Pediatr Surg Int (2010) 26:287–292
DOI 10.1007/s00383-009-2533-5

ORIGINAL ARTICLE

# The morphological evaluation of ipsilateral and contralateral vasa deferentia in a rat model of unilateral spermatic cord torsion

**Arzu Pampal · Ibrahim Onur Ozen · Gulsen Ekingen · Billur Demirogullari · Fatma Helvacioglu · Gulnur Take**

Accepted: 29 October 2009 / Published online: 18 November 2009


**Abstract**
*Aim* Spermatic cord torsion is a surgical emergency that requires early intervention to protect the effected testicle. The literature review about this ischemic reperfusion (I/R) injury reveals not only ipsilateral, but also contralateral testicular and epididymal injuries in a broad fashion. However, there is no data about vas deferens injury related with this surgical emergency. The aim of the study is to evaluate the morphological changes of the vas deferens due to testicular I/R injury.
*Materials and methods* Eighteen Wistar-Albino rats were allocated to three groups. Bilateral vasa deferentia of control group (Gr C, $n = 6$) were harvested without any surgical intervention. The torsion group was subjected to 2 h torsion and 2 h detorsion of the left testicle (Gr T, $n = 6$) and the third group underwent sham operations (Gr S, $n = 6$). Bilateral vasa deferentia of Gr T and S were harvested after surgery. The either side of the vas deferens was divided into three equal segments and these regions (adjacent to urinary bladder, medial and adjacent to testicle) were evaluated histopathologically.
*Results* The electron microscopic evaluation of bilateral vasa deferentia of Gr T revealed different degrees of degeneration on either side. The region adjacent to testicle of the contralateral vas deferens was the most effected segment when compared with the other segments.
*Conclusion* In the light of these findings, it can be said that testicular I/R injury effects not only testis and epididymis, but also the adjacent vas deferens. This effect seems to be bilateral, like the testis and epididymis injury. Moreover, it mostly seems to depend on the apoptotic processes.

A. Pampal (✉)
Department of Pediatric Surgery, Faculty of Medicine,
Ufuk University, Ankara, Turkey
e-mail: ademirtola@yahoo.com

I. O. Ozen · B. Demirogullari
Department of Pediatric Surgery, Faculty of Medicine,
Gazi University, Ankara, Turkey

G. Ekingen
Department of Pediatric Surgery,
Faculty of Medicine, Kocaeli University, Kocaeli, Turkey

F. Helvacioglu · G. Take
Department of Histology and Embryology,
Faculty of Medicine, Gazi University, Ankara, Turkey

## Introduction

Spermatic cord torsion is a surgical emergency commonly encountered in newborn and prepubertal boys. The appropriate intervention is to untwist the torsed testicle as soon as possible. As the consequences of the disease can be an orchiectomy due to the injury of ipsilateral (torsed) side or a potential infertility due to the injury of the contralateral (untorsed) side, this intervention is not only important for the effected testis, but also for the contralateral one [1].

Torsion of the spermatic cord causes a reduction in the testicular flow and leads to testicular ischemia on the effected side. The damage seen in ipsilateral testis is related to hypoxia due to ischemia and over generation of reactive oxygen and nitrogen species due to reperfusion. Even though each vas deferens is supplied independently by its own artery, unilateral torsion of the spermatic cord can lead a reduction in the contralateral blood flow [2]. It was thought to be related to a reflex to afferent stimuli. Not only the decreased blood flow, but also the formed sperm antibodies in response to testicular damage in the ipsilateral

side, or activated neurohumoral mechanisms are the blamed mechanisms for the damage of the contralateral side [3, 4]. However, the exact mechanism of contralateral injury is obscure.

The literature review about spermatic cord torsion reveals not only ipsilateral, but also contralateral testicular and epididymal injuries in a broad fashion. However, there is no data about vas deferens injury related to this surgical emergency. The aim of the study is to evaluate the morphological changes of the vas deferens due to testicular ischemic reperfusion (I/R) injury.

