# Effect of tunica albuginea incision on testicular tissue in testicular torsion

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# ABSTRACT

**BACKGROUND:** Testicular torsion is the underlying cause in 15–20% of children presenting to the emergency department with scrotal pain. Testicular torsion, which is defined as the rotation of the testis around itself at the level of the spermatic cord, may cause organ loss due to impaired circulation. It is recommended to detortion the testis within the first 6 h to prevent organ loss. The aim of the study is to examine the effect of Tunica Albuginea incision (TAI) made in addition to detorsion (DT) on the viability of the torted testis.

**METHODS:** The research has an experimental design. The study was carried out on three groups of rats (Sham, testicular torsion-(T-DT), TAI, and testicular torsion-detortion. After 10 days of follow-up, testes in the groups were taken for pathological examination. In the pathological examination, necrosis, ischemic changes, Johnsen score, edema, inflammation, and basement membrane thickening were evaluated and scored in all pathological samples.

**RESULTS:** Necrosis, ischemic changes, edema, inflammation, and basal membrane thickening were found to be significantly less in the TAI group. Furthermore, the mean Johnsen Scores were significantly different between the T-DT (7.44±0.52) and TAI (8.60±0.51) groups.

**CONCLUSION:** The results showed that the testicles in the TAI group were better preserved than the testicles, in which only DT was applied. It can be argued that in testicular torsion, the DT procedure is not sufficient to protect the testis, and it would be beneficial to add TAI to the procedure.

Keywords: Child; testicular torsion; tunica albuginea incision.

# **INTRODUCTION**

Testicular torsion is the underlying cause in 15–20% of children presenting to the emergency department with scrotal pain. Testicular torsion, which is defined as the rotation of the testicle around itself at the level of the spermatic cord, may cause organ loss due to impaired circulation. It is recommended to detortion the testicle within the first 6 h to prevent organ loss.<sup>[1]</sup> After detorsion (DT), irreversible cellular damage may occur due to edema and increased intratesticular pressure due to reperfusion damage.<sup>[2]</sup> An increase in intratesticlecular pressure causes compartment syndrome, and even if DT is performed, the pressure do not be reduced and cellular damage may develop.<sup>[3]</sup>

The duration and number of torsion are vital for the testicle to maintain its vitality, but a different approach is needed to eliminate the compartment syndrome and to normalize the circulation of the testicle in detorted testicles.

In this study, the effect of the tunica albuginea incision (TAI) applied after DT in torsioned testicles will be examined in preserving the vitality of the testicle.

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The research hypothesis is as follows; testicle with detorsion and tunica albuginea incision (DT + TAI) has less cellular damage and preserves testicular vitality more than only DT testicle.

# MATERIALS AND METHODS

The research has an experimental design. The study was carried out on three groups of rats. Rats were obtained from Balıkesir University, Experimental Animals Research Center. Thirty male Wistar Albino rats with a body weight of 230– 280 g were included in the study. The study was conducted on three groups (Fig. 1).

All surgical procedures were performed under sterile conditions with intraperitoneal anesthesia (ketamine 75 mg/kg, xylazine 10 mg/kg). After the surgical procedure, the rats continued to be fed orally.

1. Group: Sham group. The same anesthesia was applied to the rats in this group. An incision was made in the right scrotum, the incision was sutured and monitored.

2. Group: Testicular torsion group (T-DT): Right testicle of rats was torsioned 720 degrees clockwise and fixed to the scrotum. After a waiting period of 3 h, the same testicle was detorsioned and placed back into the scrotum.

3. Group: In addition to testicular torsion, TAI was made (TAI): Right testicle of rats was torsioned 720 degrees clockwise and fixed to the scrotum. After a waiting period of 3 h, the right testicle was detorsioned and a single cranial to caudal incision was made in the tunica albuginea of the right testicle and placed back into the scrotum. A full-thickness TAI was performed by a single surgeon. The incision was deepened until the underlying seminiferous tubules pro-truded.

After 10 days of follow-up, the rats were decapitated and testicles in all study groups were taken for pathological examination. Pathological examination was performed blindly by a single pathologist. In examination; necrosis, ischemic changes, Johnsen tubular biopsy score (JTBS), and edema in testicular tissues were evaluated and scored.<sup>[4–7]</sup> The scoring of inflammation and basement membrane thickening was used for the 1<sup>st</sup> time in this study, unlike the literature. Scoring is expalained as follows;

- Pathological scoring for necrosis 0 = normal (no necrosis), 1 = minimal (<5%), 2 = mild (5-25%), 3 = moderate (25-75%), and 4 = severe (>75%) used.
- Ischemic changes were evaluated according to the Cosentino scoring system (Grade I = normal, Grade 4 = coagulative necrosis).
- The JTBS has been used to evaluate spermatogenesis after testicular torsion. I–10 with 10 the best score and I the worst score, and mean score values were determined.
- In the evaluation of interstitial edema, 0 = none, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe scoring was used.
- Inflammation was scored as 0 = absent, 1 = minimal, 2 = moderate, and 3 = severe.
- Basal membrane thickening was scored as 0 = no, 1 = minimal, and 2 = mild.

