

# A longitudinally split rabbit segmental gracilis to simulate penile erectile function: anatomic basis and animal models

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**Abstract. – OBJECTIVE:** The gracilis was once applied in reconstructing erectile function but its appearance was bulky. We aimed to design a model meeting the requirements of both reducing volume and retaining function.

**MATERIALS AND METHODS:** The gracilis muscles of 6 rabbits were harvested, applied colorful vascular perfusion and modified Sihler's intramuscular nerve staining. According to their intramuscular nerves and blood vessels, 9 rabbit right gracilis muscles were then longitudinally split into two halves. The anterior muscle bundle was selected as the functional unit and blood supply.

**RESULTS:** The intramuscular nerves and vessels were simultaneously presented on a same specimen. Their relationship suggested gracilis muscle to be composed of two relatively independent subunits. The reconstructed penis survived well, simulating erectile action satisfactorily.

**DISCUSSION:** The penis model reconstructed with longitudinally split rabbit segmental gracilis myocutaneous flap had met the requirements of both restoring erectile function and improving the appearance.

## Key Words:

Gracilis, Sihler's intramuscular nerve staining, Vascular perfusion, Subunit, Erectile function.

change. An ideal surgical treatment should consist of the reconstructions of penile body, urethra, sensory function and erectile function<sup>1</sup>. So far, there have been many successful and even classical operations reported about the first three aspects, however, restoring erectile function has until now not been successfully accomplished<sup>2</sup>. The current methods of reconstructing erectile function can be categorized into two types: static and dynamic. The static methods usually obtain hardness through the use of implanted support, which can be either biological or artificial. While the dynamic methods simulate the morphological changes of erectile penis through implanted inflatable prosthesis<sup>3</sup> or transferred muscular flaps<sup>4</sup>. The dynamic reconstruction of erectile function seems more natural but is, however, relatively more difficult to achieve. After implantation of the inflatable prosthesis, the patient may encounter multiple complications such as container's leakage, restricted expansion, skin necrosis, exposure of prosthesis, etc.<sup>5,6</sup>. Besides, many patients may have a strong psychological rejection of an entirely artificial erectile launching mechanism. All the reasons mentioned above have influenced the popularization of the dynamic reconstruction of erectile function by implantation inflatable prosthesis.

In 1972, Orticochea<sup>7</sup> reported on an operation for the reconstruction of the penis with gracilis myocutaneous flap. The penile body was reconstructed with gracilis muscle and the skin above it from the dominant side. The urethra was rebuilt with the skin from the contralateral thigh. A

## Introduction

Penis reconstruction surgery is widely used in dealing with penile defects caused by trauma or surgical ablation for neoplasms, congenital malformations of gender, and female to male gender

piece of T-shaped silastic prosthesis was also implanted as support. After the operations the patient could freely raise or drop the distal end of his penis by controlling the contraction of the gracilis muscle, allowing him to achieve sexual intercourse. The result was quite satisfactory but the reconstructed penis was so bulky in appearance that this might have also influenced the popularization of the method.

Nowadays, the definition of neuromuscular compartmentalization and related research achievements has greatly inspired the authors of this study<sup>8-10</sup>. If the transferred muscle can be harvested according to the principle of compartmentalization, it will not only meet the requirements of both reducing muscular volume and retaining contractive function, but also might reduce the injuries to the donor area.

We, therefore, did a series of anatomical researches which aimed at studying the intramuscular nerve and vessel branches of rabbit gracilis muscle and then designed an animal model with longitudinally split segmental gracilis myocutaneous flap to reconstruct the erectile function.

## Materials and Methods

### Experimental Animals and Grouping

A total of 15 healthy New Zealand White rabbits of either sex, weighing between 1.80 kg and 2.82 kg were used in this experiment. Each rabbit was housed in a separate cage under a 12-hour light/dark cycle with access to food and water *ad libitum*. The rabbits were cared for in accordance to the Association for Assessment and Accreditation of Laboratory Animal Care and the Institutional Animal Care and Use Committee of the Second Military Medical University.