## Materials and methods

The study protocol was approved by the Animal Ethics Committee and performed according to the guidelines of the Research Committee of Gazi University Faculty of Medicine. The study comprised 18 male Wistar-Albino rats weighing 300–350 g. All animals were kept under controlled temperature (21 ± 2°C) and humidity (55.5%) with 14-h light and 10-h dark cycle. They were fed with standard rat chaw and free access to water. There were no water and light restrictions throughout the experiment. All animals received human care in compliance with "Principles of Laboratory Animal Care" formulated by National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health. All surgical procedures were performed by the same surgeon in the sterile conditions. Every surgical intervention was performed under 80 mg $kg^{-1}$ ketamine hydrochloride (Ketalar, Eczacibasi, Turkey) and 10 mg $kg^{-1}$ xylazine hydrochloride (Alfazyne, Ege Vet, Turkey) anesthesia.

Eighteen prepubertal rats were randomly allocated to three groups. Bilateral vasa deferentia of control group (Gr C) were harvested through a midline laparotomy incision without any surgical intervention. The experimental spermatic cord torsion was applied to the half of the rest of the animals (Gr T). After a longitudinal scrotal incision, both testicles were observed. The left testicle was dissected carefully by the surrounding fat and connective tissue and a clockwise extravaginal torsion of 720° was applied. The left testicle was fixed to the left scrotal wall with a 5/0 silk suture and the incision was stitched. After 2 h of torsion, the incision was re-entered and the testicle was untwisted and left in its original place for 2 h. After a period of 2 h torsion/2 h detorsion, the animals were subjected to laparotomy and either side of vas deferens was harvested. For the last group, after a longitudinal scrotal incision the left testicle was dissected from surrounding and was fixed to the left scrotal wall in its neutral position. The vas deferens of either side was harvested through a laparotomy after a 4-h interval (Gr S). After the removal of vasa deferentia, all rats were killed. Both ipsilateral and contralateral sides of vasa deferentia of three groups were divided into three equal segments (adjacent to urinary bladder, medial and adjacent to testicle). These three equal segments were evaluated under transmission electron microscopes by two blinded histologists and photographed.

### Histological examination

Tissues of all groups were fixed in phosphate buffered (pH 7.3) containing 2.5% glutaraldehyde (Sigma-Aldrich Co.) for 2 h at room temperature, post-fixed in 1% osmium tetraoxide (Sigma-Aldrich Co.) and dehydrated in a series of graded alcohols (50, 60, 70, 80, 90 and 100% ethanol). After passing through propylene oxide (Sigma-Aldrich Co.), the specimens were embedded in Araldite CY 212 (Ciba-Geigy), (2-dodecen-1-yl) succinic anhydride (Sigma-Aldrich Co.), benzyldimethyl amine (Poly Sciences Inc.) and dibutylphtalate (Sigma-Aldrich Co.). The semi-thin sections were stained with toluidine blue (Sigma-Aldrich Co.) and examined with a photomicroscope (BH2 Olympus, Japan). After the selection of appropriate specimens, thin sections were cut and stained with uranyl acetate (ProSciTech) and lead citrate (Sigma-Aldrich Co.). They were examined by means of an electron microscope by two blinded histologists (Carl Zeiss EM 900, Germany).

## Results

In the sham group (Gr S), alike with control group (Gr C), no evidence of histological changes was observed in all three regions of the either side.

### Left-sided vas deferens

The evaluation of the region adjacent to the left testis revealed vacuole formation and euchromatic nucleus within epithelial cytoplasm. The neutrophil predominance in the vascular lumen was thought to be related to a defense to stress. The nuclei of the smooth muscles of this region apart from the other regions were found to have dense chromatin and edema at the peripheral cytoplasm was identified in these cells (Fig. 1a, b).

The evaluation of the region adjacent to the urinary bladder revealed additional epithelial cells with electron dense cytoplasm, picnotic nuclei, numerous vacuoles and dilated GER (Fig. 2a). These cells with electron dense cytoplasm were apoptotic cells. The existence of giant neutrophils was marked even in the narrow vessels. Infiltration of eosinophils with unique granules in the muscle