The data were gathered in a database in the SPSS 23.0 program. Statistical analysis was performed on the data of all groups, but only the comparative analyzes of the T-DT and TAI groups are presented in the results section. In the analysis, descriptive statistics were calculated and Fisher exact and Freeman–Halton Fisher exact tests were used as hypothesis tests. In the interpretation of significance,  $\alpha$ :0.05 was accepted.

The research was approved by the BAUN Animal Experiments Ethics Committee (2021/7-1; 25/08/2021) and supported by BAUN BAP (2021/079).



Figure 1. Research groups and process.

# RESULTS

The study was carried out in the planned time, one of the rats in the T-DT group died during the 10-day follow-up period. Therefore, data analysis in T-DT and TAI groups was performed on 19 rats.

The regular seminiferous tubular structure and the normal structure of the basement membrane in the sham group are presented in Figure 2.

There was no or minimal necrosis, less ischemic changes, minimal inflammation, less edema, and less basement membrane thickening in the TAI group compared to the T-DT group. Pathology examination of necrosis and inflammation in the seminiferous tubules in TAI and T-DT groups is presented in Figure 3. Necrosis and inflammation were less in the TAI group. Basal mammary thickening in the seminiferous tubules is presented in Figure 4. Basal membrane thickening was minimal in the TAI group. Comparison of all variables in TAI and



Figure 2. Structure of regular seminiferous tubules (a) and basement membrane in the sham group (×100), H&E (b).



**Figure 3.** Necrosis (blue arrow) and inflammation (yellow arrow) in seminiferous tubules in T-DT (a) and TAI (b) groups (×100), H&E.



Figure 4. Basal membrane thickening in T-DT (a) and TAI (b) groups (×100), H&E.





Figure 5. Differences of all variables between groups.



Figure 6. Johnsen tubular biopsy scores of the groups.

T-DT group is shown in Figure 5. The JTBS was higher in the TAI group than in the T-DT group (Fig. 6). Variables examined in terms of testicular viability in the TAI group were significantly different compared to the T-DT group. Furthermore, the mean Johnsen tubular biopsy scores were significantly different between the T-DT ( $7.44\pm0.52$ ) and TAI ( $8.60\pm0.51$ ) groups (Table 1).

### **DISCUSSION**

In this study, the effect of TAI performed with DT in the torted testicle on preserving the vitality of the testicle was investigated. The findings of our study showed that the testicles in the TAI group were better preserved than the testi-

Table 1. Significance values of all variables according to groups	
Variables	p-value
Necrosis	0.0190
Ischemic change	0.0325
Edema	0.0495
Inflammation	0.0201
Basal membrane thickening	0.0225
Johnsen tubular biopsy scores	0.0008

cles with only DT (T-DT). Thus, the research hypothesis was confirmed.

Testicular edema is an important variable in testicular viability that develops during torsion and DT. The cause of edema is venous occlusion during torsion, and ischemia-reperfusion injury after DT.

Tunica Albuginea is a tough inelastic layer that covers the outside of the testicular tissue. In the presence of edema, Tunica Albuginea does not stretch and does not allow the testicle to expand outward. Thus, edema causes increased intratesticular pressure and compartment syndrome. Another factor that causes tissue damage is oxidative stress.<sup>[8]</sup> Compartment syndrome plays an important role in the ischemia of organs in their natural sheaths.<sup>[1,9]</sup> In animal models, it has been suggested that the increase in testicular pressure due to testicular torsion is a risk factor for spermatogenesis.<sup>[2]</sup> Acute compartment syndrome causes ischemia and subsequent necrosis in the testicular tissue.<sup>[1]</sup>

Figueroa reported that compartment syndrome after DT can be regressed with TAI.<sup>[9]</sup> Nagasawa reports that blood flow in ischemic testicles is increased significantly with TAI. <sup>[3,10]</sup> Moghimian reports that TAI can provide a protective effect for testicle in long-term ischemia in animal models.<sup>[11]</sup> Figueroa and Chu suggest that performing TAI before the decision of orchiectomy in testicles with severe ischemia due to long-term torsion can prevent testicular organ loss. <sup>[9,12]</sup> Figueroa et al.<sup>[9]</sup> reported that with TAI, 54.6% of torted testicles could be salvaged without atrophy.

The time between the onset of symptoms and admission to the hospital is vital. Tissue damage due to torsion begins in the second hour and tissue necrosis occurs at the end of the  $6^{th}$  h.<sup>[1]</sup> Occurrence of necrosis is directly related to testicular rotation angle and torsion time.

