown in Figure 1.

After detorsion, ischemic changes such as testicular swelling, discoloration, and vascular congestion were prominent in the torsion and vehicle groups, whereas these changes were notably attenuated in the mitochondria-treated group. No adverse local or systemic reactions were detected following mitochondrial injection.

Histopathological Findings

Light microscopic evaluation revealed marked degeneration of seminiferous tubules, germ cell loss, and interstitial edema in the torsion and vehicle groups. Conversely, the mitochondria-treated group demonstrated well-preserved

Table 1. Comparison of histopathological and spermatological parameters among study groups

Parameter	Sham (n=4)	Torsion (n=10)	Torsion+Vehicle (n=10)	Torsion+Mitochondria (n=10)	p
Johnsen score	9.2±0.5	6.4±1.0	3.6±0.8	9.7±0.4	<0.001[a]
Caspase-3 positivity (% cells)	12.4±3.2	54.1±8.7	58.3±9.1	18.6±4.5	<0.01[b]
Total sperm count (×10⁶/mL)	28±3.8	6±3.4	5±3.2	22±3.6	<0.05[a]
Motile sperm count (×10⁶/mL)	19.9±3.5	1.3±0.2	1.2±0.2	15±2.6	<0.05[a]
Normal morphology (%)†	92	28	24	68	<0.05[a]

[a]One-way analysis of variance with Tukey's post hoc test. [b]Kruskal–Wallis test followed by Mann–Whitney U test. †Normal morphology values are presented as group means. Values are presented as mean±standard deviation (SD).

tubular architecture, intact germinal epithelium, and restored spermatogenic organization similar to that of the sham group. Quantitative analysis based on the Johnsen scoring system showed significantly higher spermatogenic activity in the mitochondria-treated group (9.7±0.4) compared with the torsion (6.4±1.0) and vehicle (3.6±0.8) groups ($p<0.001$). There was no significant difference between the mitochondria-treated and sham (9.2±0.5) groups ($p>0.05$) (Table 1 and Fig. 1a-d).

Apoptotic Activity (Caspase-3 Immunohistochemistry)

Immunohistochemical assessment of Caspase-3 expression demonstrated intense cytoplasmic and nuclear positivity in germ cells and Sertoli cells in the torsion and vehicle groups, indicating heightened apoptotic activity. In contrast, the mitochondria-treated group showed markedly reduced Caspase-3 immunoreactivity, comparable to the sham animals (Fig. 2). Semi-quantitative scoring confirmed a significant reduction in Caspase-3 positivity in the mitochondria group relative to the torsion and vehicle groups ($p<0.01$).

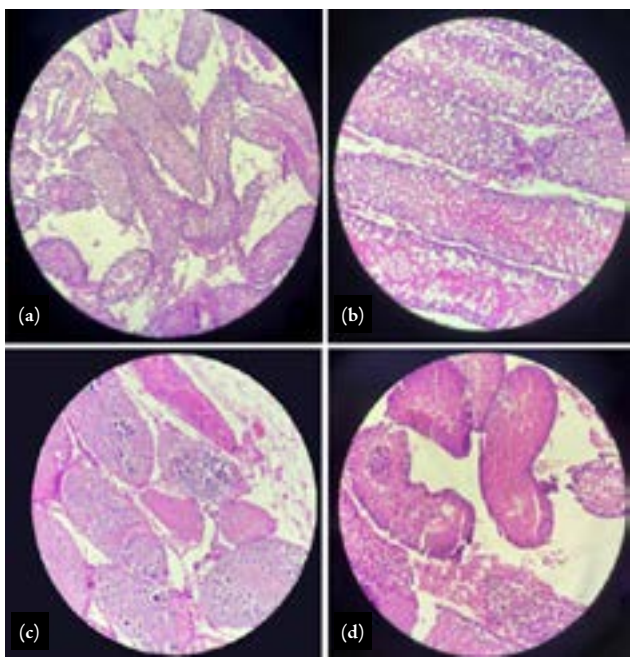

Figure 1. Representative hematoxylin and eosin-stained sections of seminiferous tubules in each group. **(a)** Sham (Johnsen Score: 9.2), **(b)** Torsion+Mitochondria (9.7), **(c)** Torsion+Vehicle (3.6), **(d)** Torsion (6.4).

