

The Effects of Transparenchymal Suture Material and Fibrine-a Tissue Adhesive-on the Contralateral Testis

Transparenkimal Geçilen Sütür Materyallerinin ve Bir Doku Yapıştırıcısı Olan Fibrin'in Kontrateral Testis Üzerindeki Etkileri

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ABSTRACT Objective: Experimental studies showed that fibrin glue (FG) which was used in orchidopexy and testicular torsion caused less tissue damage in the ipsilateral testis when compared with the transparenchymal suture materials. However, whether the usage of the FG in testis fixation causes any histological changes in the contralateral testis is not known. The aim of this study was to compare the effects of FG and other suture materials on the contralateral testis where no surgical procedure is performed. **Material and Methods:** Fifty prepubertal rats were randomly divided into 5 groups and the tunica vaginalis of all the right testes were longitudinally incised. In group 1, the incision line was closed with FG, whereas silk, catgut and polypropylene were used respectively in the groups 2, 3 and 4. Group 5 was the control group and no surgical procedure was performed on the left testes. The rats were sacrificed 3 weeks later and the diameters of the right and left testes were measured. Testes were evaluated by means of inflammation, seminiferous tubular diameter (STD), spermatogenic activity and epididymal inflammation. **Result:** In group 2, 3 and 4, spermatogenic activity, STD and the testis diameter of the ipsilateral testes decreased, whereas testicular and epididymal inflammation increased. When the contralateral testis was evaluated, STD of the FG group decreased and FG had no extra benefit on testicular histology when compared to other suture materials. **Conclusion:** Surgical procedures performed on the ipsilateral testis cause only a decrease in the contralateral STD, which is observed by light microscope, but this decrease seems to have no effect on the spermatogenic activity. Besides, the usage of FG instead of other materials in testis fixation has no extra benefit. However, it must be kept in mind that these results might vary when ultrastructural and immunological evaluations are performed.

Key Words: Testis; tissue fixation; fibrin tissue adhesive

ÖZET Amaç: Deneysel çalışmalar testis torsiyonu ve orşiopeksi sırasında fiksasyon amacıyla kullanılan fibrin yapıştırıcının, transparankimal geçilen sütür materyallerine göre ipsilateral testiste daha az hasar oluşturduğunu göstermiştir. Ancak testis fiksasyonu için kullanılan fibrin yapıştırıcının kontrateral testiste herhangi bir histolojik değişim oluşturup oluşturmadığı belli değildir. Çalışmamızda işlem yapılmayan kontrateral testiste, sütür materyallerinin oluşturduğu etkiler ile, fibrin yapıştırıcının oluşturduğu etkilerin karşılaştırılması hedeflenmiştir. **Gereç ve Yöntemler:** Çalışmamızda 50 adet preadölesan rat rastgele 10'arlı gruplara ayrılarak sağ testislerin tunika vaginalisine bisturi ile longitudinal insizyon yapılmasını takiben grup 1'de insizyon hattı fibrin yapıştırıcı ile grup 2, 3 ve 4'te sırası ile ipek, katgut ve polipropilen ile kapatıldı. Grup 5 kontrol grubu olarak planlandı ve tunika vaginalis insizyonu kapatılmadan testis yerine yerleştirildi. Sol testislere herhangi bir girişimde bulunulmadı. Üç hafta sonra sakrifiye edilen ratların sağ ve sol testislerin çapları ölçüldü. Testisler inflamasyon, seminifer tübül çapı, spermatogenezis derecesi ve epididimal yangı açısından değerlendirildi. **Bulgular:** Grup 2, 3 ve 4'te fibrin yapıştırıcı grubuna göre ipsilateral testislerde, testis çapında azalma, testiküler inflamasyon ve epididimal yangıda artış, spermatogenezis derecesinde ve seminifer tübül çapında azalma saptandı. Ancak kontrateral testisler değerlendirildiğinde, fibrin yapıştırıcı grubunda seminifer tübül çapında azalma saptandı ve sütür geçilen gruplarla kıyaslandığında fibrin yapıştırıcının ekstra bir avantaj sağlamadığı görüldü. **Sonuç:** Ipsilateral testiste yapılan işlemler, kontrateral testislerde ışık mikroskopu düzeyinde sadece seminifer tübül çaplarında azalmaya neden olmakla birlikte bu azalma spermatogenezisi etkilemiyor gibi gözükmektedir. Ayrıca fiksasyon amacıyla sütür materyalleri yerine fibrin yapıştırıcının kullanılması da ekstra bir avantaj sağlamamaktadır. Ancak ultrastrüktürel ve immunolojik bazda yapılacak değerlendirmeler ışığında sonuçların değişebileceği göz ardı edilmemelidir.

