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THE TESTES AFTER UNILATERAL INCARCERATED INGUINAL HERNIA IN PREPUBERTAL RATS*

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Incarceration of an inguinal hernia is one of the most common reasons for emergency admission of young children[1]. Incarcerated inguinal hernia causes testicular venous obstruction which may result in hemorrhagic infarction[2]. Infarcted testes which are more common than infarcted bowels[1], are followed in frequency by ipsilateral testis atrophies in 10-15 % of children[3]. The other cause for hemorrhagic infarction of testis is testicular torsion[4]. In addition to ipsilateral testicular alterations, unilateral testicular torsion also results in contralateral testicular deterioration and diminished fertility[5,6].

A recent study we conducted also revealed that unilateral incarcerated inguinal hernias cause not only ipsilateral but also contralateral testicular deterioration in adult rats[7]. Although some experimental and clinical studies have falled to reveal the contralateral damage encountered in the prepubertal period[8,9], others have suggested it[4,10-12].

Therefore, we planned this experimental study to evaluate the effect of unilateral incarcerated inguinal hernias on ipsilateral and contralateral testes in prepubertal rats. Following unilateral incarcerated inguinal hernias, the testes are also compared to the contralateral testes of prepubertal rats subjected to unilateral testicular torsion.

Material and Methods

Thirty prepubertal (30-day old) male albino rats of the Hacettepe strain were used for the investigation. They were maintained in the animal facility, five animals to a cage, under controlled temperature and dark-light cycle. The rats had access to water and rat chow ad libitum.

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General anesthesia was administered to the animals by intraperitoneal instillation of pentobarbital. Surgery was performed through identically opened and closed left abdominoscrotal incisions employing the sterile technique. The animals were randomly divided into three groups, each consisting of ten rats. The experimental groups were as follows :

Group 1. Ten animals underwent a sham operation in which the left testis and caecum were exposed and repositioned in their original locations after placing a 4-0 silk suture on the tunica albuginea.

Group 2. Ten animals underwent clockwise 720° left testicular torsion. Torsion was maintained by a 4-0 silk suture passed through the tunica albuginea and the scrotal wall. Detortion of the testes was carried out through the same incisions at 24 hours.

Group 3. Left-sided incarcerated inguinal hernias were produced in ten animals. The caecum was grasped through the inguinal canal which remains open throughout the life of rats[13]. A caecal loop was advanced through the canal down to the scrotum, and a size 8 Nelaton catheter was placed in the canal near the caecal loop. The external inguinal ring was narrowed over the caecum and the catheter by using 4-0 atraumatic silk sutures. The catheter was then carefully removed and the incision closed (Fig. 1). The incarcerated hernias were surgically reduced through the same incisions after 24 hours of incarceration.

The animals were sacrificed after 15 days. Both testes were removed and placed in Bouin's solution. The specimens were embedded in paraffin blocks and the sections were cut and stained with hematoxylin and eosin. The ipsilateral testes, which were subjected to torsion were necrotic and were not evaluated