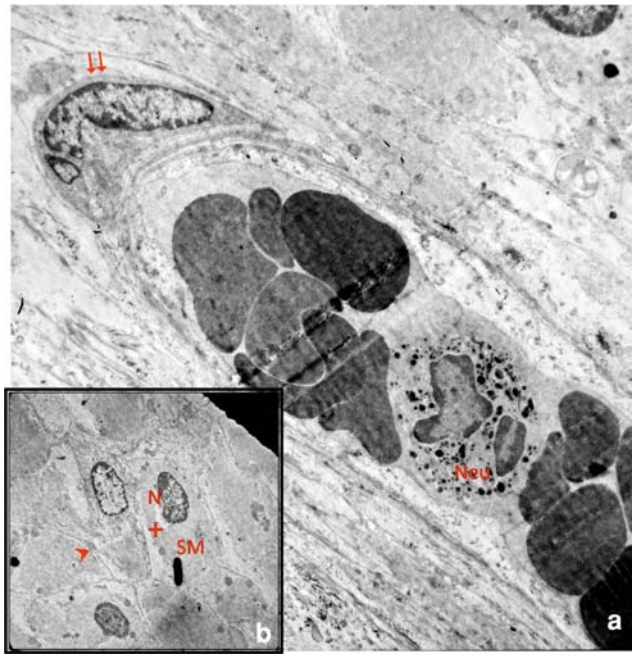


**Fig. 1** **a**, **b** Neutrophils (*Neu*) within the vessels (⇉). Small numbers of pinocytotic vesicles (*Ve*) in the endothelium cytoplasm (Fig. 1a). Typical junctional complexes (➤) in the smooth muscles (*SM*). Peripheral edema (+) in the cells with chromatin dense nuclei (*N*) (Fig. 1b)

layers was specific to this group. The eosinophil infiltration through the muscle layers were thought to be against to increased degeneration and stress (Fig. 2b, c).

The evaluation of the medial region revealed erasing of the cilia of the epithelium and prominent vacuole formation in the apical cytoplasm. Vacuoles were found dispersed within the cytoplasm of all cell kinds and the vacuole formation in the epithelium was marked in this region when compared with other groups. The aforementioned cells with electron dense cytoplasm with undistinguished cytoplasmic material were found in this region, too. The cytoplasmic material of these electron dense cells could not be distinguished. All prismatic cells were found to be edematous. Peri capillary edema and degenerative apical membrane structures were identified (Table 1).

## Right-sided vas deferens

The evaluation of the region adjacent to right testis revealed prominent prismatic epithelium with edema and patchy vacuoles (Fig. 3a). The vascular endothelium and smooth muscle cells were found to contain vacuoles, specific to this group (Fig. 3b).

The evaluation of the region adjacent to urinary bladder revealed a group of cylindrical epithelial cells with electron dense cytoplasm and both small and large vacuoles within the cytoplasm. The myelin figures in vacuoles were formed