Anahtar Kelimeler: Testis; doku fiksasyonu; fibrin doku yapıştırıcı

Testis fixation to scrotum with various suture materials is the current surgical method for orchidopexy in undescended testes and testicular torsion.¹ It is well established that transparenchymal sutures cause damage of various degrees on ipsilateral and contralateral testicular tissue.²⁻⁵ However, in some studies including undescended testis models which have been set up by fixation of the gubernaculum to abdomen without any suture, damage on the contralateral testis was not observed.⁶⁻¹⁰ Also in many studies with bilateral undescended testes models with testicular or gubernaculum suture, contralateral testis changes are considered the effects of the undescended testis. However, in undescended testis and testicular torsion models, which were set up by suturing the testis, it is difficult to evaluate whether the changes occurring on the ipsilateral and contralateral testis depend on transparenchymal sutures, torsion or undescended testis.

The negative effects of the transparenchymal suture materials on the testicular tissue led to researches on fixation without using suture material. Fibrin glue, which is a tissue adhesive, is an example. For this purpose, Sencan et al studied the effects of fibrin glue and suture materials on the ipsilateral testicular tissue, and showed that the group with the fibrin glue had the minimum testicular damage.⁵ However, it is not certain whether the fibrin glue, which is used for testis fixation, caused a histological change on the contralateral testis. This study was planned to compare the effects of different suture materials and fibrin glue on the contralateral testis.

MATERIAL AND METHODS

The study was approved by the Animal Research Ethics Committee of the Medical School of Celal Bayar University. It was carried out at the Ege University Medical School Experimental Research Center.

EXPERIMENT GROUPS

Prepubertal Wistar Albino type rats, which were 20 days old and weighed 100 to 200 grams, were

used. Five groups consisting of 10 rats were formed (50 rats totally).

In group 1, fibrin glue (Beriplast P 1 mL comby-set, Farma-Tek Medicine Industry, Istanbul, Turkey) was used as tissue adhesive along the incised tunica vaginalis line; likewise, silk for group 2, catgut for group 3 and polypropylene for group 4 were used as suture material. Group 5 was control group.

OPERATION

Right inguinal incision was performed after intramuscular xylazine hydrochloride anesthesia and under sterile conditions. An equivalent incision with lancet was made for the tunica vaginalis of the right testis in each experiment. In group 1, fibrin glue was applied with its special injector to the incision line. In groups 2, 3 and 4, superficial transparenchymal suture was applied to the upper, middle and lower pole with 4/0 atraumatic silk, 4/0 atraumatic catgut, and 4/0 atraumatic polypropylene respectively (3 sutures were applied to the incised line of each testis). In group 5, which was the control group, the testis was placed in the tunica vaginalis without closing the incision line. The left testes were left intact.

Right and left testes of the rats, which were sacrificed 3 weeks later, were excised and were fixed in Bouin solution.

HISTOPATHOLOGICAL EVALUATION

After measuring the greatest diameters of the right and left testes with linear scale, the samples taken from the epididymis and the suture line were fixed in Bouin solution for 4 hours and were embeded in paraffin blocks. Sections prepared from the tissues were 5 µm thick. The samples were stained with Hematoxyline-Eosin. All testes were histopathologically evaluated regarding inflammatory cell infiltration, spermatogenesis degree, STDs and epididymal inflammation at x 100 magnification using ocular scale.

The severity of inflammation in testes and epididymis was graded as none, mild, moderate and severe and was assigned numerical values from 0 to 3. Mild inflammatory changes were defined as

focal inflammatory infiltrates less than 25% involvement and moderate inflammatory changes were defined as greater than 25% but less than 75% involvement. Severe inflammatory changes were defined as greater than 75% involvement.

The degree of spermatogenesis was evaluated according to the percentage of the spermatozoa content of the tubulus; 0-20% (Score 1), 20-40% (Score 2), 40-60% (Score 3), 60-80% (Score 4), 80-100% (Score 5).

