

## Influence of Male Surgical Sterilization on the Copulatory Behavior and Reproduction of Brandt's Vole

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**Abstract:** The influence of sterilized male on the copulatory behavior and reproduction of Brandt's vole was studied by using the method of surgical sterilization. It was showed no influence of male surgical sterilization on the copulatory behavior of Brandt's vole. Mating times with intact mates and litter size of female Brandt's voles in male sterilized groups decreased compared with the control groups. The results supported the hypothesis of competitively reproductive interference of sterilized male. Thus, sterilization can be a viable method to control voles.

**Key words:** Brandt's vole (*Microtus brandti*); Competitively reproductive interference; Male surgical sterilization

## 雄性不育对布氏田鼠交配行为和繁殖的影响

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**摘要:** 利用雄性结扎不育方法研究雄性结扎不育对布氏田鼠交配行为和繁殖的影响。结果表明: 雄性结扎不育后不影响布氏田鼠雌雄两性的交配行为; 不育雄性的存在对雌性的正常交配产生了干扰, 降低了雌性的有效交配次数, 并导致雌性怀孕率和产仔数的下降。结果一方面说明结扎不育雄性方法可用于布氏田鼠的不育控制研究, 另一方面为不育后的竞争性繁殖干扰假设提供了实验证据。

**关键词:** 布氏田鼠; 竞争性繁殖干扰; 雄性结扎不育

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Because of many problems and side effects associated with other traditional control methods, using sterilization to control pest animals is gaining attention and favor for its long-lasting effect, safety, humanism and slight population to environment<sup>[1]</sup>. Control by contraception was first proposed by Knipling<sup>[2,3]</sup> and substantiated greatly afterward<sup>[4-8]</sup>. This method has been so far applied to the house mouse (*Mus musculus*)<sup>[9,10]</sup>, the European rabbit (*Oryctolagus cuniculus*)<sup>[11]</sup>, the brush-tail possum (*Trichosurus vulpecula*)<sup>[12]</sup> and the rat-like hamster (*Cricetulus*

*triton*)<sup>[13]</sup> and met with various levels of success. Chambers et al<sup>[9,10]</sup> found that surgical sterilization could result in 67% infertility among females and thus successfully reduce the population size and growth rate in a confined population of house mouse. This method seems to be more successful when it is used to control and reduce the European rabbit population (80%)<sup>[11]</sup> and the brush-tail possum in New Zealand (75%)<sup>[12]</sup>. Zhang et al<sup>[13]</sup> compared the effects, between imposed sterility using surgical operation and culling, on the reproduction and population

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size of the rat-like hamster in enclosures and found that fertility control can achieve a similar result as simple culling, and sterility of both sexes were more effective in reducing reproductively of hamsters. Shi *et al*<sup>[14]</sup> developed a model to investigate the effectiveness of fertility control in Brandt's vole (*Microtus brandti*) and found that fertility control is not more effective than lethal control at the same time with the same bait uptake. However, when applied in autumn, fertility control is better than lethal control in reducing the number of the voles in the following spring, summer and autumn. Despite these worthy attempts with promising effects, these studies have been focusing on the influence of surgical sterilization on the population growth and fecundity<sup>[9, 13]</sup>. The influence of surgical sterilization on social behavior, especially mating behavior, of the studied animals is still known a little. This oversight can be significant because sterilized animals can pose a strong influence on normal animals due to mate competition. This would lower the reproductive success of normal individuals in population and reduce the population size. This behavioral interference effect induced by sterilized individuals has been observed in numerous species such as gray kangaroo (*Macropus giganteus*)<sup>[14]</sup>, white tailed deer (*Odocoileus virginianus*)<sup>[15]</sup>, elk (*Cervus elaphus*)<sup>[16]</sup> and plateau pika (*Ochotona curzoni-ae*)<sup>[17]</sup>. For this, Zhang<sup>[8]</sup> proposes to use "competitively reproductive interference" to characterize the effect of sterilized individuals on normal individuals. No study, however, has so far been conducted to examine the level and significance of this behavioral interference due to mate competition.

