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Original Article

Influence of different cutting combinations of cavernous nerves, cavernous vessels, and ischiocavernosus muscles on erectile dysfunction in rat models

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Abstract: Mainstream perspective suggests that nerve and vascular injuries are the major causes of organic erectile dysfunction (ED). However, some studies have found that bilateral ischiocavernosus muscle damage can cause organic ED, resulting in the complete loss of erectile function and mating ability in male rats. Combined injuries to the cavernous nerves, cavernous blood vessels, and ischiocavernosus muscles may be major reasons for ED associated with pelvic fractures. Cavernous nerves, cavernous blood vessels, and ischiocavernosus muscles are all bilaterally distributed. How different combinations of injuries to these three tissues influence ED has not yet been reported. By cutting cavernous nerves, blood vessels and ischiocavernosus muscles of male rats in different combinations and mating the males with reproductively active female rats, this study aimed to evaluate the influence of different cutting combinations on ED. This influence was assessed using pregnancy rates of the female rats. Results showed that uni/ipsilateral cutting combinations of the cavernous nerves, blood vessels, and ischiocavernosus muscles did not cause permanent ED, but bi/contralateral cutting combinations did cause permanent ED.

Keywords: Erectile dysfunction, etiology, injury, rats

Introduction

Incidence of erectile dysfunction (ED) after pelvic fractures can be as high as 30% and up to 42% when complicated by a urethral injury, making it a difficult problem in urological treatment [1-13]. In clinical practice, most patients suffering from ED after a pelvic fracture are young adults. ED often causes male infertility and the loss of mating ability, significantly affecting family harmony. Therefore, apart from concern for a patient's erectile function, equal attention should be paid to ED-associated infertility.

Currently, the mainstream perspective suggests that nerve and vascular injuries are major reasons for organic ED [1-13]. However, previous studies have found that bilateral ischiocavernosus muscle damage causes organic ED in male rats, including a complete loss of erectile function and mating ability [14-20]. Combined injuries to nerves, blood vessels, and ischiocavernosus muscles may be the major factors for ED associated with pelvic fractures [21-25]. Diagnosis of ED often depends on measuring intracavernous pressure (ICP). Sometimes drugs are used, as well as APO or nerve stimulation [26-30], but these methods are invasive and require relatively complex operations. The present research team designed a way to diagnose ED by mating male rats with females and observing pregnancy rates of the females. This has proven to be a practical and non-invasive method of diagnosis [14-20]. Cavernous nerves, blood vessels, and ischiocavernosus muscles have a bilateral distribution, but whether different combinations of damage to these three tissues affects the severity of ED has not yet been reported. An ideal study method would be to establish a living model with pelvic fractures, then examine the features of ED caused by neurological, vascular, and myogenic injuries. However, traumatic injuries

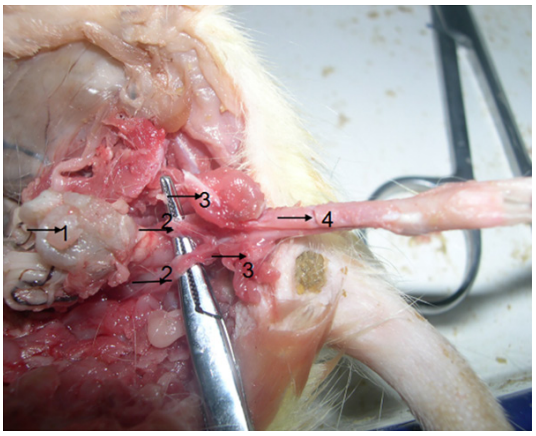

Figure 1. Anatomy of cavernous neurovascular bundles and ischiocavernosus muscles (1 bladder; 2 cavernous neurovascular bundles; 3 ischiocavernosus muscles; 4 penis).

are often combined with urethral injuries and bleeding, significantly affecting the survival of model rats with pelvic fractures. Therefore, the present research team designed different combinations of cutting the cavernous nerves, blood vessels, and ischiocavernosus muscles to simulate different types of damage after a pelvic fracture. After surgery, the male rats mated with non-pregnant females. The erectile function of males was determined by observing pregnancy rates of the females. Using this method, the current study assessed the influence of different damage combinations of the cavernous nerves, cavernous blood vessels, and ischiocavernosus muscles on ED, providing new insight for clinical diagnosis and treatment of ED after pelvic fractures.