STD was measured by taking the average of 25 tubule diameters, which were measured at the center and clock quarters 3, 6, 9, 12 of the cross section of the testis.

A single pathologist who was blinded to the experiment examined the samples.

Statistical Analysis

Statistical analysis was carried out with SPSS software (version 10.0) on a computer. Mann-Whitney U and Wilcoxon tests were used to compare data from measurements of the two dependent and independent groups. P values less than 0.05 were considered statistically significant.

RESULTS

A- TESTES DIAMETERS

The diameters of the ipsilateral testes of the catgut and polypropylene groups were significantly smaller than the other groups. Although the contralateral testes diameters decreased in both fibrin and

polypropylene groups, only the decrease in the fibrin group was statistically significant ($p < 0.001$) (Table 1).

B- TESTICULAR INFLAMMATION

The lowest inflammation score of the ipsilateral testes was in the fibrin group compared to the control group. The lowest inflammation score of the contralateral testes was observed in the silk group ($p = 0.0023$) (Table 1).

C- SPERMATOGENESIS DEGREE OF THE TESTES

The spermatogenesis degree of the ipsilateral testes all the experimental groups excluding the fibrin group was decreased compared to the control group. The spermatogenesis degrees of the contralateral testes were unaffected in all groups (Table 2).

D- STDs OF THE TESTES

Comparison of the ipsilateral STDs with those of the control group revealed that the STDs had decreased in all groups. The maximum decrease was in the silk and polypropylene groups. The STDs of the contralateral testes were unaffected only in the catgut group (Table 2) (Figure 1).

E- EPIDIDYMAL INFLAMMATION

Except for the fibrin group, significant epididymal inflammation was observed in all groups ($p < 0.05$). Although the most significant epididymal inflammation of the contralateral testes was in the silk group, the difference between groups were not significant ($p > 0.05$) (Table 3) (Figure 2).

TABLE 1: Ipsilateral and contralateral testis diameters and inflammation scores of experiment groups.

Groups	Ipsilateral Testis Diameters (cm)			Contralateral Testis Diameters (cm)			Ipsilateral Testis Inflammation Scores			Contralateral Testis Inflammation Scores		
	^a Min	^b Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median
Fibrin	1.0	1.5	1.210	1.0	1.7	1.340*	0	2	0.7	0	1	0.3
Silk	0.9	1.8	1.230	1.2	2.0	1.800	1	3	1.6	0	0	0.0♣
Catgut	0.3	1.6	1.080**	1.5	1.9	1.730	0	3	1.6	0	3	0.4
Polypropylene	0.6	1.6	1.110**	1.1	1.8	1.660	1	2	1.7**	0	2	0.6
Control	1.2	1.8	1.490	1.5	1.9	1.720	0	2	0.9	0	1	0.6

(a: Minimum, b: Maximum)

* $p < 0.05$ Comparison of the contralateral fibrin group with the contralateral control group,

** $p < 0.05$ Comparison of the ipsilateral catgut and polypropylene group with the ipsilateral control group,

♣ $p < 0.05$ Comparison of the contralateral silk group with the contralateral control group,

♣♣ $p < 0.05$ Comparison of the ipsilateral polypropylene group with the ipsilateral control group.

TABLE 2: Ipsilateral and contralateral testes spermatogenesis degree and STDs of the experiment groups.

Groups	Ipsilateral Spermatogenetic Degree Score			Contralateral Spermatogenetic Degree Score			Ipsilateral STD			Contralateral STD (µm)		
	^a Min	^b Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median
	Fibrin	4	5	4.90	4	5	4.90	15.40	20.20	18.22*	15	21
Silk	1	5	2.70*	4	5	4.90	12.00	20.00	15.60*	15	22	19.90
Catgut	1	5	3.60*	4	5	4.90	0.00	21.40	15.90*	19	23	21.04**
Polypropylene	1	5	2.30*	4	5	4.90	10.00	19.80	14.78*	15	22	20.60
Control	4	5	4.80	5	5	5.00	18.60	23.80	21.18	20	24	22.04

(a: Minimum b: Maximum), STD: Seminiferous Tubular Diameter,

*p< 0.05 Comparison of the ipsilateral silk, catgut and polypropylene groups with the ipsilateral control group,

* p< 0.05 Comparison of the ipsilateral fibrin, catgut and polypropylene groups with the ipsilateral control group,