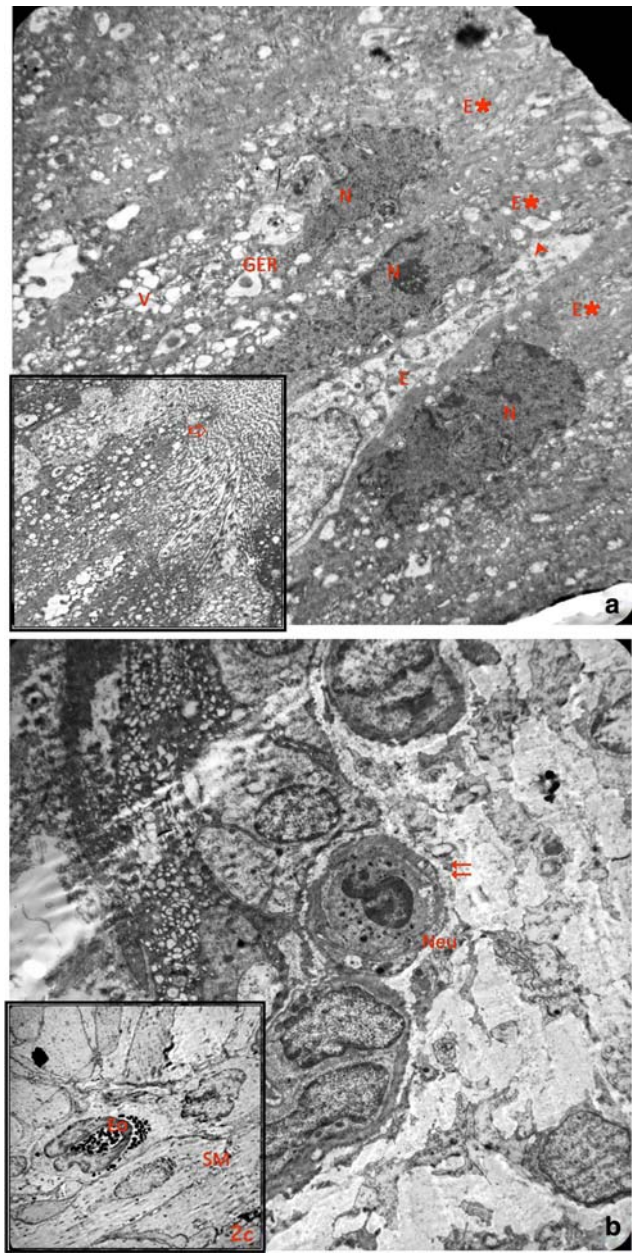


**Fig. 2** **a**–**c** Electron dense apoptotic cells (*E*✱) with picnotic nuclei (N). Separated junctional complexes (➤). Normal cilia (⇨) structure, dilated GER tubuli and numerous vacuoles (*V*). Basal membranes (*BM*) of electron lucent epithelium cells (*E*) were intact, whereas the integrity of the apical membranes (➔) were found impaired (*inset*) (Fig. 2a). Huge neutrophils (Neu) within the vessels (⇉) (Fig. 2b). Apart from the other groups, eosinophils (*Eo*) with unique granules were detected among the smooth muscle cells (Fig. 2c)

in a group of cell. These electron dense cells are thought to have severe degeneration. Electron lucent cells were found to have less vacuole formation when compared with the other cells. A group of electron lucent cells included autophagic vacuoles. Cells with their chromatin condense nuclei were clear. These findings strongly supported apoptotic pathway (Fig. 4). Connective tissue vessels were

**Table 1** The morphological findings of either side of torsion group

| | Electron dense cytoplasm | Epithelial vacuolization | Endothelial edema | Metaplastic changes in cells | WBC infiltration |
|---|---|---|---|---|---|
| L vas, adjacent to T | + | +[a] | – | – | ++[b] |
| L vas, medial | + | + | ++ | – | – |
| L vas, adjacent to UB | ++ | ++ | – | – | +[b,c] |
| R vas, adjacent to T | – | +++[d] | + | + | – |
| R vas, medial | – | + | + | – | – |
| R vas, adjacent to UB | ++ | + | – | – | ++[b] |

*WBC* white blood cells, *L* left, *R* right, *T* testis, *UB* urinary bladder, + poor, ++ mild, +++ rich

[a] Diffuse intracytoplasmic edema

[b] Infiltration of leucocytes

[c] Infiltration of eosinophils

[d] Myelin figures within vacuoles

found to be dilated when compared with control and sham groups. Neutrophil infiltration was prominent in these vessels.

The evaluation of the medial region revealed surface epithelium cells with euchromatic nuclei and marked nucleoli with minimal vacuole formation. Even edema was seen in a number of cells, the junctional complexes were normal. Minimal hypertrophy and edema was determined in the vascular endothelium (Table 1).

## Discussion

The aim of the study was to evaluate the morphological changes of the vas deferens due to testicular I/R injury. We found that the 2 h detorsion following 2 h torsion resulted variable degrees of degeneration in either side of the vas deferens. The most effected regions of either side were found to be the regions adjacent to the testicles, contralateral side being more damaged than ipsilateral side. The influence of testicular I/R injury on vas deferens seems to be bilateral like testis and epididymis and depends mostly on the apoptotic processes.