Brandt's vole is considered a pest, inhabiting in steppes from Baikal area in Russia, Mongolia, to Inner Mongolia in China<sup>[18, 19]</sup>. This species may bring severe damages to local livestock husbandry and it is known as a host and vector of several pathogens<sup>[20]</sup>. To control the vole population, chemical poisons are traditionally used. Because of numerous undesirable side effects associated with chemical control, recently a good amount of attention has been paid to fertility control as an alternative method<sup>[21]</sup>. However, much has yet to be known before this method can be routinely used to effectively control the

pest vole population.

In this study, we focused on the behavioral aspect of males after surgical sterilization. Specifically, we examined whether male surgical sterilization could influence the mating behavior and reproduction of Brandt's vole and assessed the level of competitively reproductive interference from sterilized individuals on normal individuals.

## 1 MATERIAL AND METHODS

All experimental voles were from the offspring of a wild population caught during July to August in 2000. They were reared separately in plastic boxes (28.5 cm × 17.2 cm × 15.8 cm) in the laboratory with access to food and water *ad libitum*. The photoperiod was set at 16L 8D, and light began at 05 00. Dim red light shone at all times. The temperature was maintained at 25 ± 1. Only mature and healthy individuals were selected for the experiment. Individuals were selected based on the degree of testis descent and the open condition of the vagina. This ensured that both males and females were mature and that females were in estrus and receptive. Males and females were pre-mated, and only those individuals that showed mounting and receptivity were selected for use. The individuals selected to mate were from different lineages and thus not closely related.

Surgery was performed as follows. First, animals were anesthetized (with Pentobarbital sodium (2 g) + Ethanol (16 ml) + 0.9% NaCl solution (34 ml) at the dose of 0.15 ml per 100 grams of body weight) by intraperitoneal injection. Next, the abdomen of the male voles were shaved and swabbed with iodine, followed by a small incision (20 mm) through the skin and muscle layers in the middle of the abdomen to gain access to the sperm ducts. For tubal ligation, the ducts were knotted at the place near the testis by sterile cotton thread. After tubal ligation, the muscle layer and the skin incision were stitched with the sterile cotton thread and swabbed with iodine solution. For animals undergoing the sham operation, all steps were the same as those with the experimental animals, except that the tubes were not knotted.

We conducted two experiments. In Experiment I, we selected 50 healthy mature males and 40 females subject

to three treatments: group I, one male paired with one female (1 : 1); group II, two males paired with one female (2 : 1) and group III, two males paired with two females (2 : 2). In Experiment II, we selected 30 surgically sterilized males, 20 intact males and 40 females for three treatments: group IV, one sterilized male paired with one female (1 \* : 1); group V, one sterilized male and one intact male paired with one female (2 \* : 1) and group VI, one sterilized male and one intact male paired with two females (2 \* : 2). (\* means there exists one sterilized male in this group). Each group had 10 individuals for all the six groups in this study.

We made an observation box by placing two plastic boxes (each 37 cm × 27 cm × 17 cm) together with a hole (diameter 15 cm) in the joined sides. A piece of transparent glass was used to cover the box to prevent the escape of the animals. After each observation, the box was first rinsed out using water and then wiped out with alcohol to remove residual odors. Observations were recorded through a kinescope monitor, and observation time set at an hour by following Dewsbury<sup>[22]</sup>. The fur of each individual was cut distinctively for individual identification.

A mating series in the vole includes mounting, penis insertion, pelvic thrusting and ejaculation. We collected the following data about mating behavior: (a) mating frequency (MF), referring to the number of copulations that occurred within one hour, (b) mating duration (MD), referring to the length of each copulation in seconds, (c) mating interval (MI), referring to the time between two consecutive copulations in seconds, and (d) thrust frequency (TF), referring to the number of pelvic thrust per second during a copulation in male. For all data, we did not distinguish whether the vole copulated with the same or different mates.

The normality of the data collected was tested by using a one-sample Kolmogorov-Smirnov test. Then, independent samples *t*-test (when distribution of data was normal) or Mann-Whitney test (distribution of data was unknown) was used accordingly for analyzing the significance in the difference between each two-treatment group.

