

Effects of Unilateral and Bilateral Epididymectomy on Testes of Rats

Ayfer Aktaş, Yusuf Nergiz, Murat Akkuş

SUMMARY

It is generally agreed that the testis is under endocrine control from the pituitary, and is influenced by physiological and paracrine factors within the organ.

The aim of this study is to analyze the effect of unilateral and bilateral epididymectomy on the testicular tissue growth of rats.

Twenty- one male old Sprague-Dawley rats (28 days old) were used in the study. Rats were assigned into 3 equal groups. The first group was the control group, while unilateral and bilateral epididymectomy was performed on the second and third groups, respectively. Twenty-one days after the epididymectomy, testicular tissues from each group were taken and fixed in Bouin solution. Paraffin sections were stained with Haematoxylin and Eosin, Vangiesson, PAS-Hemalun and examined by light microscopy.

Disorganization of the germinal epithelium, desquamation, degeneration and edema in interstitial tissue was seen in the testicular cross sections of the unilateral group. Arrest in spermatozoon stage in some tubules and presence of eosinophilic stained multinucler bodies were recognizable. In the bilateral group, degeneration and atrophic status in the seminiferous tubules of the bilateral group was observed precisely, and occasional interstitial edema and perforations in the basal lamina were recognizable. In addition, vasodilatation, arrest in spermatozoa stage and multinucleated bodies in some of the seminiferous tubules lumen were observed in some testicular cross sections of this group.

As a result, epididymectomy causes degeneration in the germinal epithelium and hypoplasia in Leydig cells.

It is concluded that epididymectomy causes degeneration in the germinal epithelium, interruption of spermatogenesis, and a notable decrease in the number of Leydig cells.

Key Words: Epididymectomy, Testicular, Rat

Ratlarda Unilateral ve Bilateral Epididymectomy'in Testisler Üzerine Etkisi

ÖZET

Unilateral ve bilateral epididymectomy'in sıçan testis gelişimi üzerindeki etkisini araştırmak amacıyla bu çalışma planlandı.

Çalışmada 21 adet 4 haftalık erkek Sprague-Dawley sıçan kullanıldı. Denek hayvanları 3 eşit gruba bölündü. Birinci grup kontrol, ikinci gruba unilateral epididymektomi ve üçüncü gruba ise bilateral epididymectomy uygulandı. Epididymectomy sonrası 21. günde her üç grubun testis dokuları alınarak Bouin solüsyonunda fikse edildi. Parafin kesitler Hematoksilen-Eozin, Hematoksilen-Vangiesson ve PAS-Hemalun ile boyanarak ışık mikroskopunda incelendi.

Unilateral grub testis kesitlerinde germinal epitel ayrışması, deskuamasyon, dejenerasyon ile interstisiel dokuda ödem görüldü. Bazı tubüllerde spermatozoon aşamasında arrest durum ve eozinofilik boyanan multinükler hücrelerin varlığı dikkat çekiciydi.

Bilateral grupta ise, testis tubüllerindeki dejenerasyon ve atrofik durum ileri derecede izlenirken, yer yer interstisiel ödem ve bazal laminada perforasyon dikkat çekiciydi. Yine bu grubun bazı testis kesitlerinde vazodilatasyon, spermatozoa aşamasında arrest durum ve bazı seminifer tubül lümeninde multinükler hücreler bu grupta da izlendi.

Sonuç olarak, epididymektomi testis germinal epitelinde dejenerasyon ve Leydig hücrelerinde hipoplaziye neden olmaktadır.

Anahtar Kelimeler: Epididymectomy, Testis, Rat.



INTRODUCTION

It is generally agreed that the testis is under endocrine control from the pituitary, and is influenced by physiological and paracrine factors within the organ (1).

It has been established that ligation of the vasa efferentia of the mammalian testis results in a complete and rapid atrophy of the seminiferous epithelium (2).

In many laboratories, it has been demonstrated that unilateral spermatic cord torsion has an adverse effect on the contralateral testis in various species (3-6). Recently, however, many investigators have reported that they have been unable to observe this phenomenon (7-9).

The epididymis has the functions of absorbing most of the fluid leaving the testis, and of maturing and storing the spermatozoa until ejaculation (10). However, it has also been suggested that the epididymis may have endocrine activity (11), and may be involved in absorption and transport into blood of inhibin originating from the testis (12).