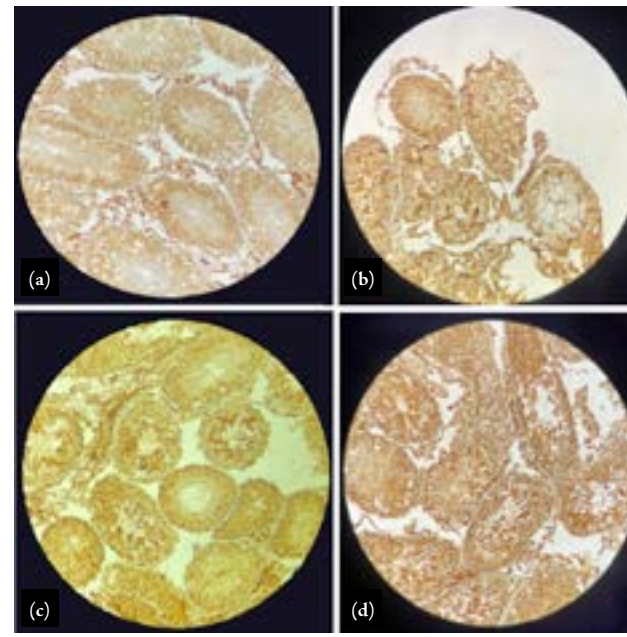

Figure 2. Immunohistochemical Caspase-3 staining in testicular tissue. **(a)** Sham, **(b)** Torsion+Mitochondria, **(c)** Torsion+Vehicle, **(d)** Torsion. Reduced apoptotic staining is observed in the mitochondria-treated group compared with untreated torsion controls.

Functional Sperm Outcomes

Epididymal spermiogram evaluation revealed significantly decreased total and motile sperm counts in the torsion and vehicle groups, whereas both parameters were markedly improved in the mitochondria-treated group (Fig. 3). When histopathological integrity, Johnsen score, Caspase-3 activity, and sperm motility were collectively evaluated, the mitochondria-treated group demonstrated the highest degree of structural and functional recovery among all ischemia-exposed groups (Fig. 4).

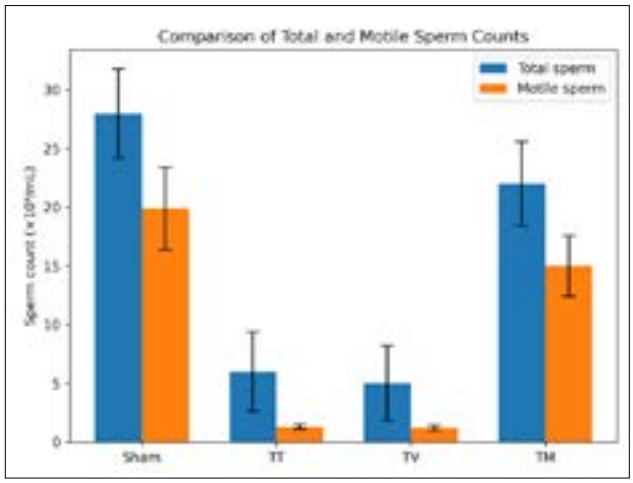

Figure 3. Comparison of total and motile sperm counts among groups. Bar graphs represent mean±standard deviation (SD) of total and motile sperm counts (×10^6/mL) in sham, torsion (TT), torsion+vehicle (TV), and torsion+mitochondria (TM) groups. The mitochondria-treated group shows significantly higher total and motile sperm counts compared with torsion and vehicle groups and demonstrates values comparable to sham controls. Statistical analysis was performed using one-way analysis of variance followed by Tukey's post hoc test.