Wilcoxon paired-sample signed ranks test was used for samples with paired data. Chi-square test was used to test if pregnancy rate was related to mating frequency. The significance level for all tests was set at  $\alpha = 0.05$ .

## 2 RESULTS

Because of the similar treatment, the following groups form natural pairs for comparing copulatory behavior of Brandt's vole: groups I and IV, groups II and V, and groups III and VI, between experiments I and II. Compared with group I, males in group IV demonstrated a higher thrusting frequency ( $t = 4.254$ ,  $df = 18$ ,  $P = 0.000 < 0.05$ ), but no difference was found in mating frequency (male:  $t = 0.365$ ,  $df = 18$ ,  $P = 0.719$ ; female:  $t = 0.365$ ,  $df = 18$ ,  $P = 0.719$ ), mating duration (male:  $t = 0.351$ ,  $df = 18$ ,  $P = 0.730$ ; female:  $t = 0.351$ ,  $df = 18$ ,  $P = 0.730$ ) and mating interval (male:  $t = 0.647$ ,  $df = 18$ ,  $P = 0.526$ ; female:  $t = 0.647$ ,  $df = 18$ ,  $P = 0.527$ ) in both males and females (Fig. 1 A). Compared with group II, the thrusting frequency of males in group V decreased significantly ( $t = 2.638$ ,  $df = 30$ ,  $P = 0.013 < 0.05$ ), but no difference was found in mating frequency (male:  $t = 0.887$ ,  $df = 38$ ,  $P = 0.381$ ; female:  $t = 0.826$ ,  $df = 18$ ,  $P = 0.420$ ), mating duration (male:  $t = 0.581$ ,  $df = 30$ ,  $P = 0.565$ ; female:  $t = 0.01$ ,  $df = 17$ ,  $P = 0.999$ ) in both male and female, and in mating interval ( $t = 1.12$ ,  $df = 28$ ,  $P = 0.272$ ) in male. Females in group V shortened its mating interval significantly ( $t = 2.934$ ,  $df = 17$ ,  $P = 0.009 < 0.05$ ) (Fig. 1 B). Compared with group III, males and females in group VI showed no difference in mating frequency ( $t = 0.208$ ,  $df = 38$ ,  $P = 0.836$ ;  $t = 0.202$ ,  $df = 38$ ,  $P = 0.841$ ), mating duration ( $t = 1.742$ ,  $df = 32$ ,  $P = 0.091$ ;  $t = 1.459$ ,  $df = 30$ ,  $P = 0.155$ ) and mating interval ( $t = 0.690$ ,  $df = 30$ ,  $P = 0.496$ ;  $t = 1.133$ ,  $df = 25$ ,  $P = 0.268$ ), whereas the thrusting frequency of males in group VI was lowered ( $t = 4.466$ ,  $df = 32$ ,  $P = 0.00 < 0.05$ ) (Fig. 1 C).

The difference in copulatory behavior between sterilized and intact males in group V and group VI was further analyzed by using Wilcoxon paired-sample signed ranks

test. Results (Fig. 2) showed that there was no difference in copulatory behavior between surgically sterilized and intact males in both groups V and VI (for group V: mating frequency:  $Z = 0.46$ ,  $P = 0.65$ ; mating duration:  $Z = 0.56$ ,  $P = 0.58$ ; thrusting frequency:  $Z = 0.70$ ,  $P = 0.48$ ; mating interval:  $Z = 0.00$ ,  $P = 1.00$ . For group VI: mating frequency:  $Z = 0.19$ ,  $P = 0.86$ ; mating duration:  $Z = 1.12$ ,  $P = 0.26$ ; thrusting frequency:  $Z = 0.42$ ,  $P = 0.67$ ; mating interval:  $Z = 0.00$ ,  $P = 1.00$ ).