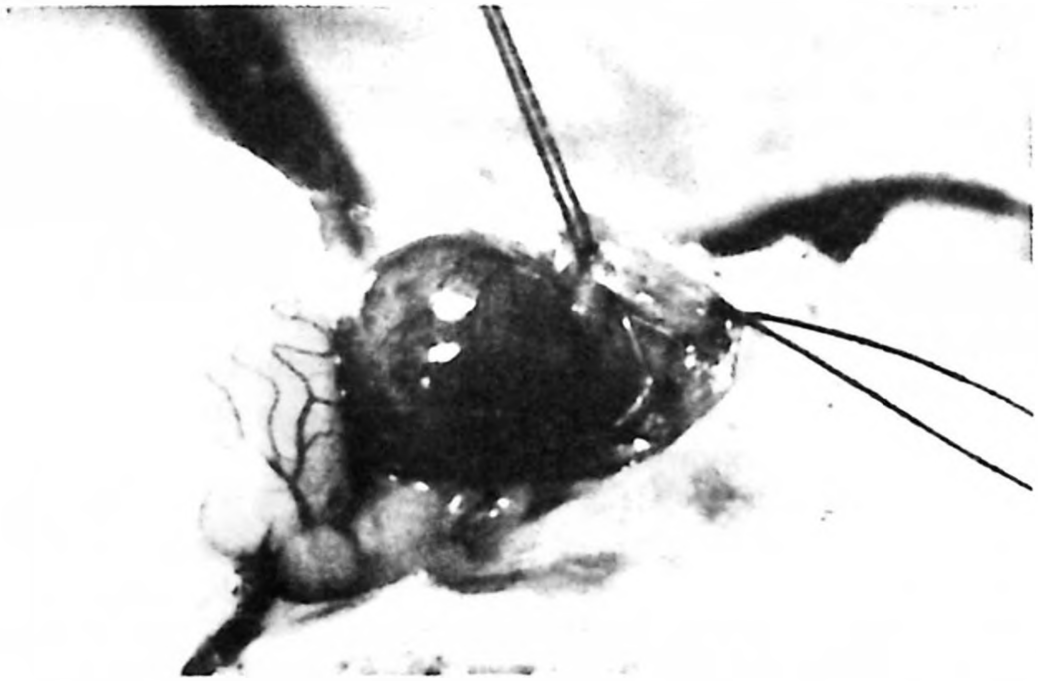

Fig.1- Prepubertal rat with an incarcerated caecal loop.

histologically. The other testicular biopsies were studied blindly and at random by the team's pathologist. Twenty-five of the most circular tubules were identified in each testicular biopsy section, and the diameters were measured with a 10x objective together with a micrometer ocular. The mean seminiferous tubular diameter (MSTD) of each testis was determined in microns. Germinal epithelium maturity was graded using a modified Johnsen testicular biopsy score[14]. By using a 40x objective, the 25 tubules were evaluated, and each tubule was given a score ranging from 1 to 10 according to the criteria shown in Table 1. The mean testicular biopsy score (MTBS) was calculated for each testis.

Statistical comparisons were made between the biopsies from the ipsilateral and contralateral testes of each group of rats using the Mann Whitney-*U*-test, and p values less than 0.05 were considered to be significantly different.

TABLE I: Testicular Biopsy Scores*

Score	Description
10	Complete spermatogenesis with many spermatozoa
9	only a few spermatozoa (5/tubule)
8	No mature spermatozoa, but late spermatids (differentiation to form spermatozoa)
7	Many spermatids without differentiation
6	Only a few spermatids (5/tubule)
5	Many spermatocytes
4	Only a few spermatocytes (5/tubule)
3	Only spermatogonia
2	Sertoli cells without germ cells
1	No cells

* If germinal epithelium is disorganized with sloughing or obliteration of the lumen, a lower score is given.

Results

Two animals in groups one and three died of unknown causes. Nine animals,each in groups 1 and 3, and ten animals in group 2 were available for evaluation at 15 days.

MSTD and MTBS are summarized in Table II. The difference between MSTD of the ipsilateral and contralateral testes was not significant in groups 1 and 3, but the ipsilateral and contralateral testes in group 3 differed significantly compared to the ipsilateral and contralateral testes in group 1.

TABLE II: Summary of Mean Seminiferous Tubular Diameters and Mean Testicular Biopsy Scores in Prepubertal Rats.

Groups	Mean Seminiferous Tubular Diameter (Micron)		Mean Testicular Biopsy Score	
	Right testes	Left testes	Right testes	Left testes
1	206.27±5.19	200.66±2.96	9.36±0.18	8.87±0.13
2	162.70±7.17		6.78±0.55	
3	176.55±5.59	168.22±6.04	7.96±0.44	6.33±0.45

*mean ± Standard deviation.

The differences between MTBS of the ispsilateral and contralateral testes were significant in groups 1 and 3. The MTBS of the ipsilateral and contralateral testes in group 3 also revealed significant differences compared to the ipsilateral and contralateral testes in group 1.

The MSTD and MTBS of the contralateral testes in group 2 differed significantly from the values of the contralateral testes in group 1, but they were not significantly different from the values of the ipsilateral and contralateral testes in group 3 (Figs. 2-4).

