**Effect of Unilateral Iatrogenic Torsion on Contralateral Testis in Rat, Prepubertal and Postpubertal**

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**Keywords:** compensatory hypertrophy; iatrogenic torsion; rat; testis; unilateral orchiectomy
ABSTRACT

**Purpose:** The present study was conducted to investigate the influence of hemicastration and age at hemicastration on the subsequent contralateral testis.

**Materials and Methods:** 64 Wistar-derived male rats divided randomly in 4 groups. Group 1 named immature intervention, group 2 immature control, group 3 mature intervention and group 4 mature control. In group 1, rats hemicastrated at 30 days of age (prepubertal). In group 2 sham surgery (midscrotal incision) was done at same age. In group 3 rats hemicastrated at 70 days of age (postpubertal) and in group 4 sham surgery was done at same age. 20 days after first surgery, contralateral orchiectomy was done in intervention groups and random orchiectomy (left or right) was done in control groups. Blood sampling for evaluation of serum testosterone was performed just before second surgery.

**Results:** Testis weight (1692 ± 26.7 in Group 1 vs 1375 ± 39.7 in Group 2; $P<0.001$ and 1760 ± 26.6 in Group 3 vs 1425 ± 44.9 in Group 4; $P<0.001$) and the mean testicular weight (mg) per 100 g of body weight (735.8 ± 82.3 in Group 1 vs 634.8 ± 84.8 in Group 2; $P=0.005$ and 652.4 ± 61.4 in Group 3 vs 572.6 ± 97.7 in Group 4; $P=0.03$) was greater in hemicastrated rats. These parameters was greater in prepubertal group than postpubertal hemicastrated rats. There was no appreciable difference in serum testosterone levels in 4 groups ($P=0.77$).

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

The removal of or injury to one of a paired organs may result in compensatory hypertrophy of the remaining organ. This phenomenon has been proven in kidney, thyroid, 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. Those who represent 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 with activation of cremasteric reflex, and rapid growth of the testis throughout puberty are prone to this medical condition. The symptoms of testicular torsion include sudden and intense scrotal pain which has been mostly started within the last six hours, vomiting, nausea, scrotal edema and erythema, fever as well as dysuria. On physical examination, tenderness of the scrotum, lack of cremasteric reflex, a higher testicular position, abnormal position of the epididymis on the anterior, thickening in the spermatic cord, testicular induration, loss of the grooves between the testis and the epididymis along with scrotal edema and erythema might be also 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 in torsion are unknown. Nevertheless, considering the recent studies it is indicated that ischemic injury is more 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, compensatory hypertrophy caused by iatrogenic torsion testis on the contralateral side is investigated in the experimental rat model prior to and following puberty and then compared with that in 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) is explored in the contralateral testis of either mature or immature rats. In doing so, a total 64 male Wistar rats with 20 days of age and an average weight of 60±8 gr was purchased from an animal library in the Mashhad University of Medical Sciences. The experimental room was automatically air-conditioned once each 3 minutes and maintained at the standard temperature of 20-22 °C (SD: ±2 °C), a humidity of 55%, and 12 h 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 (two rats from Group 1 and 4 died).

**Study Design and Procedures**

Group 1: After weighing, the immature rats in the first group underwent unilateral iatrogenic torsion in 30 days of age. A right or left iatrogenic torsion was conducted randomly. Following 20 days (at 50 days of age), their 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 by using a high-precision balance (to the nearest 0.0001 g).

Group 2: This served as the control immature rats. They were weighed on the 30th day and underwent sham surgery with scrotal incision on their skin after general anesthesia. In the same vein, blood sampling was obtained and unilateral orchiectomy were carried out random 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 detected.

Group 4: At the same time, mature rats 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 level of testosterone by a commercially available Testosterone Rat/Mouse ELISA kit.
Study proposal is approved in Mashhad University of Medical Sciences Ethics Committee and investigators had certificated to study on laboratory animals.

**Surgical technique**

Rats underwent general anesthesia. After making a unilateral incision on scrotum, iatrogenic torsion was performed and fixed by nylon 4-0 thread sutures in order to prevent detorsion. Incision sutured finally. We performed unilateral iatrogenic torsion to induce unilateral functional orchiectomy and no surgical orchiectomy was done in the first step of surgical interventions. Surgical orchiectomies only done in the last step in order to weight the testes.

Control groups underwent sham surgery with scrotal incision on their skin after general anesthesia and closure of incision by sutures.

**Statistical Analysis**

The data were collected and fed into SPSS (Version 11.5, IBM). The concentration of testosterone was compared among the groups by analysis of variance (ANOVA) and independent sample T-Test. A $P$-value less than 0.05 was considered as significant.