Detailed evaluation of sperm motility subtypes demonstrated profound alterations in progressive motility following testicular torsion. Fast and slow progressive spermatozoa (Types A and B) were completely absent in both the torsion and vehicle groups, with motility predominantly consisting of non-progressive (Type C) and immotile (Type D) spermatozoa (Table 2). Specifically, immotile sperm accounted for 78% and 82% of total spermatozoa in the torsion and vehicle groups, respectively, resulting in severely diminished total motility rates of 22% and 18% (Table 2).

Assessment of sperm morphology revealed severe structural deterioration in ischemia-exposed groups. The percentage of spermatozoa with normal morphology was reduced to 28% in the torsion group and 24% in the vehicle group, compared with 92% in the sham group (Table 1). Mitochondrial transplantation significantly improved sperm morphological integrity, with 68% normal morphology observed in the treatment group (Table 1), supporting a protective effect on spermatogenic architecture.

Post hoc Statistical Comparisons

Overall ANOVA revealed a significant between-group difference for both Johnsen scores ($F[3,30]=46.2$, $p<0.001$) and motile sperm counts ($F[3,30]=22.8$, $p<0.001$). Tukey's post hoc tests showed that the mitochondria-treated group differed significantly from the torsion and vehicle groups but not from the sham group (Table 3). Post hoc power analysis confirmed >0.80 power for detecting large effects ($f\geq0.45$) in the primary endpoints