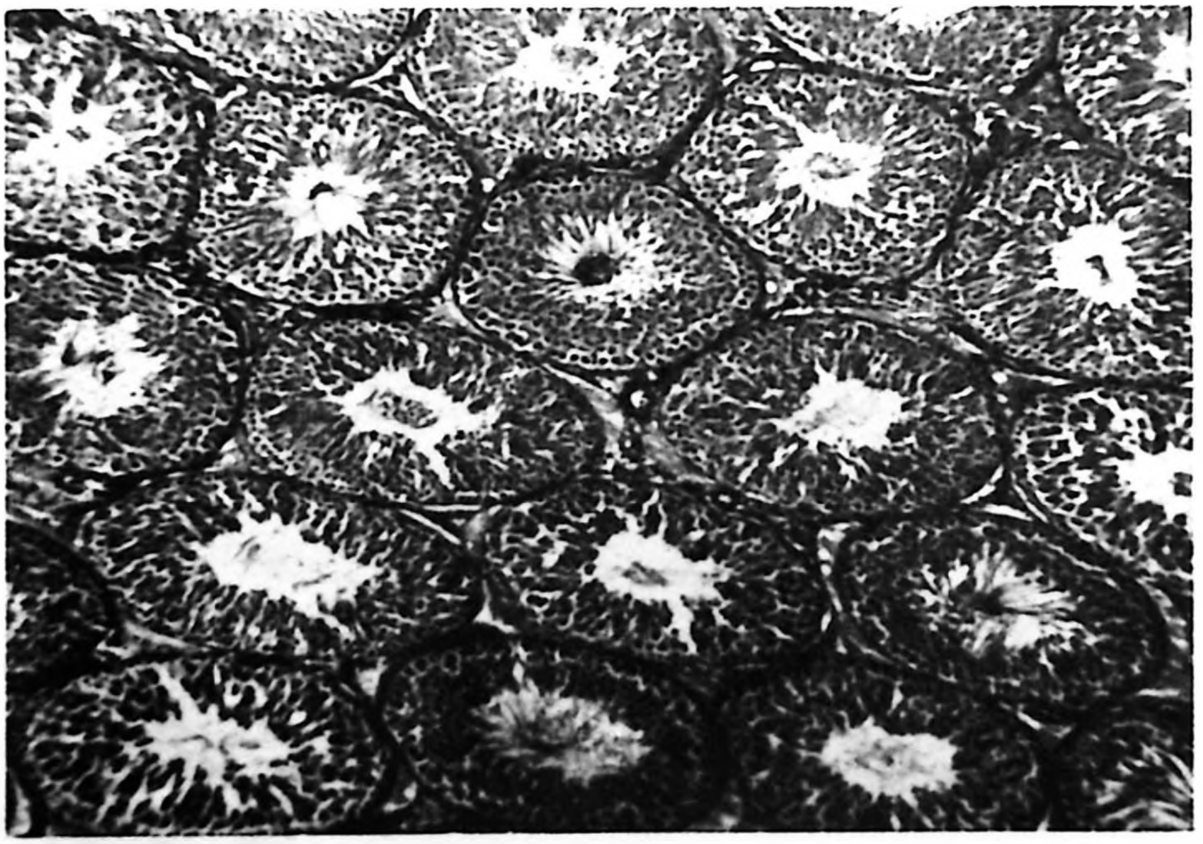

Fig.2- Contralateral testis from contol group demonstrating normal histology (H-E stain x95).

