

Effect of Unilateral Iatrogenic Testicular Torsion on the Contralateral Testis in Rats: Prepubertal and Postpubertal

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Purpose: The present study was conducted to investigate the influence of hemicastration and age at hemicastration on the contralateral testis weight and function/testosterone production.

Materials and Methods: 64 Wistar-derived male rats were divided randomly into 4 groups. Group 1 was named immature intervention, group 2 immature control, group 3 mature intervention, and group 4 mature control. In group 1, rats were hemicastrated at 30 days of age (prepubertal). In group 2, sham surgery (midscrotal incision) was performed at the same age. In group 3, rats were hemicastrated at 70 days of age (postpubertal) and in group 4, sham surgery was done at the same age. Twenty days after the first surgery, contralateral orchiectomy was performed in intervention groups and controls underwent random orchiectomy (left or right). Blood sampling for evaluation of serum testosterone was performed just before second surgery.

Results: The mean testis weight (1692 ± 26.7 in group 1 versus 1375 ± 39.7 in group 2; $P < .001$ and 1760 ± 26.6 in group 3 versus 1425 ± 44.9 in Group 4; $P < .001$) and the mean testicular weight (mg) per 100 g of body weight (735.8 ± 82.3 in group 1 versus 634.8 ± 84.8 in group 2; $P = .005$ and 652.4 ± 61.4 in group 3 versus 572.6 ± 97.7 in group 4; $P = .03$) were significantly greater in hemicastrated rats as to their controls. Also, these parameters were greater in prepubertal group than postpubertal hemicastrated rats. There was no appreciable difference in serum testosterone levels across the 4 groups ($P = .77$).

Conclusion: Our research demonstrated that hemicastration results in compensatory hypertrophy of the remaining testis and it decreases as the animals age. Hemicastration does not lead to reduction in serum testosterone levels and the remaining testis can retrieve a normal serum testosterone level.

Keywords: compensatory hypertrophy; iatrogenic testicular torsion; rat; testis; unilateral orchiectomy

INTRODUCTION

In paired organs, the removal of or injury to one organ may result in compensatory hypertrophy of the remaining organ. This phenomenon has previously been proved in the kidneys, thyroid glands, and ovaries⁽¹⁻³⁾. The time of unilateral testis removal (prior to and following puberty) due to undescended testis (UDT), trauma, and testicular abscess affects the amount of compensatory hypertrophy. That is, compensatory hypertrophy of the testis occurs more frequently before puberty. Torsion of the spermatic cord is associated with restriction and interruption of testicular blood flow. Anatomical abnormalities (the bell-clapper deformity caused by lack of normal attachment of the epididymis to the tunica vaginalis which leads to incomplete fixation of the testis and the epididymis to the scrotum or abnormally high attachment of the testis to the epididymis), cold weather, sudden movements or trauma which activate the cremasteric reflex, and rapid growth of the testis throughout puberty make individuals prone to this medical condition. The symptoms of testicular torsion include a sudden and intense scrotal

pain which has started within the last six hours, vomiting, nausea, scrotal edema and erythema, fever as well as dysuria. On physical examination, tenderness of the scrotum, absence of cremasteric reflex, a higher testicular position, abnormal position of the epididymis on the anterior, thickening of the spermatic cord, testicular induration, loss of the grooves between the testis and the epididymis along with scrotal edema and erythema might also be observed^(4,5). In order to diagnose testis torsion, radionuclide scanning, color doppler ultrasound (CDUS), and high resolution ultrasound (HRUS) can be used⁽⁶⁻⁸⁾. The presence of sexual findings is an indication for surgical exploration with detorsion and fixation as the treatment of choice⁽⁹⁻¹¹⁾. The prognosis and long-term outcomes of torsion are unknown. Nevertheless, considering the recent studies it is indicated that ischemic injury is likely to occur quickly, even if the testis appears viable during detorsion^(11,12). As testicular development in humans is akin to rats, it is assumed that the use of an experimental model in rats provides beneficial evidence for further research on the morphology and histology of the testis⁽¹³⁾. In the present study, com-

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Table 1. Comparison of body weights across groups before the intervention.