Materials and methods

Experimental animals

A total of 600 Wistar rats (SPF degree; males and females) were purchased from the Experimental Animal Center of the Third Military Medical University. All animal operations were approved by the Animal Management Committee of Zunyi Medical College.

Animal grouping

Reproductive-age male rats (n = 300, age = 4-6 months, weight = 200-300 g) were randomly assigned to 15 groups: 1) Unilateral cavernous nerve cutting group (Uni-N cutting); 2) Unilateral cavernous blood vessel cutting group (Uni-V cutting); 3) Unilateral ischiocavernosus muscle cutting group (Uni-ischio cutting); 4) Ipsilateral cavernous nerve + blood vessel cutting group (Ipsi N + V cutting); 5) Ipsilateral cavernous nerve + ischiocavernosus muscle cutting group (Ipsi N + ischio cutting); 6) Ipsilateral cavernous blood vessel + ischiocavernosus muscle cutting group (Ipsi V + ischio cutting); 7) Ipsilateral cavernous nerve + blood vessel + ischiocavernosus muscle cutting group (Ispi N + V + ischio cutting); 8) Bilateral cavernous nerve cutting group (Bi-N cutting); 9) Bilateral cavernous blood vessel cutting group (Bi-V cutting); 10) Bilateral ischiocavernosus muscle cutting group (Bi-ischio cutting); 11) One cavernous nerve + contralateral cavernous blood vessel cutting group (One N + Contra V cutting); 12) One cavernous nerve + contralateral ischiocavernosus muscle cutting group (One N + Contra ischio cutting); 13) One cavernous blood vessel + contralateral ischiocavernosus muscle cutting group (One V + Contra ischio cutting); 14) Sham group; and 15) Control group. Each experimental group consisted of 20 rats. After surgery, the male rats were separately mated with reproductively active females (n = 300, age = 4-6 months, weight = 200-300 g) to determine the severity of ED.

Surgical equipment

The anesthetic used was 20% chloral hydrate. It was injected intraperitoneally, at a dose of 0.1 mL/100 g, while 0.5% povidone-iodine was used as disinfectant. Surgical tools included a vas deferens-separating clamp, pair of ophthalmic scissors, pair of suture scissors, a needle holder, and scalpels. Other tools used for surgery were two leather clamps, two forceps, 4 mosquito clamps, one small round needle, one triangular needle, and #1 and #4 sutures.

Model establishment and observation

Rats in each experimental group were weighed, anesthetized, and placed on the operating table. Using the principles of aseptic surgery, cavernous nerves, blood vessels, or ischiocavernosus muscles were cut, according to the group's surgical requirements (**Figure 1**). Cavernous nerves and blood vessels were cut through the abdominal cavity (**Figure 2**), while ischiocavernosus muscles were cut through the perineum (**Figure 3**). The surgical procedure was simulated in the sham group, but no cut-

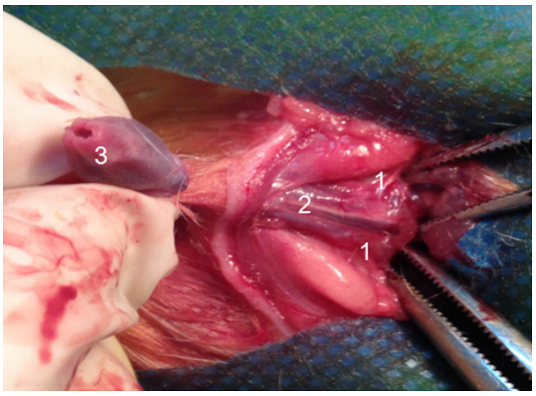

Figure 2. Bilateral cavernous nerves and blood vessels exposed through the abdominal cavity (1 cavernous nerves and vessels; 2 penis base, 3 exposed penis).