**RESULTS**

In this study, a total of 62 rats were randomly divided into four groups, mature and immature ones undergoing unilateral iatrogenic torsion addition to their corresponding controls. Tables 1 and 2 show the mean weights of the study groups prior to and following the intervention. There was no remarkable difference between all groups ($P > 0.05$) (Table 1).

It was revealed that a unilateral iatrogenic torsion did not impact on body growth and rats’ weight at prepubertal age ($P = 0.17$). On the contrary, performing this surgery following puberty was found to significantly accelerate body growth and increase rats’ weight from $250 \pm 22.2$ g in Group 4 to $271 \pm 17.5$ g in Group 3 ($P = 0.02$).

Moreover, the weight of the contralateral testis was remarkably on the rise in Groups 3 more than Groups 1 ($271 \pm 17.5$ g vs $231 \pm 19.4$ g; $P < 0.001$), and Group 4 more than Group 2 ($250 \pm 22.2$ g vs $217 \pm 15.2$ g; $p < 0.001$). Therefore, it could be concluded that unilateral iatrogenic torsion
causes hypertrophy in the contralateral testis. Also, unilateral iatrogenic torsion is associated with higher degree of compensatory hypertrophy in the contralateral testis at pubertal age (Table 2).

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 < 0.001). The similar increase was evident in Groups 3 and 4 (from 1425 ± 44.9 mg to 1760 ± 26.6 mg). Pairwise comparisons between Groups 1 and 3, as well as Groups 2 and 4 indicated no statistically significant difference (P = 0.52, P = 0.75) (Table 3).

As the weight of the testis is contingent on the body weight of the animal, the ratio of testis weight (mg) to bodyweight in each rat was measured in order to minimize probable risks in estimating compensatory hypertrophy of the remaining testis. This ratio was defined as testis weight (mg) per 100 gram of body weight. Table 4 summarizes the ratio of the remaining testis to body weight of the rats.

Groups 1 and 3 presented a notably higher ratio than the corresponding control (Groups 2 and 4, in order) (P < 0.05) (Table 4). Furthermore, this ratio was also significantly increased in Group 1 more than Group 3 (p = 0.02) however the other two groups demonstrated comparable ratios (P = 0.15). Additionally, the plasma testosterone levels were shown statistically alike by the study groups (P = 0.77) (Table 5). Thus, unilateral iatrogenic torsion and time of the surgery had no effect on the level of testosterone, thus, the remaining testis can afford to compensate for desirable testosterone levels.

**DISCUSSION**

The removal of or injury to one of a paired organs may result in compensatory hypertrophy of the remaining organ. This phenomenon has been proven in kidney, thyroid, and ovaries (2,3,15). The unilateral testis removal due to undescended testis (UDT), trauma, and testicular abscess causes great difficulties in the future. It is hypothesized that the unilateral testis removal ends up compensatory hypertrophy in the contralateral testis. However, this occurs in the normal condition where 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 it is after
Orchiectomy is recommended for prepubertal patients who are afflicted with UDT, torsion, and testicular trauma and whose probability of testicular loss is high. Today, some surgeons, although assuring the testicular loss, still insist on retaining the appearance of the testes. The removal of an injured testis which 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 hypofertility in future \(^{12,17}\). 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. 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 contralateral testis, which is in agreement with Putra, Lin, Simorangkir, Sane Fuji, and yet contradicts Romero’s findings \(^{17,19,20,22,23}\). Also, a unilateral functional orchiectomy came up with compensatory hypertrophy of the contralateral testis, which is in agreement with Putra, Lin, Simorangkir, Sane Fuji, and yet contradicts Romero’s findings \(^{17,19,20,22,23}\). 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, Tusti where as other scholars have reported the opposite effect (Thompson, Romero, Frankel) \(^{24}\) \(^{19,25-28}\). More to the point, a unilateral functional orchiectomy is not correlated to the plasma level of testosterone, with the remaining testis being able to compensate for diminishing the testosterone concentrations. In spite of Ahmadi’s results, this is confirmed by Furuya \(^{25}\) \(^{29}\). 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 are some limitations in our study included: absence of histopathologic study, absence of fertility status assessment and also there was no evaluation of testis weight before the intervention. Compensatory hypertrophy could be the consequence of multiple factors including the unilateral torsion and we did not assess the role of other possible actors.
CONCLUSION

Our research demonstrated that hemicastration resulted in compensatory hypertrophy of the remaining testis and it decreased as the animals aged. Hemicastration does not lead to reduction in serum testosterone levels and remaining testis can retrieve a normal serum testosterone level. It is recommended for future studies to investigate on histopathologic changes, fertility status and also on other possible factors which could lead into compensatory hypertrophy of the contralateral testis. This study paved the way for further research on larger animals such as dogs, cats, rabbits, and goats.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

The authors report no conflict of interest.