Group	Mean Weight (SD), g	Minimum Weight, g	Maximum Weight, g	P-value
G1 (n = 15)	151 (7.3)	140	165	.94 ^a
G2 (n = 16)	149 (13.1)	117	165	
G3 (n = 16)	231 (12.3)	208	259	.34 ^b
G4 (n = 15)	224 (13.1)	195	241	

Abbreviations: G: group; M: mean; SD: standard deviation, g: gram.

a: Presents the comparison of body weights before the intervention between Group1 and Group 2

b: Presents the comparison of body weights before the intervention between Group3 and Group 4

pensatory hypertrophy caused by iatrogenic torsion of the testis on the contralateral side is investigated in an experimental rat model prior to and following puberty and then compared with the control group.

MATERIALS AND METHODS

Study Population

In this investigation, the effect of iatrogenic torsion (in order to do a unilateral functional orchiectomy, but not surgical orchiectomy) was explored in the contralateral testis of either mature or immature rats. A total of 64 male Wistar rats with 20 days of age and an average weight of 60 ± 8 gr were purchased from an animal library in Mashhad University of Medical Sciences. The experimental room was automatically air-conditioned once each 3 minutes and maintained in the standard temperature of 20-22 °C (SD: ± 2 °C), a humidity of 55%, and an 12 hour day-night cycle. The rats spent one week for quarantine and acclimation in the room before the start of the experiment. They were subsequently assigned into four groups (n = 16) at random.

Study Design and Procedures

Group 1: After weighing, the immature rats in the first group underwent a unilateral iatrogenic torsion in 30 days of age. Either a right or left iatrogenic torsion was conducted randomly. After 20 days (at 50 days of age), their testis and body weight was measured again and general anesthesia was induced before collecting a supraorbital blood sample from the cavernous sinus to determine the plasma testosterone. Thereafter, the remaining testis was also removed and weighed⁽¹⁴⁾ using a high-precision balance (readable to the nearest 0.0001 g).

Group 2: This group served as control immature rats. They were weighed on the 30th day and underwent sham surgery with scrotal incision on their skin after general anesthesia. Blood samples were obtained from the same vein and unilateral orchiectomy were carried out randomly before the second weight measurement of the testes.

Group 3: Akin to Group 1, all mature rats in this group underwent a unilateral iatrogenic torsion on the 10th

week. On the 90th day, they were weighed and underwent general anesthesia, blood sampling, as well as contralateral orchiectomy. In the long run, the weight of the testis was evaluated.

Group 4: At the same time as group 3, mature rats in this group were treated with sham operation. A right (n = 8) or left (n = 6) orchiectomy was randomly performed after 20 days of sham surgery and weight of the testes was measured thereafter.

An ELISA assay was used to determine the plasma level of testosterone by a commercially available Testosterone Rat/Mouse ELISA kit.

This study was approved by Mashhad University of medical Sciences ethics committee and investigators had been certified to study on laboratory animals.

Surgical technique

All rats underwent general anesthesia before surgery. After making a unilateral incision on the scrotum, iatrogenic torsion was performed and the testis was subsequently fixed by 4-0 nylon thread sutures in order to prevent detorsion. Incisions were sutured finally. We performed unilateral iatrogenic torsion to induce unilateral functional orchiectomy without any surgical orchiectomy intervention in the first step. Surgical orchiectomies were only carried out in the last step in order to weigh the testes.

Both of the control groups underwent sham surgery with scrotal incision on their skin after general anesthesia and then incisions were closed by sutures.

Statistical Analysis

The data was collected and entered into SPSS (Version 11.5, IBM, Chicago, IL, USA). The concentration of serum testosterone was compared among groups by analysis of variance (ANOVA) and independent sample t-test. A P-value of less than .05 was considered significant.

RESULTS

In this study, rats were randomly divided into four groups, mature and immature ones underwent unilateral iatrogenic torsion in addition to their corresponding controls. During the study period, two rats from Group

Table 2. Comparison of body weights a across groups after the intervention.