The rabbits were divided into the anatomy group (6 rabbits) and the operation group (9 rabbits) through the use of a table of random numbers. Within the operation group, the right-side gracilis muscle was used to construct the model penis, while the left gracilis served as control. The operated rabbits were also randomly divided into 3 groups (each group consisted of 3 rabbits), which should apply neuromuscular electrophysiology experiments respectively at week 1, week 2 and week 4 after operation.

### Anatomical Study

After the rabbits were sacrificed with an overdose of intravenous anesthesia (20% chloral hy-

drate solution, 2 ml/kg via peripheral ear vein), the skin of lower extremities was stripped. The abdominal aorta and inferior vena cava were dissected through an abdominal midline incision, ligated proximally and cannulated distally, and rinsed with normal saline thoroughly. A mixture made of transparent liquid silica gel and red toner according to particular proportion, as is described in greater detail in a research paper we previously published<sup>11</sup>, was then perfused via the abdominal aorta gently and slowly. The presence of a diffuse red color within the distal vessels of lower extremities and small cutaneous vessels implied the filling adequacy and suggested the termination of perfusion. After the perfusate was coagulated, the gracilis muscles from both sides were harvested intact and then subjected to a modified Sihler's staining<sup>12</sup>.

At first they were fixed in 10% un-neutralized formalin for 3 weeks, depigmented in 3% aqueous potassium hydroxide for 2 weeks. Then the specimens were macerated in Sihler's solution I for decalcification for 3 weeks and stained by modified Sihler's solution II for 3 weeks. Later on, the muscles were destained with modified Sihler's solution I for about 12 hours, neutralized by 0.05% lithium carbonate for nearly 1 hour, and cleared with increasing concentration of glycerin solutions (40%, 60%, 80%, and 100% glycerin, each for 3 to 4 days). At last the gracilis muscles were placed on a horizontally laid negatoscope for observation and photography.

### Construction of Animal Models

After the rabbits were anaesthetized with chloral hydrate solution (10%, 2.3 ml/kg) via the ear vein, they were fixed supine on the operating board with lower extremities stretched and thighs shaved.

The incisions were given along the visible anterior and posterior margins of right gracilis muscle beneath the skin. The longitudinal midline of the gracilis was marked in advance to identify the range of skin elevation needed. The skin above the posterior portion of the muscle was raised, while the connection between the anterior portion and the skin remained intact. The muscle was dissociated and lifted slightly from below, taking special caution not to hurt its blood vessels and dominant nerve branches. A glass needle was placed along the longitudinal axis of the muscle and penetrated it from the deep side, in the center of two points from where two adjacent primary nerve branches enter the gracilis. The puncture site was then extended towards both ends with an



**Figure 1.** The operative procedure in constructing a model penis is shown. **A**, Rabbit gracilis muscle was almost completely split into halves. The skin above the posterior muscle bundle had been raised and began shrinking. **B**, A silastic stick with 2 paralleled silver electrodes was implanted below the anterior muscle bundle. The nerve trunk and its anterior branches were dragged through and limited between the electrodes intact. **C**, A notch was left in the middle of skin incisions to let the blood pedicle pass through after operation.

ophthalmic scissors along the muscle fibers which gradually caused the gracilis to be split into two halves completely (Figure 1A). After its tendon was detached from its point of insertion, the muscle could be elevated distally to ensure that the branch of the femoral artery and of the dominant nerve which is derived from the obturator nerve, together with its anterior 3 primary branches remained intact and still connected to the anterior muscle bundle. The posterior muscle bundle can be removed to enhance range of motion of the anterior bundle, it is however recommended to retain the external muscular segments of its nerve branches for traction later on.