REFERENCES


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### Tables

**Table 1. A comparison of weights among the groups before the intervention**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (M ± SD), g</th>
<th>Minimum Weight, g</th>
<th>Maximum Weight, g</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (n = 15)</td>
<td>151 ± 7.3</td>
<td>140</td>
<td>165</td>
<td>0.94a</td>
</tr>
<tr>
<td>G2 (n = 16)</td>
<td>149 ± 13.1</td>
<td>117</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>G3 (n = 16)</td>
<td>231 ± 12.3</td>
<td>208</td>
<td>259</td>
<td>0.34b</td>
</tr>
<tr>
<td>G4 (n = 15)</td>
<td>224 ± 13.1</td>
<td>195</td>
<td>241</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** G: Group; M: Mean; SD: Standard Deviation  

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

**Table 2. A comparison of weights among the groups after the intervention**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (M ± SD), g</th>
<th>Minimum Weight, g</th>
<th>Maximum Weight, g</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (n = 15)</td>
<td>231 ± 19.4</td>
<td>194</td>
<td>263</td>
<td>0.17a</td>
</tr>
<tr>
<td>G2 (n = 16)</td>
<td>217 ± 15.2</td>
<td>191</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>G3 (n = 16)</td>
<td>271 ± 17.5</td>
<td>248</td>
<td>310</td>
<td>0.02b</td>
</tr>
<tr>
<td>G4 (n = 15)</td>
<td>250 ± 22.2</td>
<td>203</td>
<td>285</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** G: Group; M: Mean; SD: Standard Deviation  
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. A comparison of the weight of the remaining testis of the rats after the intervention**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (M ± SD), mg</th>
<th>Minimum Weight, mg</th>
<th>Maximum Weight, mg</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (n = 15)</td>
<td>1692 ± 26.7</td>
<td>1540</td>
<td>1860</td>
<td>&lt; 0.001a</td>
</tr>
<tr>
<td>G2 (n = 16)</td>
<td>1375 ± 39.7</td>
<td>1060</td>
<td>1650</td>
<td></td>
</tr>
<tr>
<td>G3 (n = 16)</td>
<td>1760 ± 26.6</td>
<td>1540</td>
<td>1900</td>
<td>&lt; 0.001b</td>
</tr>
<tr>
<td>G4 (n = 15)</td>
<td>1425 ± 44.9</td>
<td>980</td>
<td>1680</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** G: Group; M: Mean; SD: Standard Deviation  
a: Presents the comparison of the remaining testis weight after the intervention between Group1 and Group 2  
b: Presents the comparison of the remaining testis weight after the intervention between Group3 and Group 4
Table 4. A comparison of the ratio of the remaining testis weight (mg) per 100g of body weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Ratio (M ± SD), mg testis weight/100g of body weight</th>
<th>Minimum Ratio, mg testis weight/100g of body weight</th>
<th>Maximum Ratio, mg testis weight/100g of body weight</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (n = 15)</td>
<td>735.8 ± 82.3</td>
<td>618.5</td>
<td>853.7</td>
<td>0.005a</td>
</tr>
<tr>
<td>G2 (n = 16)</td>
<td>634.8 ± 84.8</td>
<td>495.3</td>
<td>774.5</td>
<td></td>
</tr>
<tr>
<td>G3 (n = 16)</td>
<td>652.4 ± 61.4</td>
<td>496.8</td>
<td>746.0</td>
<td>0.03b</td>
</tr>
<tr>
<td>G4 (n = 15)</td>
<td>572.6 ± 97.7</td>
<td>343.9</td>
<td>704.4</td>
<td></td>
</tr>
</tbody>
</table>

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. A comparison of the level of testosterone

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (M ± SD), ng/mL</th>
<th>Minimum Concentration, ng/mL</th>
<th>Maximum Concentration, ng/mL</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (n = 15)</td>
<td>3.5 ± 1.9</td>
<td>1.0</td>
<td>7.2</td>
<td>0.77</td>
</tr>
<tr>
<td>G2 (n = 16)</td>
<td>3.4 ± 2.4</td>
<td>0.8</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>G3 (n = 16)</td>
<td>3.1 ± 1.9</td>
<td>0.7</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>G4 (n = 15)</td>
<td>3.9 ± 2.4</td>
<td>0.6</td>
<td>9.5</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: G: Group; M: Mean; SD: Standard Deviation