Group	Mean Weight (SD), g	Minimum Weight, g	Maximum Weight, g	P-value
G1 (n = 15)	231 (19.4)	194	263	.17 ^a
G2 (n = 16)	217 (15.2)	191	248	
G3 (n = 16)	271 (17.5)	248	310	.02 ^b
G4 (n = 15)	250 (22.2)	203	285	

Abbreviations: G: group; M: mean; SD: standard deviation, g: gram.

a: Presents the comparison of body weights after the intervention between Group1 and Group 2

b: Presents the comparison of body weights after the intervention between Group3 and Group 4

Table 3. Comparison of the contralateral testis weight of the rats after the intervention.

Group	Mean Weight (SD), mg	Minimum Weight, mg	Maximum Weight, mg	P-value
G1 (n = 15)	1692(26.7)	1540	1860	< .001 ^a
G2 (n = 16)	1375(39.7)	1060	1650	
G3 (n = 16)	1760(26.6)	1540	1900	< .001 ^b
G4 (n = 15)	1425(44.9)	980	1680	

Abbreviations: G: group; M: mean; SD: standard deviation, mg: milligram.

a: Presents the comparison of the remaining testis weight after the intervention between Group 1 and Group 2

b: Presents the comparison of the remaining testis weight after the intervention between Group 3 and Group 4

1 and 4 died, thus 62 were included in the final analysis. Table 1 and Table 2 show the mean body weight of the study groups prior to and following intervention. There was no remarkable difference across the groups before intervention ($P > .05$) (Table 1). It was revealed that a unilateral iatrogenic torsion did not have any impact on body growth and rats' weight at prepubertal age ($P = .17$). On the contrary, performing this surgery following puberty was found to significantly accelerate body growth and increase rats' weight from 250 ± 22.2 g in Group 4 to 271 ± 17.5 g in Group 3 ($P = .02$).

Moreover, the weight of the contralateral testis was remarkably higher in Group 3 compared with Group 1 ($1760(26.6)$ mg vs. $1692(26.7)$ mg; $P < .001$), and Group 4 was also higher than Group 2 ($1425(44.9)$ mg vs. $1375(39.7)$ mg; $p < .001$). Therefore, it could be concluded that unilateral iatrogenic torsion possibly causes hypertrophy in the contralateral testis and is associated with a higher degree of compensatory hypertrophy at postpubertal age (Table 2& Table 3).

A significant difference was observed in the mean weight of the remaining testis in Group 1 when compared to Group 2 (1692 ± 26.7 vs. 1375 ± 39.7 ; $P < .001$). A similar increase was evident in Groups 3 and 4 (from 1425 ± 44.9 mg to 1760 ± 26.6 mg; $P < .001$). Pairwise comparisons between Groups 1 and 3, as well as Groups 2 and 4 indicated no statistically significant difference ($P = 0.52$ and $P = 0.75$, respectively).

As the weight of the testis is contingent on the body weight of the animal, the ratio of testis weight (mg) to total body weight was measured in each rat in order to minimize any possible errors in estimating compensatory hypertrophy of the remaining testis. This ratio was defined as the mean testis weight (mg) per 100 gram of body weight. Table 4 summarizes the results of this analysis. As shown, groups 1 and 3 presented with a notably higher ratio than their corresponding controls ($P < .05$). Furthermore, this ratio was significantly higher in Group 1 compared to Group 3; $P = .02$, however, the other two groups demonstrated comparable ratios ($P = .15$). Additionally, the plasma testosterone levels were statistically shown to be alike across the study groups

($P = .77$) (Table 5). Thus, unilateral iatrogenic torsion and the time of which it is induced does not seem to have an effect on the level of testosterone as evident by the remaining testis being able to compensate for desirable testosterone levels.