In our study, the testicles were torsioned according to the twisting time and number, which is consistent with the literature. Although torsion was applied for <6 h, which is the limit time for tissue necrosis, tissue necrosis and edema were found to be more severe in the T-DT group compared to the TAI group. This shows the importance of the torsion time for the vitality of the testicle.

Despite the improved medical facilities and the increase in health literacy, organ loss due to testicular torsion is still high. Testicular torsion is an acute urological emergency problem with a rate of 1/4000 in men under the age of 25.<sup>[8]</sup>

Gultekin et al. found that reducing tissue pressure with TAI reduces edema, thus increasing tissue perfusion and decreasing the amount of free oxygen radicals, Gultekin et al. found that ischemia-reperfusion injury decreased with multiple and transverse incisions, but unlike Gultekin et al.,<sup>[1]</sup> a single (craniocaudal) incision was applied in our study. Another idea is

that Vitamin C supplementation before TAI contributes to the reduction of tissue damage by reducing oxidative stress.<sup>[13]</sup>

## Conclusion

In accordance with the literature, our study showed that edema, ischemic changes, necrosis, and inflammation occur less with TAI compared to the T-DT group, and tissue damage is less. The high JTBS s in the TAI group is a positive condition for the continuity of spermatogenesis.

The result of testicular torsion with orchiectomy is not only an organ loss but also a traumatic process in terms of men's social roles and reproductive functions. In the literature, it is stated that TAI can be an alternative technique before orchiectomy in only selected cases of testicular torsion. Pathological evaluations also support less tissue damage in the TAI group.

Unlike the literature, we used inflammation and basement membrane thickening scoring for the first time in our study. Since these factors are the precursors of tissue damage, they provided a more sensitive evaluation opportunity in the detection of damage.

As a result of the pathological examination, it was determined that only DT was insufficient to prevent testicular damage. Adding TAI to DT has proven effective in preserving testicular tissue.

For these reasons, DT causes ischemia-reperfusion injury in cases presenting with testicular torsion and causes compartment syndrome and thus tissue damage to continue.

Since the aim of treatment in testicular torsion is to prevent tissue damage in the testis, our study showed that DT alone is not sufficient, and the TAI procedure should be added to the process.

**Ethics Committee Approval:** This study was approved by the Balıkesir University Animal Experiment Ethics Committee (Date: 25.08.2021, Decision No: 2021/7-1).

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#### Conflict of Interest: None declared.

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### DENEYSEL ÇALIŞMA - ÖZ

### Testis torsiyonunda, tunica albuginea insizyonunun testis dokusuna etkisi

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AMAÇ: Acil servise skrotal ağrı nedeniyle başvuran çocukların %15–20'sinde altta yatan neden testis torsiyonudur. Testisin spermatik kord düzeyinde kendi etrafında dönmesi olarak tanımlanan testis torsiyonu dolaşımın bozulması nedeniyle organ kaybına neden olabilir. Organ kaybının önlenmesi için ilk altı saat içinde testisin detorsiyone edilmesi önerilmektedir. Çalışmanın amacı, detorsiyona ek olarak yapılan Tunica Albuginea insizyonunun torsiyone olmuş testisin canlılığına etkisini incelemektir.

GEREÇ VE YÖNTEM: Araştırma deneysel tasarıma sahiptir. Çalışma 3 grup (sham, testis torsiyonu [T-DT] ve tunica albuginea insizyonu [TAI]) sıçan üzerinde uygulanmıştır. On günlük izlem sonrasında gruplardaki testisler patolojik inceleme için alınmıştır. Patolojik incelemede tüm patolojik örneklerde, nekroz, iskemik değişiklikler, Johnsen skoru, ödem, enflamasyon ve bazal membran kalınlaşması değerlendirilmiş ve skorlanmıştır.

BULGULAR: TAI ve T-DT grubu değişkenler bakımından karşılaştırıldığında; nekrozun, iskemik değişikliğin, ödemin, enflamasyon ve bazal membrane kalınlaşmasının TAI grubunda anlamlı seviyede az olduğu saptanmıştır. Aynı zamanda, ortalama Johnsen Scores T-DT (7.44±0.52) ve TAI (8.60±0.51) grupları arasında anlamlı olarak farklıdır.

TARTIŞMA: Bulgular TAI grubundaki testislerin sadece detorsiyon uygulanan testislere göre daha iyi korunabildiğini göstermiştir. Testis torsiyonunda, detorsiyon işleminin testisi korumak için yeterli olmadığı, TAI'nın işleme eklenmesinin yararlı olacağı ileri sürülebilir. Anahtar sözcükler: Çocuk; testis torsiyonu; tunica albuginea insizyonu.

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