The effects of testicular I/R injury on either testis are well documented in the literature. Even though the presence of some earlier reports about no injury related to testicular torsion/detorsion [5], the general acceptance is that of bilateral injuries during this I/R process. However, the exact mechanism of the bilateral injury is obscure. It is accepted that the injury related to I/R injury of the ipsilateral side is due to tissue hypoxia, over generation of reactive oxygen and nitrogen spices and necrosis of germinal cells [6, 7]. In terms of the contralateral injury, there are some asserted hypotheses. In two different studies, it was found that the contralateral testicular blood flow, but not the testicular oxygenization decreased during testicular torsion [2, 8]. Even though each vas deferens is supplied by its own artery derived from superior vesical artery, this finding was explained by a reflex to afferent stimuli [2]. However, the decreased blood flow does not seem to be related to a classic cell injury by oxidative free radicals [9]. Probable neural or vascular responses bring forth such tissue damages of the contralateral side. There are some data in the literature suggesting contralateral testicular damage related to immune mechanisms, release of acrosomal enzymes or neurohumoral mechanisms [4, 10].

The effects of testicular I/R injury on either epididymis are also studied in the literature. [11, 12] The ipsilateral damage was shown to be related to either over generation of free-oxygen radicals or inflammatory processes. Contralateral damage was found to depend mostly on the apoptosis due to I/R injury.

Duration <1 h of 720° testicular torsion in rat model is proved to be not adequate to demonstrate the permanent spermatogenesis disruption in the ipsilateral testis [13]. In the light of these findings, to evaluate a damage of the vas deferens due to testicular I/R injury, a protocol of 2 h ischemia following 2 h reperfusion was considered and we found that 2 h reperfusion following 2 h ischemia affected bilateral vasa deferentia morphologically in a broad fashion, being diverse from the suggestions of different authors about the duration of testis injury [14, 15.] The cause of this early impairment might be the reason for the limited vascular supply of the vas deferens when compared with testis.

The vas deferens is a muscular tube that contracts unidirectional during ejaculation to emit the seminal fluid to ejaculatory duct. This contractile activity is biphasic in the rodents and mediated by autonomic nervous system. The first phase is a twitch-like contraction mediated by the synaptic release of adenosine 5′-triphosphate and the second phase is a tonic contraction mediated by the synaptic