DISCUSSION

The removal of or injury to one pair of a paired organ may result in compensatory hypertrophy of the remaining organ. This phenomenon has been previously proved in the kidneys, thyroid glands, and ovaries^(2,3,15). Removal of unilateral testis due to undescended testis (UDT), trauma, and testicular abscess causes great difficulties in future. It is hypothesized that the unilateral testis removal ends up in compensatory hypertrophy of the contralateral testis. However, this occurs under normal circumstances when the testis is palpable in the usual position⁽³⁾. On the other hand, testicular removal prior to and following puberty is influential on the degree of compensatory hypertrophy. If the testis is removed at prepubertal age, more compensatory hypertrophy occurs than after puberty⁽¹⁶⁾. Orchiectomy is recommended for prepubertal patients who are afflicted with UDT, torsion, and testicular trauma and whose probability of testicular loss is high. Nowadays, some surgeons, in spite of testicular loss, still insist on retaining the appearance of the testes. The removal of an injured testis which has lost its viability and spermatogenesis is more likely to increase FSH and then cause compensatory hypertrophy of the contralateral testis^(17,18). Furthermore, contralateral testicular injury on affinity of anti-sperm antibody may induce damage to the healthy testis and cause hypofertility in future^(12,17). As testicular development in humans is akin to rats, it is assumed that the use of an experimental rat model provides beneficial evidence for further research on the morphology and histology of the testis. Considering the results of the present study it is concluded that a unilateral functional orchiectomy at postpubertal age leads to an increase in body growth, which is corroborated by Putra et al.⁽¹⁹⁾ Also, a unilateral functional orchiectomy came up with compensatory hypertrophy of the

Table 4. Comparison of the contralateral testis weight (mg) per 100g of body weight after the intervention.

Group	Mean testis weight (mg)/100g of body weight (SD)	Minimum testis weight (mg) / 100g of body weight	Maximum testis weight (mg) / 100g of body weight	P-value
G1 (n = 15)	735.8(82.3)	618.5	853.7	.005 ^a
G2 (n = 16)	634.8(84.8)	495.3	774.5	
G3 (n = 16)	652.4(61.4)	496.8	746.0	.03 ^b
G4 (n = 15)	572.6(97.7)	343.9	704.4	

Abbreviations: G: group; M: mean; SD: standard deviation

a: Presents the comparison of the ratio of the remaining testis weight (mg) per 100g of body weight between Group 1 and Group 2

b: Presents the comparison of the ratio of the remaining testis weight (mg) per 100g of body weight between Group 3 and Group 4

Table 5. Comparison of the serum level of testosterone (ng/mL) across groups after the intervention.

Group	Mean serum level (SD)	Minimum serum level	Maximum serum level	P-value
G1 (n = 15)	3.5 (1.9)	1.0	7.2	.77
G2 (n = 16)	3.4 (2.4)	0.8	9.5	
G3 (n = 16)	3.1(1.9)	0.7	7.2	
G4 (n = 15)	3.9 (2.4)	0.6	9.5	

Abbreviations: G: group; M: mean; SD: standard deviation

contralateral testis, which is in agreement with Putra, Lin, Simorangkir, and SaneFuji's findings yet contradicts Romero's findings^(3,17,19,20,21,22,23). Having a unilateral functional orchiectomy prior to puberty is associated with more compensatory hypertrophy in the contralateral testis. This is supported by Putra, Furuya, Cunningham, and Tusti where as some researchers have reported the opposite effect^(19,24,25-28). Besides, in this study, a unilateral functional orchiectomy was not correlated to the plasma level of testosterone, with the remaining testis being able to compensate for diminished testosterone concentrations. In spite of Ahmadi's results, this is confirmed by Furuye et al.^(25,29) It has been reported that the size of the testis is directly related to the amount of spermatogenesis. Given the occurrence of compensatory hypertrophy in the contralateral testis, it would be expected that normal spermatogenesis and fertility can be preserved⁽²¹⁾.

There were some limitations to our study including absence of histopathologic study, and fertility status assessment. Also there was no evaluation of testis weight before the intervention. Compensatory hypertrophy could be the consequence of multiple factors including unilateral torsion and we did not assess the role of other possible factors.

CONCLUSIONS

Our research demonstrated that hemicastration results in compensatory hypertrophy of the remaining testis and the degree of compensation is inversely associated with the age of rats (prepubertal or postpubertal). Hemicastration did not lead to reduction in serum testosterone levels and the remaining testis retrieved a normal serum testosterone level. It is recommended that future studies investigate histopathologic changes, fertility status and also other possible factors which could lead to compensatory hypertrophy of the contralateral testis. This study paved the way for further research on larger animals such as dogs, cats, rabbits, and goats.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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