Although epididymectomy has been recommended for chronic painful lesions of the epididymis, few data are available to support the indications for this procedure or its outcome (13).

MATERIALS and METHODS

Animals

As experimental design, twenty-one male Sprague-Dawley rats (28 days old) were housed in groups of three under natural light-dark conditions and fed ad libitum on a standard laboratory diet and water. Before we start study, we took report of ethic committee. During the experimental process We carefully respected the criteria about the animal rights which were defined by NIH.

Surgery Procedure

In 7 of these rats, under ether anaesthesia, the testes and epididymis were exposed through scrotal incisions, the right efferent duct was ligated and the right epididymis was then removed. In a second group of 7, the other epididymis was removed. In addition to the procedure mentioned above. A third group

consisting of 7 rats served as sham-operated controls.

All experimental animals were sacrificed 21 days after surgery. The rats were weighed and their testes were removed, weighed again and fixed in 4% formaldehyde for 2h. A central longitudinal slice of 3 mm thick was transferred to 20% formaldehyde for further 24 h. and paraffin sections (5 µm) were cut and stained with periodic acid schiff (PAS)-Hemalun, Haematoxylin Eosin and Vangiesson (14).

Testicular tissue was evaluated by light microscope. In every section, the diameters of ten seminiferous tubules selected randomly were measured. Selected tubular sections were measured with an ocular micrometer. Only those tubules with roughly circular outlines were selected.

Statistical Analysis

The body and testes were analysed by Kruska Wallis and tubular perimeter by one-way ANOVA.

RESULTS

Body weight

Statistically a significant difference was seen between the three groups. According to Kruska-Wallis variance any significant difference was not found between the three groups in terms of body weight and weight of testicle ($p>0.05$).

Testis Weight

Kruska-Wallis variance analysis was performed statistically. As a result, statistically, any significant difference was not found between the three groups in terms of body weight and weight of testicle ($p>0.05$).

Tubular perimeter

Statistically a significant difference was seen between the three groups. According to Tukey HSD; The difference between unilateral-bilateral, unilateral-control, and bilateral-control groups was observed to be significant ($p<0.05$).

According to Dunnett test, there was also a significant difference between control-unilateral and control-bilateral groups ($p<0.005$).

Table 1. Effects of unilateral and bilateral epididymectomy on testicular weight body weight and tubular perimeter of seminiferous tubules

Days after surgery	Body weight			Testis weight			Tubular perimeter		
	Control (n:5)	ULE* (n:6)	BLE** (n:6)	Control (n:5)	ULE* (n:6)	BLE** (n:6)	X±SD		
21	195 gr	212.5gr	19 gr	1.080µm	1.01µm	0.97µm	Control (st:150)	ULE (st:150)	BLE (st:150)
							21.28±2.403µm	22.06±2.406µm	18.34±2.430µm

* ULE: Unilateral epididymectomy group

**BLE: Bilateral epididymectomy group

st: Seminifer tubule

Tubular Histology

Control group: normal testicular tissue was observed (Figs. 1-2).

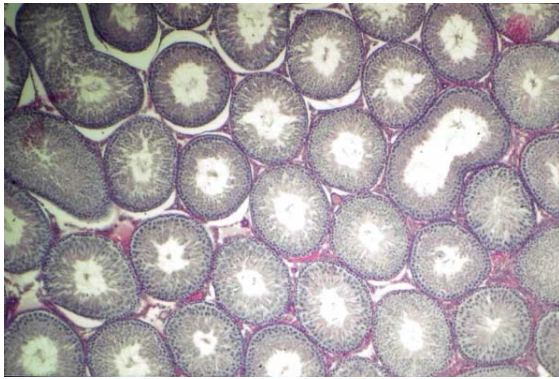


Figure 1. Control Group:View of normal seminiferous tubules and leydig cells (PAS-Hemalun staining,original magnification X41)

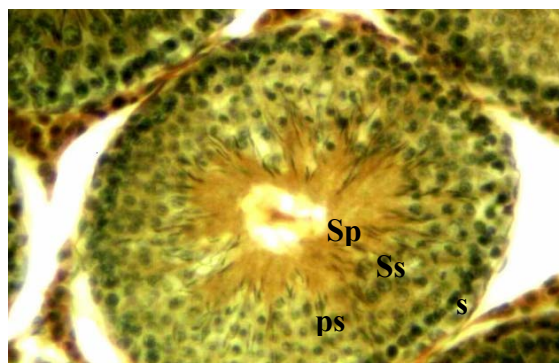


Figure 2. Control Group:Note the presence of all cellular types and their connect arrangement in the germinal epithelium, **s**:spermatogonia **ps**:primer spermatosit,**ss**:sekonder spermatosit **Sp**:spermatozoon (Vangieson staining,original magnification X164)