Figure 3. Ischiocavernosus muscles exposed through the perineum (1 ischiocavernosus muscles; 2 penis).

ting was performed. After surgery, long-acting penicillin was intraperitoneally injected at a dose of 125,000 units/rat to prevent infections. One week later, the model males were housed individually with non-pregnant female rats. Male rats in the control group (which did not receive surgery) were also mated with non-pregnant females. After 3 months of housing, the death of males and pregnancy and delivery times of the females in each group were recorded. Upon completion of the experiment, the rats were sacrificed by cervical dislocation.

Standard of erectile function in male rats

Male rats were bred with female rats. Erectile function was inferred indirectly by the fertilization of female rats. In addition, the failure of the paired female rats to conceive indicated that the male rats had erectile dysfunction. Delayed conception of the female rats indicated that the male rats had temporary erectile dysfunction. Pregnancy of the female rats at the normal time indicated that the male rats did not have erectile dysfunction. Normal erectile function and fertility of the male rats and their paired female rats were taken as references.

Statistical analysis

Statistical analysis was performed using SPSS 18.0 software. Inter-group comparisons of pregnancy rates were analyzed by x^2 test, while pairwise comparisons were made using x^2 test, with subgroupings. The time of first pregnancy and delivery are presented as mean ± SD. Time comparisons were made using Bonferroni's *t*-test. Statistical significance is defined as $P < 0.05$.

Results

Deaths of male rats

In each experimental group, 2-5 rats died after surgery. Reasons for the deaths included anesthesia, surgical trauma, and postoperative infections. Data of the males that died more than 7 days after surgery was included in statistical analysis. The pregnancies of females mated to these rats were also observed. The number and time of deaths among the groups are shown in **Table 1**. No rats died in the control group.

Successfully mated female rats and pregnancy rates

The term successfully mated female rats refers to female rats that were mated with control males or males that underwent surgery and survived more than a week after surgery. Results (see **Table 2**) showed that none of the females mated to males with bilateral or contralateral injuries were pregnant (pregnancy rate = 0). It is noteworthy that pregnancy rates of females mated to males with Ipsi-N + ischio cutting, Ipsi-V + ischio cutting, and Ipsi-N + V + ischio cutting were 88.9%, 83.3%, and 94.1%, respectively, while females mated with males in the uni-cutting, sham, and control groups all had pregnancy rates of 100%. Overall differences were significant ($P < 0.05$). Pairwise comparisons between non-pregnant and pregnant groups were also significant ($P < 0.05$). However,

Table 1. The number and time of death of male rats by group

Group	Number of deaths	Time of deaths (after surgeries)
Uni-N Cutting	4	8 h; 12 h; 24 h; 36 h
Uni-V Cutting	2	12 h; 16 h
Uni-Ischio Cutting	3	12 h; 16 h; 5 d
Ipsi-N + V Cutting	2	12 h; 18 h
Ipsi-N + Ischio Cutting	4	12 h; 3 d; 9 d; 11 d
Ipsi-V + Ischio Cutting	5	8 h; 24 h; 10 d; 12 d; 16 d
Ipsi-N + V + Ischio Cutting	4	24 h; 48 h; 5 d; 10 d
Bi-N Cutting	3	12 h; 24 h; 9 d
Bi-V Cutting	2	24 h; 5 d
Bi-Ischio Cutting	3	8 h; 6 d; 15 d
One N + Contra-V Cutting	2	24 h; 8 d
One N + Contra-Ischio Cutting	5	12 h; 16 h; 8 d; 11 d; 15 d
One V + Contra Ischio Cutting	5	8 h; 16 h; 9 d; 13 d; 16 d
Sham Group	2	16 h; 3 d
Control Group	0	

Annotation: The bold type stands for the male rats that died 7 days after surgery and were counted as successful mating in **Table 2**.

no statistically significant differences were found between the non-pregnant groups ($P > 0.05$).

