The Effect of Iatrogenic Vas Deferens Injury on Fertility in an Experimental Rat Model

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The long-term result of accidental crushing of the vas deferens during inguinal surgery is unpredictable. It is stated that even a slight disturbance in the muscular layer or mucosa may endanger fertility. This study was designed to investigate the early and late histopathologic changes of iatrogenic injury on the vas and its effect on fertility in a rat model. Both vasa deferentia of 54 male rats, divided into three groups (n = 18 each), were subjected to an operative manipulation. These manipulations consisted of digital compression for 45 seconds in group A, grasping with a mosquito clamp for 2 seconds in group B, and grasping with a mosquito clamp for 2 minutes in group C. In five rats from each group, the vasa were removed on the third and 21st postoperative day for histological evaluation. The remaining 13 rats in each group were allowed to mate for a period of 4 months. Bilateral vasa were analyzed for patency and histology. A spermatic granuloma was observed in 79% of the vas deferens in vitro, whereas a statistical significance was present between these two groups and group A (P < 0.001). It can be said that type rather than time of injury is important in this experimental model, because the results of the patency test alone are sufficient to prove the possibility of functional damage in vas deferens. The authors conclude that inguinal contents should be handled carefully and contralateral exploration in asymptomatic cases older than 1 year with inguinal pathology should be avoided.

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INDEX WORDS: Vas deferens, injury, fertility.

MOST INFANT SURGERY deals with inguinal pathology. During these surgical procedures the vas deferens and vessels are involuntarily manipulated. The accidental crushing of inguinal contents is at times unavoidable and the result unpredictable. It may not be as harmful as iatrogenic separation, but some sort of disturbance in the continuity of the vas may have a long-lasting functional effect.

The aim of this study was to determine the effects of accidental clamping of the vas deferens on early and late histopathologic changes and fertility in an experimental rat model.

MATERIALS AND METHODS

Fifty-four male Wistar-Albino rats (200 to 250 g) were equally divided into three groups. All rats were proven fertile by a 3-week fertility test in which pregnancy was observed in any of three females. Under intraperitoneal xylazin hydrochloride (Rompun), 9 mg/kg, anesthesia, bilateral inguinal contents were exposed through a lower abdominal incision. Both vasa deferentia in each group were subjected to a different operative manipulation. These manipulations were digital compression for 45 seconds in group A, clamping with a mosquito clamp in fully locked fashion for 2 seconds in group B, and identically clamping for 2 minutes in group C. The nonvascular areolar tissue adjacent to the manipulation area was tagged with a 5-0 silk suture for future identification. The inguinal contents were replaced, and the abdominal incision was repaired. The rats did not receive any medical treatment, those that suffered from infection were discarded from the study and replaced.

In five rats from each group, the vasa deferentia were removed consecutively from one side on the third day and the other side on the 21st day postoperatively. The specimens were fixed in 10% buffered formalin solution, embedded in paraffin blocks and sectioned longitudinally at 4 to 5 μm. Sections were stained with hematoxylin and eosin (H&E) for gross changes in structure. Specimens were additionally sectioned and stained with Masson’s trichrome to show fibrotic activity.

The remaining 39 rats, 13 in each group, were tested for fertility starting from 6 weeks after the initial procedure. Each male was placed in a cage with three adult females. Any male that failed to produce pregnancy after three different intervals during a period of four months was considered infertile.

The study was terminated 6 months after the initial procedure for each rat. Bilateral vasa and testes were removed for histological evaluation as described above. Each vas was tested for degree of patency in fertile rats. The vas deferens was divided, so that the specimen contained the crushed site and any spermatic granuloma present. Under magnification the vas was cannulated using a 24-gauge angiocath. The cannula was advanced in the lumen until 1 cm proximal to the crushed site, and the distal end resected 1 cm distal to the crushed site. High-pressure flow rate was determined by collecting 0.9% saline solution flowing through the vas from a height of 90 cm into a beaker for 2 minutes. The volume of solution in the beaker was used to calculate the flow per minute (in mL/min). The average of three repeated tests was accepted as the high flow rate for each vas. This procedure was repeated identically from a height of 40 cm and accepted as the low flow rate for each vas.

Statistical evaluation of fertility was analyzed using the χ² test, and patency was analyzed using Student’s t test, with P = 0.05 considered statistically significant.

RESULTS

Histological study findings showed that digital compression leads to only mild inflammatory changes with normal architecture (columnar epithelium, three layers muscular coat, and thin external areolar coat) and mini-
IATROGENIC VAS DEFERENS INJURY: EFFECT ON FERTILITY

Fig 1. Spermatic granuloma formation in vas deferens 21 days after grasping with a mosquito clamp for two minutes. (H&E, original magnification ×40.)

ma1 to moderate inflammation in the surrounding tissue after 3 and 21 days in group A. There was a minimal periductal fibrosis 6 months after the initial manipulation. In groups B and C, muscular disruption in all layers and spermatic granuloma were present in all specimens after 3 and 71 days (Fig 1). Additionally, there was a full-thickness disruption in 20% (2 of 10) of vasa in group C.

We observed muscular disruption and reorganization in all specimens 6 months after initial manipulation in groups B and C (Fig 2). Additionally, there was 73% spermatic granuloma and 12% full-thickness disruption and reorganization with fibrosis in group B (Fig 3). There was 85% spermatic granuloma and 19% full-thickness disruption and reorganization with fibrosis in group C. Histopathologic study results of the testes showed minimal focal testicular atrophy in 31% and 42% of groups B and C, consecutively, at the end of the study (Table 1).