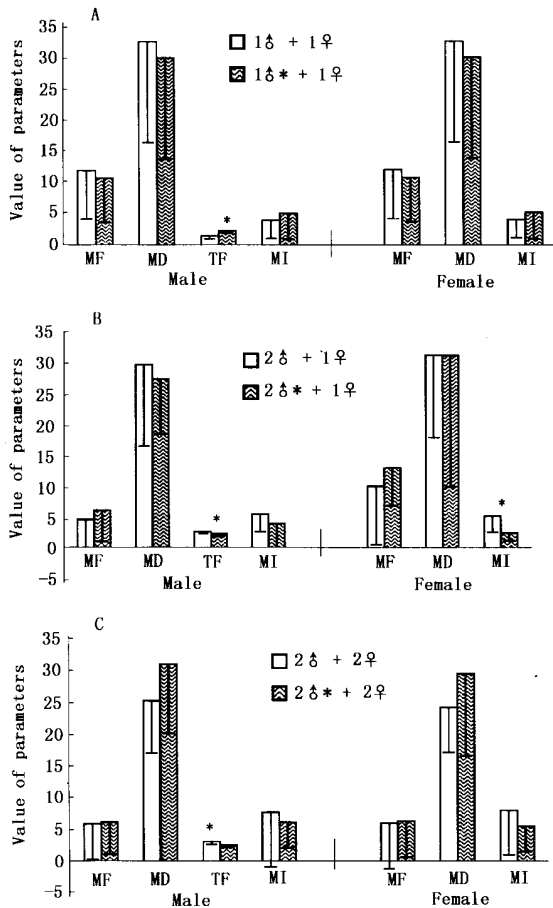


Fig. 1 Influence of male surgical sterilization on the copulatory behavior of Brandt's vole between the paired groups in experiment I and experiment II. A: group I - group IV; B: group II - group V; C: group III - group VI. MF: Mating frequency; MD: Mating duration; TF: Thrusting frequency; MI: Mating interval. The value if MI has been converted from seconds to minutes for convenience of drawing. Significant differences are indicated: \*  $P < 0.05$ ; \* means there exists one sterilized male in this group.

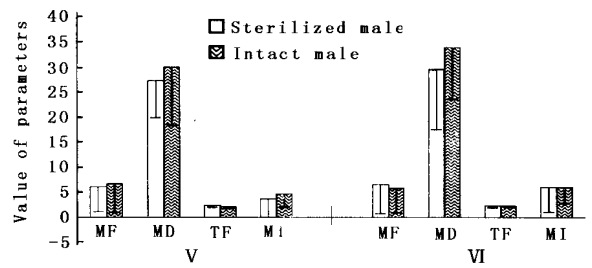


Fig. 2 Comparisons of copulatory behavior of surgical sterilized males and intact males respectively in group V and group VI. MF: Mating frequency; MD: Mating duration; TF: Thrusting frequency; MI: Mating interval. The value if MI has been converted from seconds to minutes for convenience of drawing.

Valid mating of females was defined as the mating with intact males. In experiment I, all matings were valid mating for females, while in experiment II, only those that happened between females and intact males were valid mating for females. The reproduction success was measured by litter size (LS) in each group. Compared with experiments II and I, our results (Fig. 3) showed that females in group IV had no valid mating and its litter size is zero. Compared with group II, valid mating frequency ( $t = 0.99$ ,  $df = 18$ ,  $P = 0.34$ ), mating duration ( $t = 0.36$ ,  $df = 15$ ,  $P = 0.72$ ) and mating interval ( $t = 0.26$ ,  $df = 14$ ,  $P = 0.80$ ) of female in group V all decreased but not significantly, but the litter size decreased significantly in group V ( $t = 2.638$ ,  $df = 28$ ,  $P = 0.013 < 0.05$ ) (Independent samples  $t$ -test). Compared with group III, valid mating frequency of female in group VI decreased significantly ( $t = 2.17$ ,  $df = 38$ ,  $P = 0.036 < 0.05$ ), whereas the valid mating duration increased remarkably ( $t = 3.025$ ,  $df = 27$ ,  $P = 0.005 < 0.05$ ). However, no difference was found in the valid mating interval ( $t = 0.051$ ,  $df = 22$ ,  $P = 0.96$ ) or in the litter size in group VI (Mann-Whitney  $U = 1.132$ ,  $P = 0.258$ ). Our results also showed the pregnancy rate was much lower when the valid mating frequency of females was five or less than when it was more than five ( $\chi^2 = 25.771$ ,  $df = 1$ ,  $P = 0.00 < 0.05$ , Fig. 4).