Delivery time of successfully mated female rats

The delivery time of successfully mated female rats (as defined above) refers to the number of days from the start of the paired-housing to the end of the first delivery. Non-pregnant female rats were excluded from **Table 3**, which presents the mean ± SD for delivery time by surgical group. Overall, group differences in delivery times were significant ($P < 0.05$). Pairwise comparisons also identified groups that differed significantly ($P < 0.05$). Females mated to males with ipsilateral cuttings (any combinations of cavernous nerves, blood vessels, or ischiocavernosus muscles) showed delayed delivery times, ranging from 5 to 12 days.

Discussion

The present study evaluated the erectile function of male rats by cutting off bilateral cavernous nerves, cavernous vessels, and ischiocavernosus muscles in different combinations. They were bred with female rats. Erectile function was inferred indirectly by the fertilization of female rats. In addition, failure of the paired female rats to conceive indicated that the male rats had erectile dysfunction. Delayed conception of the female rats indicated that the male rats had temporary erectile dysfunction. Pregnancy of the female rats at the normal time indicated that the male rats did not have erectile dysfunction. Normal erectile function and fertility of the male rats and their paired female mice were taken as references. According to results, female rats in both the normal group and sham group were pregnant. There were no statistically significant differences in the time of conception and rate of conception (all $P > 0.05$). After cutting off any combination of the cavernous nerve of the penis, the cavernous blood vessel of the penis, and the ischiocavernosus muscle of the male rats on the same side, postnatal delay of the male rats was found. When male rats were cut off from any combination of penile cavernous nerve, penile cavernous vessel, and ischiocavernosus muscle in different sides, their paired female mice were not pregnant. Differences were statistically significant, compared with the normal group and sham operation group (all $P < 0.05$). Results suggest that temporary erectile dysfunction occured after the male rats were cut off from any combination of the cavernous nerve of the penis, cavernous blood vessels of the penis, and ischiocavernosus muscles in the same side. Permanent erectile dysfunction can occur when males on different sides are cut off from any combination of the cavernous nerves of the penis, cavernous blood vessels of the penis, and ischiocavernosus muscles in different sides.

As in previous experiments, the current study observed the interesting phenomenon where male rats with bilateral injuries exhibit chasing or back-climbing behaviors towards female rats. However, because their penis could not be erected and inserted into the vagina, these rats eventually failed to mate. This is in line with the

Table 2. The number and rate of pregnant females in different groups

Group	Pregnant (n)	Non-pregnant (n)	Total	Pregnancy rate (%)
Uni-N Cutting	16	0	18	100*
Uni-V Cutting	18	0	18	100*
Uni-Ischio Cutting	17	0	17	100*
Ipsi-N + V Cutting	18	0	18	100*
Ipsi-N + Ischio Cutting	16	2	18	88.9*
Ipsi-V + Ischio Cutting	15	3	18	83.3*
Ipsi-N + V + Ischio Cutting	16	1	17	94.1*
Bi-N Cutting	0	18	18	0#
Bi-V Cutting	0	18	18	0#
Bi-Ischio Cutting	0	18	18	0#
One N + Contra-V Cutting	0	19	19	0#
One N + Contra-Ischio Cutting	0	18	18	0#
One V + Contra Ischio Cutting	0	18	18	0#
Sham Group	18	0	18	100
Control Group	20	0	20	100
Total	154	115	269	57.25

Pairwise comparisons between data labeled with "*" and "#", $P < 0.05$.