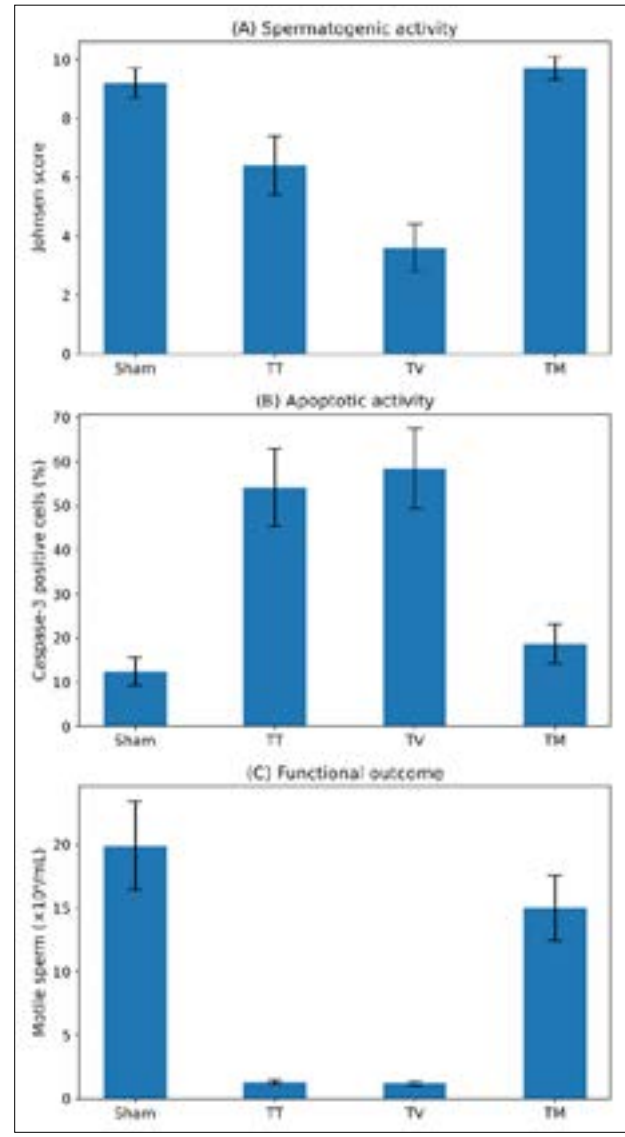

Figure 4. DStructural and functional recovery following mitochondrial transplantation. Composite figure summarizing the structural and functional effects of mitochondrial transplantation in experimental testicular ischemia–reperfusion injury. The mitochondria-treated group demonstrates coordinated recovery across histopathological, apoptotic, and functional sperm parameters compared with torsion and vehicle-treated groups. **(a)** Spermatogenic integrity (Johnsen score). Mean Johnsen scores (± SD) are shown for each experimental group. Mitochondrial transplantation significantly preserved seminiferous tubular architecture and spermatogenic activity compared with torsion and vehicle groups (****p<0.001). **(b)** Apoptotic activity (Caspase-3 expression). Percentage of Caspase-3–positive cells in testicular tissue sections. Mitochondria-treated animals exhibit a marked reduction in apoptosis relative to ischemia and vehicle controls. **(c)** Functional outcome (Sperm motility). Mean motile sperm counts (×106/mL±SD) across groups. The mitochondria-treated group shows significant functional recovery compared with torsion and vehicle groups, with values comparable to sham controls (p>0.05 vs. Sham). Statistical analysis was performed using one-way analysis of variance with Tukey's post hoc test or non-parametric equivalents where appropriate.

Table 2. Distribution of sperm motility subtypes (%)

Group	Fast progressive (A) (%)	Slow progressive (B) (%)	Non-progressive (C) (%)	Immotile (D) (%)	Total motility (%)
Sham	53	18	14	15	71
Torsion	0	0	22	78	22
Vehicle	0	0	18	82	18
Mitochondria	35	21	17	27	73

Table 3. Post hoc pairwise comparisons of key parameters

Comparison	Johnsen score p	Caspase-3 p	Total sperm p-value	Motile sperm p
TT versus TV	0.042	0.310	0.21	0.18
TT versus TM	<0.001	<0.01	<0.001	<0.001
TV versus TM	<0.001	<0.01	<0.001	<0.001
TT versus Sham	0.006	<0.01	<0.001	0.003
TM versus Sham	0.688	0.770	0.15	0.03

Pairwise comparisons were conducted using Tukey's post hoc or Mann–Whitney U tests as ap-propriate. The significance threshold was set at p<0.05. TT: Torsion, TV: Torsion+Vehicle, TM: Torsion+Mitochondria.

DISCUSSION

The present study demonstrates that intratesticular mitochondrial transplantation significantly preserves spermatogenic activity and reduces apoptotic cell injury in a rat model of TTD. Animals receiving mitochondria before torsion exhibited higher Johnsen scores, improved sperm motility, and reduced Caspase-3 expression compared with untreated torsion and vehicle groups. These findings indicate that exogenous mitochondria can exert a potent cytoprotective and anti-apoptotic effect in I/R injury, contributing to the preservation of testicular function.

I/R injury following testicular torsion remains one of the most destructive events in reproductive physiology. The pathogenesis involves rapid generation of ROS, mitochondrial depolarization, and activation of intrinsic apoptotic pathways within Sertoli and germ cells.[2] Mitochondria play a pivotal role in maintaining testicular energy homeostasis through oxidative phosphorylation and regulation of calcium flux. Therefore, mitochondrial dysfunction following torsion may directly compromise spermatogenesis and Leydig cell steroidogenesis. The current study was designed to evaluate whether targeted mitochondrial transplantation could restore bioenergetic balance and mitigate apoptotic cell death after TTD injury. Mitochondrial delivery was intentionally performed before torsion induction to evaluate its prophylactic cytoprotective capacity and to isolate mitochondrial effects from reperfusion-related confounders. Although pretreatment does not directly reproduce the clinical scenario of testicular torsion, this experimental design has been widely used in previous studies investigating the mechanisms of I/R injury and the protective effects of pharmacological agents. Pretreatment with compounds such as ternatin, L-alanyl-glutamine, and α-lipoic acid has been shown to attenuate testicular I/R injury in rat torsion-detorsion models. Consistent with these proof-of-concept studies, our objective was to determine whether transplantation of healthy mitochondria could confer resistance to subsequent ischemic injury. Future investigations should evaluate mitochondrial transplantation administered at the time of detorsion or during early reperfusion to better reflect the clinical management of testicular torsion.[23-25]