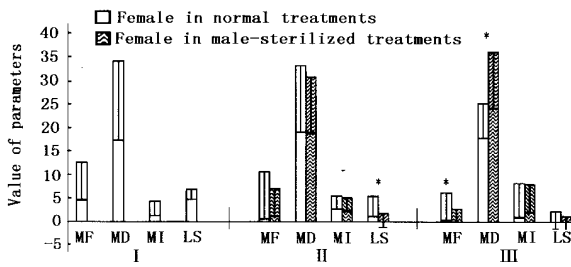


Fig. 3 Comparisons of valid mating and reproduction success of female Brandt's voles between the paired groups in experiment II and experiment I. I: group I - group IV; II: group II - group V; III: group III - group VI. MF: Mating frequency; MD: Mating duration; MI: Mating interval; LS: Litter size. The value if MI has been converted from seconds to minutes for convenience of drawing. Significant differences are indicated: \*  $P < 0.05$ .

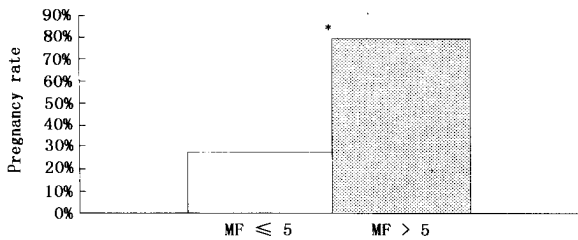


Fig. 4 Correlation of valid mating times of female Brandt's vole and pregnancy rate. MF: mating frequency. Significant differences are indicated: \*  $P < 0.05$ .

### 3 DISCUSSIONS

Influence of sterilization on the social behavior was reported in some animals. For instance, the sterilized female white-tailed deer moved more actively than the normal female<sup>[15]</sup> and the sterilized plateau pika shows more amicable behavior and less agnostic behavior<sup>[17]</sup>. No influence of sterilization, however, was found in the social behavior such as foraging, grooming, resting and moving in gray kangaroo<sup>[14]</sup> and elk<sup>[16]</sup>. So sterilization may have different effect on different animal. Our study showed that male surgical sterilization had no significant influence on the mating ability of male Brandt's vole, and the sterilized males could compete for mating opportunities with intact males and thus decreased the valid mating and reproduction success of female Brandt's voles. Therefore, the competitively reproductive interference of sterilized individuals<sup>[8]</sup> seems to be substantial in this species. For this reason, the method of male surgical sterilization has the

potential to be used effectively in fertility control of Brandt's vole. It is, however, still difficult to explain why thrusting frequency of males decreased in group V and group VI when compared respectively with males in group II and group III, but increased in group IV compared with males in group I. If the reason for the increase in thrusting frequency in group IV was only a mating compensation, how can we explain the decrease in thrusting frequency in group V and VI? Perhaps it was influenced by surgery on male Brandt's voles. Thus, further study is needed to clarify this issue. The difficulty incurred here in the explanation may be due to lack of long-term observation after the animals were sterilized in our study. In the rat-like hamster, Zhang *et al*<sup>[23,13]</sup> had a long-term observation and found that the body growth rate of sterilized females is faster than intact females or sterilized males. Body weight influences the status of animals, and thus may influence the mating success<sup>[24]</sup>. Thus, long-term study is necessary to clarify the effect of sterilization.

In any case, based on our results, the sterilized males not only competed for resources such as food and space, they also competed for mating opportunities with normal males in the same population. In natural conditions, when the population density is high, the interaction frequency of males and females increases and thus, mating competition also intensifies. Therefore, if a good number of sterilized individuals are added into a population, it is expected to see that the population size will decrease in the ensuing year due to numerous invalid matings.

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