Table 3. Delivery time (days) of successfully mated female rats in the different groups

Group	Delivery time (days)	
	Mean	Standard deviation
Uni-N Cutting (n = 16)	32	3
Uni-V Cutting (n = 18)	31	3
Uni-Ischio Cutting (n = 17)	37	5
Ipsi-N + V Cutting (n = 18)	38	4
Ipsi-N + Ischio Cutting (n = 16)	36	4
Ipsi-V + Ischio Cutting (n = 15)	32	5
Ipsi-N + V + Ischio Cutting (n = 16)	33	4
Sham Group (n = 18)	28	3
Control Group (n = 20)	26	5

Pairwise comparison, $P < 0.05$.

diagnosis of ED in clinical settings. On the other hand, rats in the control, sham, uni-, and ipsilateral injury groups all exhibited normal mating behaviors. The females became pregnant and delivered live offspring, indicating that these male rats had not developed ED. Instead of using the internationally recognized ICP method [21-24], this study adopted a mating method to indirectly evaluate the erectile function of male rats by observing the pregnancy of females. However, whether this strategy is as accurate as the ICP measure requires verification.

Cavernous blood vessels include the cavernous arteries and cavernous veins. Cavernous arteries supply major blood flow for penile erection. They pass through the base of the penis, then enter the corpus cavernosum. Pubic or ischial fractures are likely to damage cavernous arteries, leading to vascular ED. The anatomy of male rats shows that the cavernous blood vessels are adjacent to cavernous nerves. Both are wrapped within a thin connective sheath of tissue that forms the corpus cavernosum neurovascular bundle. When creating the nerve and vascular ED models, rat bladders and bladder necks were exposed. The corpus cavernosum neurovascular bundle could be seen clearly behind the lateral sides of the bladder. The bundle passes through the bladder necks and the pelvic cavity, finally entering into the base of the penis. Based on surgical requirements, the corpus cavernosum neurovascular bundle was opened and the cavernous blood vessels and nerves were carefully isolated, cut, and ligated (**Figure 1**). This is the major technical difficulty with this surgery.

This study involved many test groups, making it difficult to read and understand the results. Briefly, the groups can be classified into 4 types: control, sham, uni/ipsilateral cutting, and bi/contralateral cutting groups. The uni/ipsilateral cutting groups included the unilateral cavernous nerve cutting group, unilateral cavernous blood vessel cutting group, unilateral ischiocavernosus muscle cutting group, ipsilateral cavernous nerve + blood vessel cutting group, ipsilateral cavernous nerve + ischiocavernosus muscle cutting group, ipsilateral cavernous blood vessel + ischiocavernosus muscle cutting group, and ipsilateral cavernous nerve + blood vessel + ischiocavernosus muscle cutting group. The bi/contralateral cutting groups included the bilateral cavernous nerve cutting group, bilateral cavernous blood vessel cutting group, bilateral ischiocavernosus muscle cutting group, one cavernous nerve + contralateral cavernous blood vessel cutting group, one cavernous nerve + contralateral ischiocavernosus muscle cutting group, and one cavern-

ous blood vessel + contralateral ischiocavernosus muscle cutting group.

Results showed that most, or all, of the female rats mating with males in the uni/ipsilateral cutting groups were pregnant. Their rates of pregnancy did not differ significantly ($P > 0.05$), compared with the rates of sham and control groups. This indicates that neither uni- nor ipsilateral injuries cause permanent ED. This can be explained by the fact that the cavernous nerves, cavernous blood vessels, and ischiocavernosus muscles are all bilaterally distributed. Hence, when one side is injured, compensation by the other side can be activated to maintain erectile function [15, 18, 19]. Though no significant differences were observed in pregnancy rates, delivery times of females mating with males in the uni/ipsilateral groups were 5-12 days longer than those mating with males in the sham and control groups ($P < 0.05$). This suggests that transient ED might occur during the early stages of compensation. As for females mating with males with bi/contralateral cuttings, none of them were pregnant. Differences in pregnancy rates were significant, compared with the sham and control groups ($P < 0.05$). This suggests that bi/contralateral damage caused permanent ED. Since the cavernous nerves, blood vessels, and ischiocavernosus muscles are all bilaterally distributed, contralateral injuries may disturb the function of undamaged tissues, resulting in the loss of erectile function [14, 18, 19].