Our findings are consistent with those of Ekşi et al.,[26] who reported that experimental I/R injury in rat testes causes severe germ cell loss, tubular atrophy, and elevated oxidative stress markers, including malondialdehyde and nitric oxide. In their study, antioxidant treatment partially attenuated these effects, but structural recovery remained incomplete. Similarly, Chung et al.[23] demonstrated that mitochondrial dysfunction contributes significantly to testicular damage in torsion models, emphasizing the need for therapeutic approaches targeting mitochondrial integrity. Hu et al.[27] further showed that exogenous mitochondrial therapy can improve organ viability in ischemic conditions by stabilizing ATP production and decreasing cytochrome c release. Our results extend these observations by providing direct histological and functional evidence that intratesticular mitochondrial delivery restores

spermatogenic architecture and improves fertility indices.

The observed improvement in Johnsen scores and sperm parameters aligns with the hypothesis that mitochondrial transplantation replenishes the injured germ cell environment with functional organelles capable of restoring oxidative phosphorylation. Several preclinical studies have demonstrated the feasibility of mitochondrial transfer as a therapeutic tool in different ischemic models. For instance, McCully et al.[19] successfully used autologous mitochondrial injection to reduce myocardial infarct size and improve cardiac contractility following ischemia in porcine models. Similarly, Gollihue et al.[28] reported improved neuronal survival and axonal regeneration following mitochondrial transplantation in spinal cord injury. The current study represents, to our knowledge, the first evidence of this approach applied to testicular I/R injury, highlighting its potential translational relevance to male reproductive preservation.

Apoptosis plays a central role in testicular degeneration following torsion. Our immunohistochemical findings demonstrate a marked reduction in Caspase-3 expression in the mitochondria-treated group compared with untreated controls. This observation suggests that exogenous mitochondria may inhibit the intrinsic apoptotic pathway, possibly through restoration of mitochondrial membrane potential and suppression of cytochrome c release. Previous research has established that torsion induces mitochondrial swelling, loss of cristae, and depolarization of the inner membrane, leading to caspase activation and germ cell apoptosis.[3,17,21] The introduction of healthy mitochondria likely counteracts this process by restoring electron transport chain function and scavenging free radicals.

Another key observation in our study was the significant improvement in sperm motility and total count in the mitochondria-treated animals. The epididymal spermiogram revealed that mitochondrial therapy preserved both quantitative and qualitative sperm parameters, indicating functional recovery beyond mere histological preservation. This is particularly important because motile sperm production reflects the cumulative health of germ cells, Sertoli cell metabolism, and epididymal maturation. Wei et al.[4] also reported a strong correlation between oxidative stress markers and sperm motility decline following torsion, further supporting the hypothesis that mitochondrial damage underlies post-torsion subfertility.

The improved outcomes observed in our model can also be attributed to the metabolic support provided by the transplanted mitochondria. Exogenous mitochondria are capable of transiently integrating into host cells, restoring local ATP concentrations, and stabilizing membrane potential.[27] Studies in hepatic and renal ischemia models have demonstrated that transplanted mitochondria can maintain bioenergetic stability and reduce necroptosis through direct interaction with host organelles.[12,13] Although the persistence of transplanted mitochondria within testicular cells remains to be fully elucidated, previous fluorescence-tracking studies have confirmed mitochondrial uptake by parenchymal cells within hours after administration.

Our study differs from previous approaches primarily in the route and timing of mitochondrial delivery. While the recent studies performed systemic mitochondrial infusion, which may limit local concentration at the injury site,[10,11] our intratesticular injection ensured direct parenchymal distribution and immediate availability of functional organelles before ischemic insult. This strategy contributed to the pronounced preservation of seminiferous architecture observed in histological analyses. Moreover, the use of freshly isolated mitochondria from skeletal muscle – a metabolically robust source – enhanced the likelihood of transplant viability and functional competence.