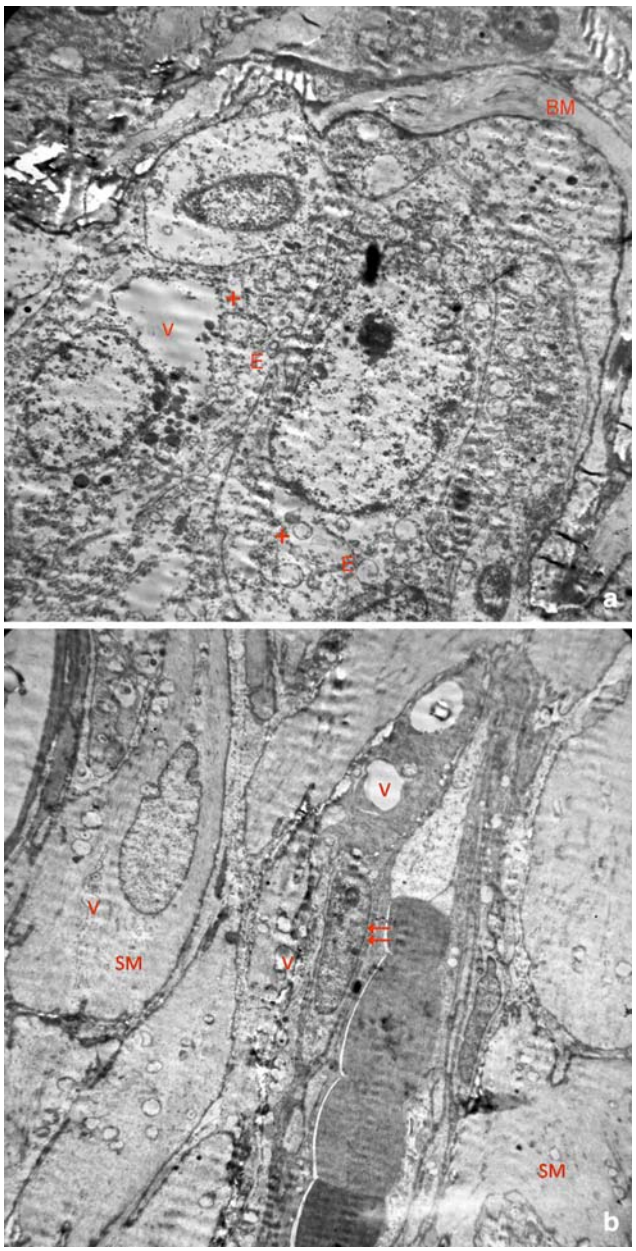


**Fig. 3** **a**, **b** Edema (+) and patchy irregular vacuoles (*V*) in the epithelium (*E*). Normal basal cells (*B*) and basal membrane (*BM*) (Fig. 3a). Huge vacuoles (*V*) in the vascular endothelium (⇉) and smooth muscle cells (*SM*) (Fig. 3a, b)

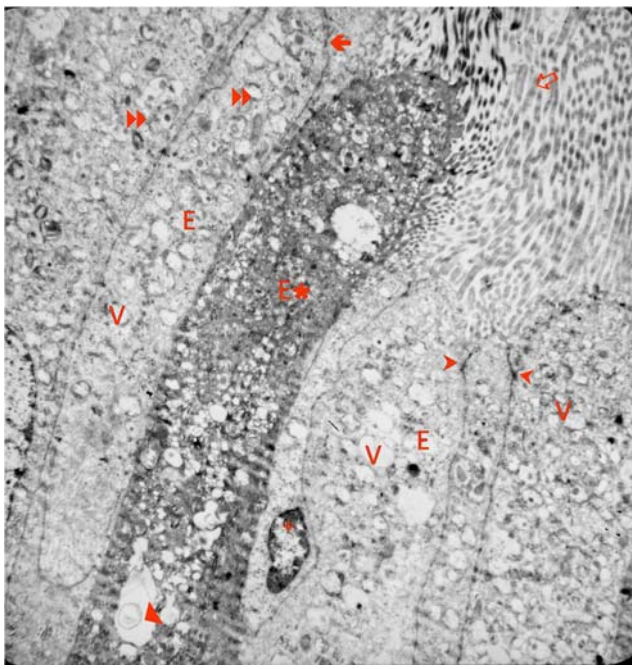


**Fig. 4** Surface epithelium (*E*), cilia structure (⇨), junctional complexes (➤), autophagic vacuoles (⏭) and differentiation at the apical surfaces (➔) in some electron lucent epithelium cells (*E*). Both large and small vacuoles (*V*) and myelin figures (◗) in electron dense cells (*E*✱). The epithelial cells were converted to apoptotic cells

release of noradrenaline. In two different studies, evaluating the motility of vas deferens, Onur et al. and Barun et al. studied the effects of two antioxidant agents on contractile responses of vas deferens following unilateral testis torsion/detorsion [16, 17]. However, their findings related to the vas deferens impairment do not correlate, the reason why is the methodology of either study. Each researcher has chosen separate species to evaluate the vas deferens motility. Onur et al. demonstrated that the unilateral testis I/R injury caused a significant inhibition in both phases of contractile activity of either vas deferens. These effects, however, seemed to be prejunctional, they also showed that KCl-induced vas deferens contractions were found to be decreased in the ipsilateral side, suggesting a post-junctional damage. Similarly, Barun et al. revealed a significant reduction in both phases of contractile activity of the ipsilateral side in the early and late stages of the reperfusion. Apart from the aforementioned study, Barun et al. demonstrated a significant inhibition on the second phase of contractile activity on the contralateral side in either stages of reperfusion and stated this inhibition was due to post-junctional damage. Moreover, they interconnected this altered contraction to the humoral factors released from the ipsilateral side as a result of I/R injury. However, it is emphasized by the authors that the way of interaction of these tissues in the same neighborhood (testis and adjacent vas deferens) might not be comparable. The presented morphological changes due to apoptosis in our study can be the reason for the reduction in the postsynaptic receptors or decreased receptor sensitivity and that might be the cause of altered contractions of the either vas deferens after unilateral torsion in the abovementioned studies by Ozen et al. and Barun et al.