In the ipsilateral cavernous nerve + ischiocavernosus muscle cutting group, 16 of the 18 successfully mated female rats were pregnant. The pregnancy rate was 88.9% in this group. Moreover, 15 out of 18 females (83.3% pregnancy rate) were pregnant in the ipsilateral cavernous nerve + blood vessel + ischiocavernosus muscle cutting group and 16 out of 17 female rats were pregnant (94.1% pregnancy rate). In these 3 groups, a total of only 6 female rats were not pregnant. Records showed that all of them were mated to males that died shortly after mating. Thus, the absence of pregnancy in these females might be attributed to failure of effective intercourse. However, the possibility of congenital fertility problems in the males and females and the influence of anesthesia, trauma, and infection on the males sexual function, even the decompensation of the contralateral cavernous nerves and blood vessels and ischiocavernosus muscles, should be considered. However, it should be noted that this study used a randomized design. Thus, the influence of the above factors should have been evenly distributed among all groups. These factors did not have a significant effect on overall results.

Based on the above results, this study concludes that uni/ipsilateral combinations of cutting the cavernous nerve, cavernous blood vessels, and ischiocavernosus muscles do not cause permanent ED. However, bi/contralateral combinations of cutting these tissues do cause permanent ED. This suggests that, in a clinical setting, the development of ED after a pelvic fracture is probably caused by combined bilateral injuries of the cavernous nerves, cavernous blood vessels, and/or ischiocavernosus muscles. If surgery could repair one side of the injury, making it a uni/ipsilateral injury, the symptoms of ED might be improved. Previous studies have accumulated some evidence about repair of ischiocavernosus muscles [17]. Present results are relevant for diagnosis of ED and the prevention of ED when conducting radical prostatectomy or radical cystectomy. During surgery, injuries to the bilateral cavernous nerves and blood vessels should be avoided to protect patients from permanent ED [31].

An additional point which should be noted about this study is that the delivery time of the successfully mated female rats was not the same as the gestation time. The delivery time was calculated as the sum of non-pregnant time and gestation time. The average gestation time for normal female rats is 21 days, thus the delivery time would be longer than that. According to present results, the delivery time of females ranged from 26-38 days, in line with expectations. Moreover, differences in delivery times between the groups were statistically significant ($P < 0.05$). Many factors could have affected the delivery times of the female rats, including different cutting combinations of their mates, such as the time of estrus and successful intercourse, as well as individual differences in gestation periods.

Male rats died in all experimental groups. Results were in accord with previous studies. It was concluded that anesthesia and surgical

trauma are the likely causes of early deaths after surgery, whereas infections were likely to cause later deaths after surgery [14-20].

The present study had some limitations, however. This study adopted a mating method to indirectly evaluate the erectile function of male rats by observing the pregnancy rates of females, instead of using the internationally recognized ICP method. However, whether this strategy is as accurate as the ICP measure requires verification. Due to racial differences, whether present conclusions can be applied to humans requires further verification.

In conclusion, this experiment suggests that temporary erectile dysfunction can occur after male rats are cut off from any combination of the cavernous nerve of the penis, cavernous blood vessels of the penis, and ischiocavernosus muscles in the same side. Permanent erectile dysfunction can occur when males on different sides are cut off from any combination of the cavernous nerves of the penis, cavernous blood vessels of the penis, and ischiocavernosus muscles in different sides. This study may be used as a guide for diagnosis of erectile dysfunction after pelvic fractures, as well as pelvic surgery, especially radical cystectomy and radical prostatectomy. This study may aid in avoiding damage to bilateral erectile nerves and erectile vessels, aiming to retain sexual function.

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Disclosure of conflict of interest

None.

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