** p> 0.05 Comparison of the contralateral catgut group with the contralateral control group.

DISCUSSION

Many articles published on unilateral testicular torsion or undescended testis reported that decreased fertility.¹¹⁻¹⁵ This decrease in fertility suggests that the contralateral testis is somehow affected by the pathology of the unilateral testis. However, the effect on the contralateral testis of surgical operations (transparenchymal sutures during testicular fixation) is not completely clear. Especially in the experimental unilateral undescended testis or testicular torsion models, the changes in the contralateral testis differ according to the fixation method of the testis. Undescended testis model in some studies were formed by suturing the testis and others composed the model by without suturing.^{8-11,16} However, the results of such studies are interpreted as a contralateral effect produced by unilateral testicular pathologies without regarding this difference in methodology. Yet, the changes of the contralateral testis in the unilateral undescended testis or testicular torsion models formed with suturing the testis may not depend only on the effect of torsion or undescended testis. Suturing the testicular parenchyma only without forming any additional pathology, may affect ipsilateral and contralateral testis tissue.

Bellinger et al examined histological changes associated with the tunica albuginea sutures in adult rat testes and showed severe inflammatory reactions associated with the suture.³ They recom-

mended that a fine nonabsorbable suture should be placed when an adjunct to dartos pouch fixation is necessary. Ideal suture is a suture that would have minimal inflammatory and parenchymal changes. The parenchymal injury caused by transparenchymal sutures on the testicular tissue led researches to improve new fixation methods without using suture material. Fibrin glue is commonly used in clinical practice, especially in Pediatric Urology. In addition to being a good tissue adhesive, its homeostatic effect and being a natural product should be considered advantages. However, it also has disadvantages such as being expensive compared with other suture materials and although very rare, some viruses may infect via the agent.¹⁷⁻²⁰ Sencan et al studied the effects of fibrin glue and suture materials on the ipsilateral testicular tissue, and showed that the testicular damage was minimal in the group of testes on which fibrin glue was used.⁵ We planned this study to investigate whether fibrin glue used for testis fixation had an effect on the contralateral testis, and if so, to evaluate its difference from the effects of other suture materials.

Our initial hypothesis was that fibrin glue being a natural product caused less fibrosis compared to suture materials. Since it would not create transparenchymal trauma, the contralateral testis would not be affected, meaning an advantage compared to suture materials. However, the STDs of the contralateral testes decreased in the fibrin group. Similar histological signs were detected in groups in which



FIGURE 1: The seminiferous tubular diameters (STD) of the contralateral testes are shown. No significant change in the STD of the catgut group was observed, whereas STD decreased in the fibrin group, (catgut group A, fibrin group B, control group C) (HE x 200).

suture materials were used. This effect on the contralateral testes in the transparenchymally sutured groups (group 2, 3 and 4) may depend on the damaging effect of the superficial vascular structure on the testicular parenchyma during suturing because the intragonadal arteries of the testis are localized just under the tunica vaginalis. The release of cytokines, which are formed due to the trauma to the systemic circulation, may be affecting seminiferous tubulus diameters of the contralateral testes. Besides, the development of antisperm antibodies due to the breakdown of the testis-blood barrier by sutures may be responsible for the negative effects on the contralateral testis. A study of Tanyel et al revealed that the blood flow through the two testes was independent from each other.²¹ However, contralateral blood flow may vary as a reflex to an afferent stimulus in unilateral testis pathologies. The decrease of the seminiferous tubulus diameters of the contralateral testes in the presented study may have a similar mechanism. A clinical study with 387 cryptorchid males that investigated the factors influencing fertility revealed that the testicular struc-

TABLE 3: Epididymal inflammation scores of the ipsilateral (operated) and the contralateral (unoperated) testes of the experiment groups.

Groups	Ipsilateral Epididymal Inflammation Score			Contralateral Epididymal Inflammation Score		
	Min	Max	Median	Min	Max	Median
Fibrin	0	2	0.70	0	1	0.30
Silk	1	3	1.60*	0	0	0.00
Catgut	0	3	1.60*	0	3	0.40
Polypropylene	1	2	1.70*	0	2	0.60
Control	0	2	0.90	0	1	0.60

*p< 0.05 Comparison of the ipsilateral silk, catgut and polypropylene groups with the ipsilateral control group.

ture was an independent determinant of infertility. When the factors related to infertility were analyzed, the infertility rate was higher in patients who had orchidopexy with transparenchymal testicular suture.¹² Thus, we suggest that testicular fixation using paratesticular suture will be more useful than transparenchymal suturing.