At the end of the patency test, we observed one obstructed lumen among all specimens obtained from fertile rats. There was a statistically significant difference between groups A and B and groups A and C (P < .001), regarding high and low flow rates. There was no statistical significance between groups B and C (P > .05; Table 2).

All rats in group A (100%) were able to produce conception in any of three females. Eleven rats (85%) in group B and ten rats (77%) in group C were able to produce conception. Statistical analysis showed no significant difference among the three groups. Histopathologic changes observed in the vasa deferentia of the five infertile rats are seen in Table 3.

DISCUSSION

Human vas deferens has a thick muscular coat, which constitutes three fourths of its total diameter. This anatomical structure is specific for the ejaculation process, which enables the sperm to be projected at high speed through the urethra. Any slight disturbance in its muscular layer or mucosa will jeopardize fertility.

Digital compression of the vasa resulted in minimal histological change at the end of 6 months, and all animals preserved fertility. Collection of sperm through a leak in the mucosa after trauma or vasovasostomy may lead to spermatic granuloma. There is no statistical significance in the formation of a spermatic granuloma between groups B and C because of duration of injury (Table 1). The adverse effect of such a spermatic granuloma on fertility is unclear.1,2 Our result is consistent with previously published series on the effect of spermatic granuloma in fertility. We observed a spermatic granuloma.

Table 1. Histopathologic Changes in Vasa Deferentia and Testes of Groups B and C After 6 Months

<table>
<thead>
<tr>
<th></th>
<th>Muscular Coat Disruption</th>
<th>Spermatic Granuloma (%)</th>
<th>Full-Thickness Disruption (%)</th>
<th>Minimal Focal Testicular Atrophy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>20/20</td>
<td>19/26 (73)</td>
<td>3/26 (12)</td>
<td>8/26 (31)</td>
</tr>
<tr>
<td>Group C</td>
<td>28/28</td>
<td>22/26 (85)</td>
<td>5/26 (19)</td>
<td>11/26 (42)</td>
</tr>
</tbody>
</table>

NOTE. *P > .05.
loma in 79% of fertile rats and 80% of infertile rats in groups B and C. From this perspective, we can deduce that the type of injury rather than time is important in this experimental model. It is also impossible to correlate an adverse effect on fertility with muscular disruption because the frequency of muscular disruption is identical in groups B and C.

A full-thickness disruption was observed in 2% of the vasa of 21 fertile rats and 70% of the vasa of five infertile rats from groups B and C. There was a statistically significant difference between fertile and infertile rats ($P < .001$).

There was no statistical difference between groups B and C in terms of high and low flow rates in vitro, whereas a statistical significance was present between these two groups and group A (Table 2). Carey et al. observed a $2.5 \pm 0.3$ mL/min high flow rate and $1.0 \pm 0.1$ mL/min low flow rate with spermatic granuloma, compared with $2.6 \pm 0.2$ mL/min and $1.2 \pm 0.1$ mL/min consecutive flow rates without spermatic granuloma after vasovasostomy in bilateral vasectomized rats. We can conclude that the injury we created had an adverse effect on the flow rate of the vas, possibly caused by spermatic granuloma or defective healing and fibrotic tissue formation at the injury site. Disturbances in patency did not have an adverse effect on the fertility of rats in the study. This may not be the case in human beings because Matsuda et al. state that 27% of subfertile cases repaired for inguinal hernia during childhood present with vas deferens obstruction. Muscular coat disruption alone may have an adverse effect on the projectile function of the vas in vivo. Histopathologic study of the testes showed minimal focal testicular atrophy in 36% of fertile and 40% of infertile rats. Chehval et al. demonstrated significant alterations in both the obstructed (vasectomy) testis and contralateral testis in 40% of immature rats and 90% of mature rats, although Flickinger et al. observed normal histological differentiation in testes after obstruction of the vas deferens in immature rats. Also in the previously published reports, vasectomy performed in rats and dogs was said to create degeneration in seminiferous tubules and decrease in bilateral testicular weight. Fischer and Mumenthaler have found 1% testicular atrophy in a series of 587 pediatric-age inguinal herniorrhaphy.

Fertility analysis showed statistical insignificance for all three groups in rats with vasa deferentia devoid of full-thickness disruption. We are cautious to interpret the results of this study because anatomy and physiology of the vas deferens in a rat model partially differs from that of human beings. Even though this is an animal model, the results of the patency test alone are sufficient enough to prove the possibility of functional damage in vas deferens. We agree with Janik and Shandling that great care should be taken in handling inguinal contents and to avoid contralateral exploration in asymptomatic cases older than 1 year.

### REFERENCES


### Table 2. Patency of the Vas Deferens After 6 Months in Fertile Rats

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>High flow rate, mL/min</td>
<td>3.41 ± 0.14*</td>
<td>2.56 ± 0.12†</td>
<td>2.55 ± 0.151</td>
</tr>
<tr>
<td>Low flow rate, mL/min</td>
<td>1.45 ± 0.16*</td>
<td>1.09 ± 0.101</td>
<td>1.08 ± 0.211</td>
</tr>
</tbody>
</table>

NOTE. Data are expressed as mean ± SEM.
*Group A v Group B and Group A v Group C, $P < .001$.
†Group B v Group C, $P > .05$.

### Table 3. Histopathologic Changes in Vasa Deferentia of Infertile Rats

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Group</th>
<th>Muscular Coat Disruption</th>
<th>Spermatic Granuloma</th>
<th>Full-Thickness Disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Unilateral</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Bilateral</td>
<td>Unilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Unilateral</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>Bilateral</td>
<td>Unilateral</td>
<td>Unilateral</td>
</tr>
</tbody>
</table>