A prefabricated silastic stick was then implanted underneath the anterior muscle bundle as a means of support. There was a notch made at the root of the silastic stick which allowed the dominant nerve trunk and its branches to pass through. Two parallel silver electrodes, 1 mm apart from each other, were inserted across the notch and vertical to the axis of the silastic stick. The distal electrode was fully inserted while the proximal only inserted half way through. It should be pushed in place after the dominant nerve trunk and its anterior branches are also inserted through and limited between the two electrodes (Figure 1B).

The muscle bundle was then stretched and fixed with non-absorbable sutures to the distal end and lateral margins of the support, covering it like a hood<sup>7</sup>. The distal end of the silastic stick should be slightly elevated under adequate tension but should not be curved too tight lest it could result in tearing of the muscle attachment from the support. Finally, the flap above gracilis was stretched backwards and downwards to cover the muscle bundle and the support, rolled into a tube and sutured. Yet a gap was left in the middle of the incisions to let the blood pedicle pass through (Figure 1C).

### **Electroneurophysiology**

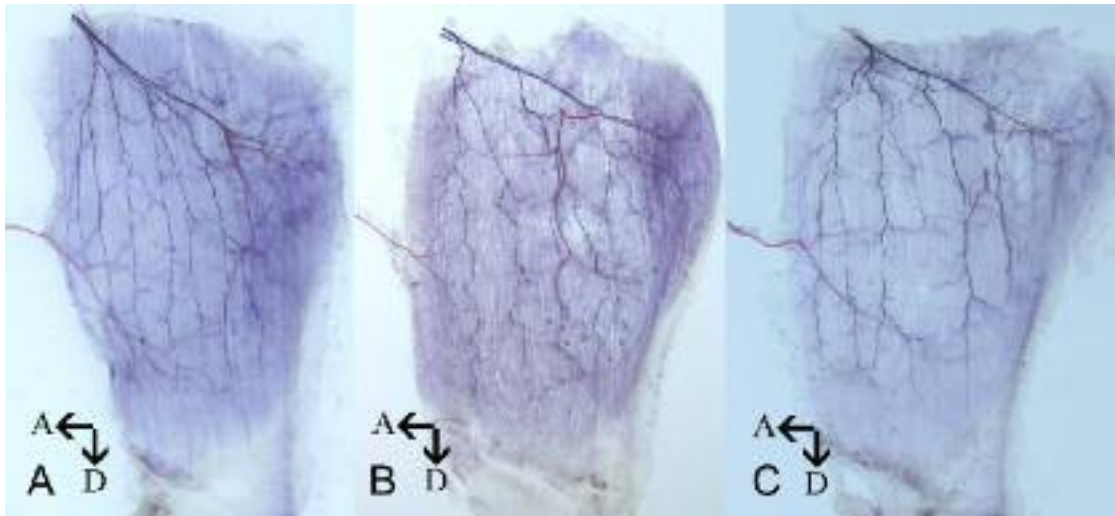
After the rabbits were anaesthetized and prepared as described before, the proximal part of the previous incisions were dissected to expose the dominant nerve at the root of the reconstructed penis. Since the nerves and electrodes were tightly surrounded by connective tissues, the two hooks of stimulating electrode were respectively hanging on the parallel silver electrodes. The positive, negative recording electrodes were also respectively inserted into the muscle at the distal end and in the middle of reconstructed penis, thus, forming a standard “positive-negative-negative-positive” circuit for electrodiagnosis<sup>13</sup>.

A multi-channel physiological signal acquisition and processing system (RM-6240BD, Chengdu instrument factory, China) was used to test the reaction of the muscle bundle when its dominant nerve was electrically stimulated. At first, the stimulation mode was single stimulation: The nerve was stimulated by single positive constant voltage at 5.0 V, pulse width of 5 ms, stimulus interval of 5 s, repeated for 10 times. Then the stimulation mode was changed to frequency ascending mode: The nerve was stimulated by a series of positive constant voltage at 5.0 V, pulse width 5 ms and stimulus interval 5 s. The initial stimulation frequency was 1 Hz, then increased by 1Hz each time, till tetanic contractions occurred in the muscle. Both the CMAP (compound muscle action potential) of gracilis muscle bundle and the morphological changes of the reconstructive penis was observed and recorded.