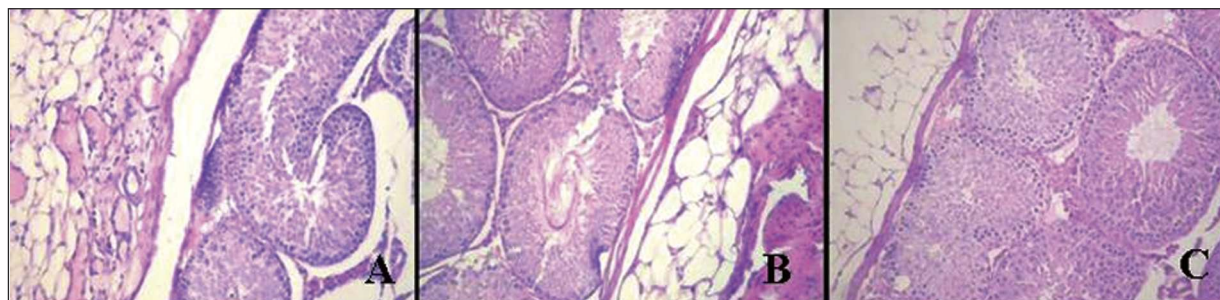


FIGURE 2: The inflammation in the contralateral testes is shown. No inflammation was observed in the silk group and the inflammation was minimal in the fibrin group (fibrin group A, silk group B, control group C) (HE x 200).

In clinical practice, Nöske reported that there was no relapse after using fibrin glue for testicular fixation of the extravaginal cord torsion in 100 adult patients.²² However, the same study did not include any data on the effects of fibrin glue on ipsilateral and contralateral testes. In another study, partial ovariectomy was performed in female rats and the effects of fibrin glue and suture materials were investigated. Minimum fibrosis and atrophy were observed in the fibrin glue group. However, there was no data on the effects of fibrin glue on contralateral ovaries.²³ In the presented study, transparenchymal suturing or usage of fibrin glue significantly decreased the STD of the ipsilateral testes. Spermatogenesis degree in the contralateral testes seemed not be affected. This may be due to the heterogeneity of spermatogenesis in testes. In addition, the finding that there was no histological damage under the light microscope does not mean that there was no injury to the testes. In other words, the effects of suture materials used for fixation of the testis on the contralateral testes and consequently on fertility may be considered minimal or none.

In an experimental undescended testis model with fixation of the gubernaculum into the abdomen without touching the testis, while no negative changes on the contralateral testes was present under the light microscope, basal membrane thickening, decrease in the number of spermatogenesis cells, vacuolization, monocell apoptosis, hyperplasia and hypertrophy were observed under electron microscope. How these ultra structural changes occur in the contralateral testis is unclear; a possible mechanism may be an immunological response to trauma.²⁴ Srinivas et al found that fertility,

contralateral testicular weight, contralateral testicular maturation score, haploid cell population, STDs in undescended testis model formed by direct fixation of the testis were significantly reduced compared to undescended testis model formed by gubernaculum fixation.⁹ They also showed that the maturation of the tubules, which contain primary spermatocides stopped and plasma antisperm antibodies increased in the undescended testis model formed by direct fixation of the testis. Besides, no histological abnormality was observed in the contralateral undescended testis group, which was formed by fixing the gubernaculum into the abdomen. Increasing plasma antisperm antibodies may be attributed to the loss of immunologic tolerance due to suturing of the testis directly.¹⁰ This study highlights the importance of transparenchymal sutures on the testes. However, it would be hard to evaluate which differences were associated with undescended testis and which ones with the suture used.

In conclusion, according to results of our study; 1- Surgical procedures on the ipsilateral testis only reduces the STDs of the contralateral testes under the light microscope, 2- Using the fibrin glue instead of transparenchymal sutures on the incised tunica vaginalis for fixation reduces the STDs similar to the suture materials, 3- In animal studies, while forming testicular torsion or undescended models, it is important to take care to form the model without transparenchymal suturing in order not to cause an additional pathology which may affect the results of the present model.

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