However, our study has several limitations. The sample size, while statistically adequate for large effect detection, remains relatively small, reflecting ethical constraints inherent to animal research. Furthermore, long-term outcomes such as hormonal profiles, fertility rates, and offspring viability were not evaluated. Future studies should include assessments of mitochondrial integration, lifespan, and genetic stability within recipient testicular tissue, potentially using labeled or fluorescently tagged organelles. In addition, while Caspase-3 expression serves as a reliable apoptotic marker, inclusion of complementary assays such as TUNEL or Western blotting for Bax/Bcl-2 ratio would strengthen mechanistic conclusions. Another limitation is that this study utilized allogeneic mitochondria derived from skeletal muscle, which may elicit mild immunologic responses. However, no inflammatory or fibrotic changes were histologically evident in our specimens, suggesting acceptable biocompatibility. Future investigations may explore the use of autologous mitochondria isolated from less ischemia-prone tissues such as adipose or buccal mucosa, which could enhance translational applicability. Although a full spermatogenic cycle in rats lasts approximately 50 days, early spermatogenic disruption and recovery markers, including Johnsen score and epididymal sperm parameters, have been shown to reflect acute testicular injury and functional trends in short-term torsion models.

A limitation of the present study is that the cellular localization of transplanted mitochondria within the testis was not evaluated. Although our previous studies demonstrated efficient cellular uptake of exogenous mitochondria in kidney and liver tissues.[29,30] whether transplanted mitochondria are internalized by Sertoli, Leydig, or germ cells in the testis remains to be elucidated. Future studies using fluorescently labeled mitochondria together with cell-specific markers will be required to define their cellular fate following intratesticular administration.

Clinically, our results support the potential of mitochondrial therapy as an adjunctive strategy in testicular torsion, especially in cases where surgical detorsion alone may not fully

restore spermatogenic function. Given the narrow therapeutic window after torsion onset, rapid mitochondrial delivery at the time of surgical exploration could serve as a feasible intervention to preserve fertility potential. Advances in organelle isolation and storage technology may further facilitate bedside applications of mitochondrial transplantation.

In addition to testicular torsion, the implications of this approach may extend to other urological and andrological pathologies involving oxidative stress and mitochondrial injury, such as varicocele, cryptorchidism, and chemotherapy-induced gonadotoxicity. Indeed, Costa et al.[8] emphasized that mitochondria-targeted therapeutics might represent a unifying strategy for conditions characterized by energy imbalance and apoptotic dysregulation in male reproductive tissues.

CONCLUSION

Our findings suggest that intratesticular mitochondrial transplantation may offer structural and functional protection against I/R injury. The observed increase in Johnsen score, reduction in Caspase-3 expression, and improvement in sperm motility indicate a potential beneficial effect of this approach in preserving testicular viability. However, to validate these preliminary results and support clinical translation, larger prospective randomized studies incorporating mitochondrial tracking, molecular imaging, and long-term fertility outcomes are needed.

Acknowledgment: The authors would like to thank Saki Yenilli Experimental Animals Production and Research Laboratory for their technical support during the experimental phase of this study.

Ethics Committee Approval: All experimental procedures were approved by the Saki Yenilli Experimental Animals Production and Research Laboratory Local Ethics Committee (Date: 02.12.2022, Decision No: 14).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: C.Ö., G.B.K., S.G., Ü.Ö.; Design: A.S., U.D.M., M.S., C.Ö.; Supervision: G.B.K., A.M.A., G.Ş., M.M.A.; Resource: C.Ö., G.B.K., S.G., Ü.Ö.; Materials: A.S., U.D.M., M.S., M.M.A.; Data collection and/or processing: G.S.K., A.M.A., G.Ş., M.M.A.; Analysis and/or interpretation: C.Ö., G.B.K., S.G., Ü.Ö., U.D.M.; Literature review: S.G., C.Ö., G.S.K., M.M.A.; Writing: A.S., U.D.M., M.S., A.M.A.; Critical review: G.B.K., G.Ş., C.Ö., S.G., Ü.Ö.