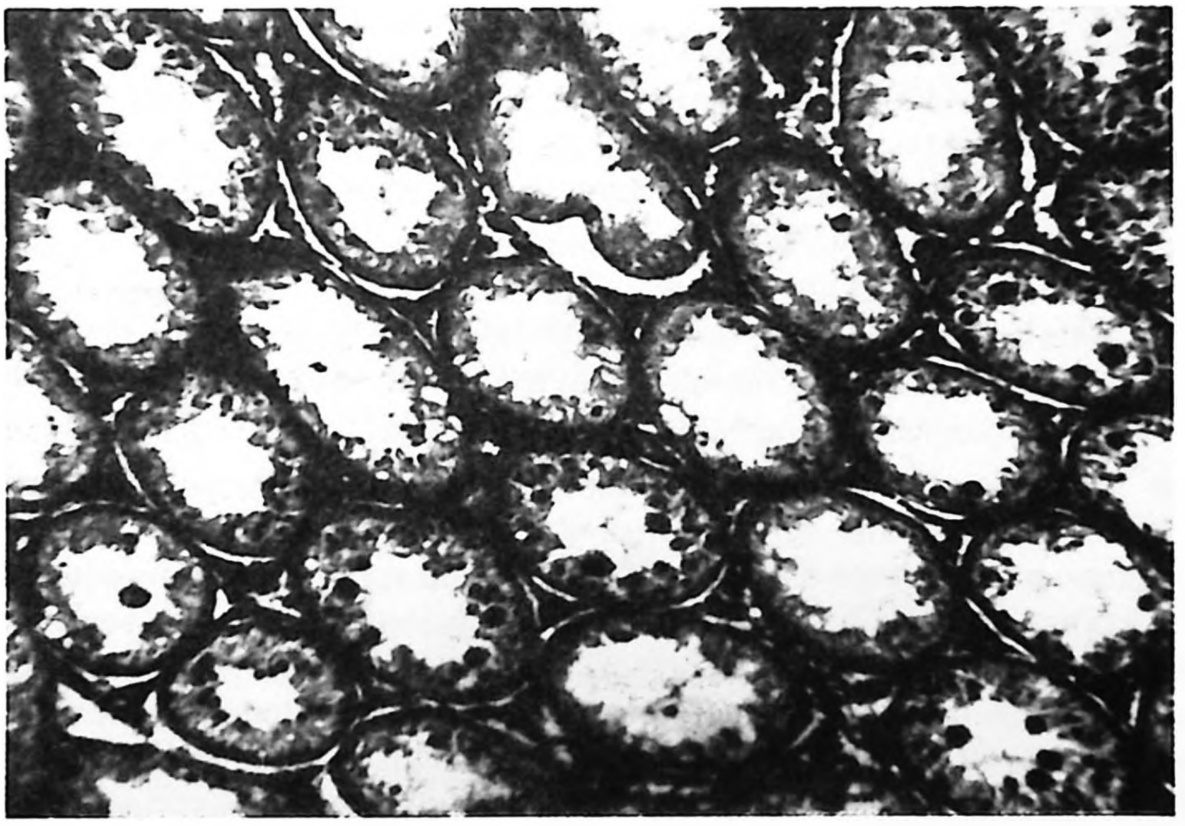

Fig.3- Microscopic appearance of a contralateral testis from torsion-detorsion group (H-E stain x95).

Fig.4- The contralateral testis of a rat from the incarcerated inguinal hernia group (H-E stain x95).

Discussion

Since incarcerated inguinal hernias are commonly seen in prepubertal children[1], we performed the present experiment on sexually immature animals[13]. Although the representativeness of 30-day-old rats as a prepubertal model has been opposed[15], we believe that it resembles the situation of prepubertal children[4].

In experimental studies evaluating the contralateral testicular effects of unilateral testicular torsion, MSTD and the presence or absence of spermatozoa within a seminiferous tubule have been employed[15]. However, the testicular biopsy score has been suggested to be a more reliable method for evaluating the status of a testis[14]. Therefore, MSTD and MTBS have been used for testicular assessment.

Similar to previously reported alterations[16], our sham operation affected the maturity of the germinal epithelium of the ipsilateral testes compared to the contralateral ones, but the MSTD did not differ and spermatogenetic activity was still well preserved. We found that experimentally induced unilateral incarcerated inguinal hernias caused a decrease in MSTD and a depression in MTBS among the ipsilateral testes compared to controls. Although spermatogenetic activity was better in the contralateral testes compared to the ipsilateral testes, contralateral testicular histologic deterioration also accompanied ipsilateral testicular histologic alterations following unilateral incarcerated inguinal hernia. Contralateral testicular alterations, which were expressed in terms of decreased MSTD and depressed MTBS were observed after unilateral testicular torsion. The histologic alterations encountered in the ipsilateral and contralateral testes of the prepubertal rats subjected to unilateral incarcerated inguinal hernia and in the contralateral testes of the prepubertal rats subjected to unilateral testicular torsion were similar to the utilized parameters.

Experimentally induced unilateral incarcerated inguinal hernia results in histologically deteriorated ipsilateral and contralateral testes in prepubertal rats. The effect of unilateral inguinal hernia on subsequent fertility remains to be clarified.

Summary

The ipsilateral and contralateral testes after unilateral incarcerated inguinal hernia were evaluated, and compared to the contralateral testis after unilateral testicular torsion in 30 prepubertal rats. Control, torsion and detorsion at 24 hours, and incarcerated inguinal hernia and reduction in the 24 hour groups, each consisting of ten rats were established. The testes were harvested after 15 days. Mean seminiferous tubular diameters (MSTD) and mean testicular biopsy scores (MTBS) were determined and compared.

A decrease in MSTD and depression in MTBS, which was more prominent in the ipsilateral testes, were found in both ipsilateral and contralateral testes following

unilateral incarcerated inguinal hernia. The testicular damage encountered after unilateral incarcerated inguinal hernia was similar to the contralateral testicular damage following unilateral testicular torsion with the utilized parameters.

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