Unilateral group: In addition to normal seminiferous tubules, atrophic tubules and vasodilatation in the veins were seen in the testicular cross sections of the unilateral group. Moreover, disorganization of the germinal epithelium, desquamation, degeneration and edema in the interstitial tissue were seen. Arrest was observed in spermatozoon stage in some tubule (Figs. 3- 4).

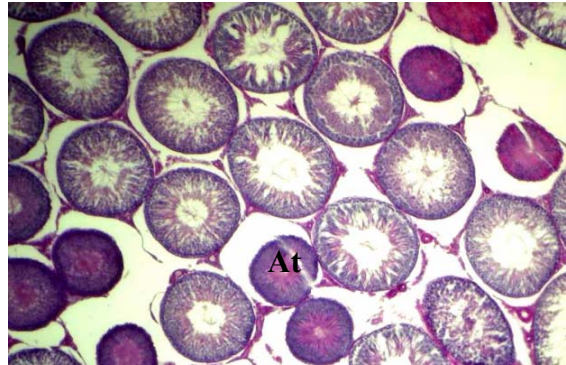


Figure 3. Unilateral Group: Note the distruption of the seminiferous epithelium and atrophic tubules(At).PAS-Hemalun staining original magnification,X41)



Figure 4. Unilateral Group: Interstitiel oedema (öd) and the loss of spermatids,(PAS-Hemalun staining original magnification,X82)

Bilateral group: degeneration and atrophic stage in the seminiferous tubules of the bilateral group were observed thoroughly, and occasional interstitial edema and perforations in the basal lamina were recognizable. Also in this group, vasodilatation, arrest stage in spermatozoa stage and multinucleated bodies were observed in some of the seminiferous tubules (Figs. 5-6).



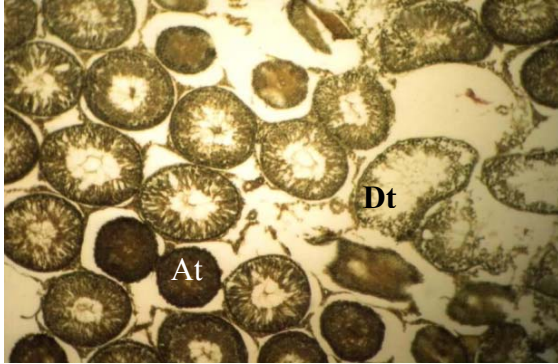


Figure 5. Bilateral Group: Extensive atrophic tubules (At) degenerated tubules (Dt) seen, (Vangiesson staining original magnification, X41)

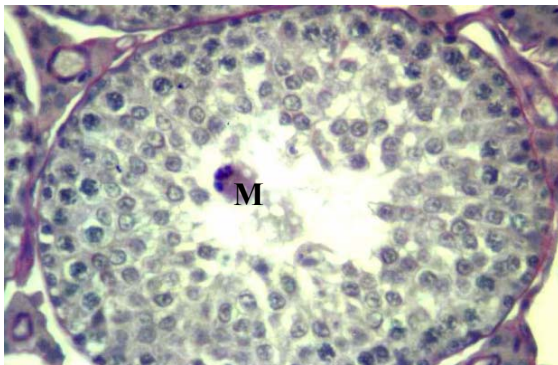


Figure 6. Bilateral Group: Note multinucleated bodies (M) rest in the lumen of seminiferous tubule (PAS-Hemalun staining original magnification, X164)

DISCUSSION

The present results show that either unilateral or bilateral epididymectomy is followed by a marked increase in degeneration of seminiferous epithelium.

There have been a number of qualitative or semiquantitative of histological effects of epididymectomy on the testis (15), and in some studies it has been that there are linear increases in testis weight as a percentage of body weight following bilateral ligation of the efferent ducts and removal of part of the epididymis (15- 16).