Informed Consent: As this study involves only experimental animals, informed consent is not required.

Conflict of Interest: The authors declare that they have no conflicts of interest.

Financial Disclosure: This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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DENEYSEL ÇALIŞMA - *ÖZ*

Mitokondriyal transplantasyon, sıçanlarda testis torsiyonu-detorsiyonu yaralanması sonrası apoptozu azaltıcı ve spermatogenez fonksiyonunu iyileştirici etkisi

AMAÇ: Testis torsiyonu, zamanında cerrahi detorsiyon yapılmasına rağmen geri dönüşümsüz iskemi-reperfüzyon hasarına ve spermatogenez fonksiyonunda uzun süreli bozulmaya yol açabilen bir ürolojik acil durumdur. Mitokondriyal disfonksiyon, torsiyon sonrası oksidatif stresin neden olduğu germ hücresi hasarında merkezi bir rol oynar. Bu çalışma, testis torsiyonu-detorsiyon hasarı olan sıçan modelinde izole intratestiküler mitokondriyal transplantasyonun koruyucu etkilerini araştırmayı amaçlamıştır.

GEREÇ VE YÖNTEM: Otuz altı yetişkin erkek Sprague–Dawley sıçanı rastgele dört gruba ayrıldı: sham (n=4), torsiyon (n=10), torsiyon+araç (n=10) ve torsiyon+mitokondri (n=10). Testis torsiyonu, 2 saat boyunca 720° rotasyon ile indüklendi, ardından cerrahi detorsiyon uygulandı. Torsiyon indüksiyonundan önce izole edilmiş mitokondriler intratestiküler enjeksiyon yoluyla uygulandı. Histopatolojik değerlendirme hematoksilen-eozin boyaması kullanılarak yapıldı ve Johnsen skorlama sistemi ile ölçüldü. Apoptotik aktivite, Caspase-3 immünohistokimyası ile değerlendirildi. Fonksiyonel sonuçlar, toplam sperm sayısı, hareketli sperm sayısı ve ayrıntılı sperm hareketliliği alt tip analizi ile değerlendirildi.

BULGULAR: Mitokondri tedavisi uygulanan sıçanlar, tedavi uygulanmayan kontrol grubuna kıyasla önemli ölçüde daha yüksek Johnsen skorları (9.7±0.4'e karşı 6.4±1.0, p<0.001), azalmış Caspase-3 ekspresyonu ve iyileşmiş toplam ve hareketli sperm sayıları sergilemiştir. Sonuçlar, mitokondriyal tedavi sonrasında testis yapısının ve spermatogenik fonksiyonun korunduğunu göstermektedir. Mitokondri ile tedavi edilen sıçanlar, tedavi edilmeyen iskemik kontrol grubuna kıyasla önemli ölçüde daha yüksek Johnsen skorları, azalmış Caspase-3 ekspresyonu, korunmuş sperm konsantrasyonu ve morfolojisi ve ilerleyici sperm hareketliliğinin geri kazanıldığını göstermiştir.

SONUÇ: Mitokondriyal transplantasyon, testis torsiyonuna bağlı iskemi-reperfüzyon hasarında hem testiküler yapıyı hem de spermatogenik fonksiyonu anlamlı düzeyde korumuştur. Mitokondri ile tedavi edilen grupta sperm konsantrasyonu, morfolojisi ve özellikle ilerleyici sperm hareketliliğinin korunması, bu yaklaşımın fertiliteyi sürdürmede potansiyel bir organel temelli tedavi stratejisi olabileceğini göstermektedir.

Anahtar sözcükler: Apoptoz; iskemi-reperfüzyon hasarı; mitokondriyal transplantasyon; spermatogenez; testis torsiyonu.

Ulus Travma Acil Cerrahi Derg 2026;32(10):1171-1179 DOI: 10.14744/tjtes.2026.02503