The findings of our study demonstrate the morphological changes of the post-junctional area, and these morphological changes might be the reason for the affected motility of the vasa deferentia. As the unilateral spermatic

cord torsion is accepted to lead to infertility due to the damage of the contralateral side, this infertility can be the consequence of not only testicular and/or epididymal injury, but also the consequence of the altered vas motility due to vas deferens injury after testicular I/R. Owing to our histological findings, it is obvious that apoptosis is the main reason for these changes, and the I/R injury seems to lead to this apoptotic process. As bilateral vasa deferentia were evaluated under electron microscopy all the way through, we had no chance to evaluate the tissues in another manner. It is probable that evaluating the nature of this damage will be the subject for other studies and will give us enlightenment about the changes in the surrounding tissue due to testicular I/R injury.

## References


1. Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A (2004) Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia–reperfusion injury. Mol Aspects Med 25(1–2):199–210
2. Tanyel FC, Büyükpamukçu N, Hiçsönmez A (1989) Contralateral testicular blood flow during unilateral testicular torsion. Br J Urol 63(5):522–524
3. Rodriguez MG, Rival C, Theas MS, Lustig L (2006) Immunohistopathology of the contralateral testis of rats undergoing experimental torsion of the spermatic cord. Asian J Androl 8(5):576–583
4. Wallace DM, Gunter PA, Landon GV, Pugh RC, Hendry WF (1982) Sympathetic orchiopathia: an experimental and clinical study. Br J Urol 54(6):765–768
5. Becker EJ Jr, Turner TT (1995) Endocrine and exocrine effects of testicular torsion in the prepubertal and adult rat. J Androl 16(4):342–351
6. Akgür FM, Kilinç K, Aktuğ T (1993) Reperfusion injury after detorsion of unilateral testicular torsion. Urol Res 21(6):395–399
7. Sarica K, Küpeli B, Budak M, Koşar A, Kavukçu M, Durak I, Göğüş O (1997) Influence of experimental spermatic cord torsion on the contralateral testis in rats: evaluation of tissue free oxygen radical scavenger enzyme levels. Urol Int 58(4):208–212
8. Klotz T, Vorreuther R, Heidenreich A, Zumbé J, Engelmann U (1996) Testicular tissue oxygen pressure. J Urol 155(4):1488–1491
9. Blank ML, O'Neill PJ, Steigman CK, Cobb LM, Wilde RA, Havenstein PJ, Chaudry IH (1993) Reperfusion injury following testicular torsion and detorsion in prepubertal rats. Urol Res 21(6):389–393
10. York JP, Drago JR (1985) Torsion and the contralateral testicle. J Urol 133(2):294–297
11. Oh SJ, Kwak C, Baek M, Kim CS, Kim KS, Choi H (2004) Histologic and molecular changes in the ipsilateral and contralateral epididymides of the rat in response to unilateral testicular torsion followed by detorsion. Fertil Steril 81(Suppl 1):882–887
12. Ozturk H, Ozturk H, Dokucu AI (2008) The role of cell adhesion molecules in ischemic epididymal injury. Int Urol Nephrol 40(1):137–142
13. Turner TT, Brown KJ (1993) Spermatic cord torsion: loss of spermatogenesis despite return of blood flow. Biol Reprod 49(2):401–407
14. Cosentino MJ, Nishida M, Rabinowitz R, Cockett AT (1986) Histopathology of prepubertal rat testes subjected to various durations of spermatic cord torsion. J Androl 7(1):23–31
15. Koc A, Narci A, Duru M, Gergerlioglu HS, Akaydin Y, Sogut S (2005) The protective role of erdosteine on testicular tissue after testicular torsion and detorsion. Mol Cell Biochem 280(1–2):193–199
16. Ozen IO, Vural IM, Moralioğlu S, Barun S, Ercan ZS, Sarioğlu Y (2006) Effects of mexiletine on electrical field stimulation-induced contractile responses in the ipsilateral and contralateral vasa deferentia after unilateral testicular torsion/detorsion. Eur Surg Res 38(4):423–429
17. Barun S, Ekingen G, Mert Vural I, Türkyilmaz Z, Başaklar C, Kale N, Sevim Ercan Z, Sarioğlu Y (2005) The effects of melatonin on electrical field stimulation-evoked biphasic twitch responses in the ipsilateral and contralateral rat vasa deferentia after unilateral testicular torsion/detorsion. Naunyn Schmiedebergs Arch Pharmacol 371(5):351–358