The present results show that epididymectomy is not followed by a marked increase in testicular and body weight by 21 days after the operation. There is a rapid

decrease in the diameter of some tubules, associated with degeneration of the seminiferous epithelium. The present observations indicate that degeneration of the germinal elements following epididymectomy occurs far more rapidly than has been previously reported. Tamura (1), for instance, reported that only partial degeneration had occurred 70-90 days after removal of the entire epididymis in the mouse. Histologic studies showed that degeneration of the seminiferous epithelium start within 1 day after ligation. The state of necrosis was maximal at 21 days, when the majority of tubules were lined by only a single row of sertoli cells and occasional spermatogonia lying along basement membran.

Our study showed that multinucleated bodies formed by coalescence of degenerating spermatids were common. This manner, as well as binucleate cells containing spermatocyte nuclei has been claimed to result from an instability of damaged cytoplasmic membran. Normally spermatids between stages 9 and 15 either became pyknotic or underwent nuclear swelling but never appeared to form multinucleated bodies. These observations are in complete agreement with those reported earlier (1).

It is concluded that epididymectomy causes degeneration in the germinal epithelium, interruption of spermatogenesis, and a marked decrease in the number of Leydig cells.

REFERENCES

1. Smith, G. The effects of ligation of the vasa efferentia and vasectomy on testicular function in the rat. *J. Endocrin.* 1996, 223: 385-399.
2. Bedford, J. M. Maturation, transport and fate of spermatozoa in the epididymis. In "Handbook of Physiological Soc". (Eds D. W. Hamilton and R. D. Greep.). 1975, 7: 303-317.
3. Cosentino M.J., Rabinowitz R. Experimental aspects of torsion. *Dialog Pediatr Urol.* 1985, 8: 6-8.
4. Cosentino M.J., Nishida M., Rabinowitz R., Cockett A.T.K. The effect of prepubertal spermatic cord torsion on subsequent fertility in rats. *J Androl.* 1984, 75: 93-98.

5. Cosentino M.J., Nishida M., Rabinowitz R., Cockett A.T.K. Histological changes occurring in the contralateral testes of prepubertal rats subjected to various durations of unilateral spermatic cord torsion. *J Urol.* 1985, 133: 906-911.
6. Cosentino M.J., Nishida M., Rabinowitz R., Cockett A.T.K. Histopathology of prepubertal rat testes subjected to various durations of spermatic cord torsion. *J Androl.* 1986, 7: 23-31.
7. Turner T.T. Acute experimental testicular torsion. *J Androl.* 1985, 6: 65-72.
8. Turner T.T. On unilateral testicular and epididymal torsion: no effect on contralateral testis. *J Urol.* 1987, 138: 1285-1290.
9. Nagler H.M., de Vera White R. The effect of testicular torsion on the contralateral testis. *J Urol.* 1982, 128: 1343-1348.
10. Turner, T. T. On the epididymis and its function. *Invest. Urol.* 1979, 16: 311-321.
11. Hamilton D.W. Structure and function of the epithelium lining the ductuli efferentes, ductus epididymidis and ductus defferens in the rat. In "Handbook of Physiology".(Eds D.W.Hamilton and R.D.Greep.) Sect. 7, V, pp. (American Physiological Soc.: Washington, D.C.) 1975, 143-172.
12. Le Lannou, D., Chambon, Y., and Le Calve, M. Role of the epididymis in reabsorption of inhibin in the rat. *J. Reprod. Fertil. Suppl.* 1979, 26: 117-121.
13. Goldstein, M. Surgery of male infertility and other scrotal disorders. In: *Champbell's Urology*, 6th ed. Philadelphia: W. B. Saunders Co. 1992, 3: 3114-3149.
14. Sam F., Reitmen S., Sonnenwirth A., Growoh'l: *Clinical laboratory methods and diagnosis.* 1970, 1: 935- 960.
15. Soler C., Blazquez J., Pertusa M., Nunez J and Nunez A. A Comparison of the effects of bilateral efferent duct ligation and partial epididymectomy on the testes of rats. *Reprod. Fertil. Dev.* 1990, 2: 321-326.
16. Abou-Haila, A., and Fain-Maurel, M. A. Regional differences of the proximal part of mouse epididymis: morphological and histochemical characterization. *Anat. Rec.* 1984, 209: 197-208.
17. Ramos, A. S., and Dym, M. Fine structure of the monkey epididymis. *Am. J. Anat.* 1997, 149: 501-532.

Correspondence Address

Ayfer AKTAŞ
Department of Histology and Embyology, Medical
School, Dicle University, Diyarbakır
E-mail: aaktas@dicle.edu.tr