### **Results**

#### **Anatomical Study**

After colorful vascular perfusion and modified Sihler's staining, the rabbit gracilis muscle



**Figure 2.** Rabbit gracilis muscle after vascular perfusion and Sihler's staining. The intramuscular nerves appeared dark blue or black and blood vessels appeared red. Notice the varied number of the branches from the profunda femoris which enters the rear part of the gracilis from the deep side at the junction of the proximal and distal two-thirds. (A: anterior; D: distal).

showed a transparent or semitransparent appearance. The intramuscular nerves were counterstained with dark blue or black, arteries turned into red, and muscle fibers appeared light blue. The intramuscular nerves and arteries could be distinguished from each other well and easily above a bright background (Figure 2).

There were mainly three vascular pedicles supplying the rabbit gracilis muscle. From proximal to distal, they were derived from the obturator artery, the profunda femoris and the femoral artery respectively. The branch of obturator artery was the thinnest pedicle and entered the gracilis muscle from the deep side near its anterior origin. The branch of profunda femoris was the thickest one and often considered to be the primary pedicle of the muscle<sup>14</sup>. It can appear as a single branch (7 in 12 muscles, 58.3%, Figure 2A), double branches (3 in 12 muscles, 25%, Figure 2B) or three branches (2 in 12 muscles, 16.7%, Figure 2C). Regardless of its variable number, this pedicle always entered the rear part of the gracilis from the deep side at the junction of the proximal and distal two-thirds. The branch of femoral artery had the longest dissectible pedicle and was often considered to be the secondary nourishing vessel<sup>15</sup>. It entered the gracilis horizontally around the center of the anterior margin. The different pedicles were communicated with each other by a microcirculation network through intramuscular vessel branches while their perfusion ranges were affected by their di-

ameters and the locations from where they entered the muscle.

Like human, the rabbit gracilis muscle was also innervated by the anterior branch of the obturator nerve which reached the proximal and anterior part of the muscle from deep side<sup>16</sup>. It divided into 5-6 primary branches before entering the muscle. The intramuscular nerve branches then generally ran parallel to the muscle fibers distally. Yet the most posterior branch also sent out 1-2 smaller branches to control the proximal muscular segment. The intramuscular nerve branches were well distributed and most of them were accompanied by corresponding vessel branches.

According to the concept of neuromuscular compartmentalization<sup>10</sup>, the rabbit gracilis muscle seemed to be composed of two relatively independent subunits. The anterior 1/3-1/2 of the muscle was nourished by the branch of the femoral artery and innervated by 3 anterior primary nerve branches, whereas the posterior 1/2-2/3 of the muscle was nourished by the branches of the profunda femoris and innervated by 2-3 posterior primary nerve branches. Thus, the rabbit gracilis was suggested to be divided into two segments: the anterior and posterior muscle bundle.

### **Construction of Animal Models**

All the reconstructed penis built with the longitudinally split segmental gracilis myocutaneous flap survived well at the different time points of this experiment (Figure 3), except for one which





**Figure 3.** The reconstructed penis survived with a good appearance 4 weeks after operation.

developed partial skin necrosis 5 days after the operation because of a too tight local tension. Yet the whole silastic support remained beneath the skin all along and the function of the muscle bundle maintained as in other models.

When their dominant nerves were electrically stimulated, the anterior muscle bundles contracted, leading to the “erection” of the distal end of the reconstructed penis. In the frequency ascending mode test, when there was stimulation with gradually increasing frequency, tetanic contractions finally occurred causing the distal end of the reconstructed penis rose to the highest point and maintained the maximum height for several seconds. Thus, the erectile action of a normal penis was simulated satisfactorily (Figure 4).

#### **Electroneurophysiology**

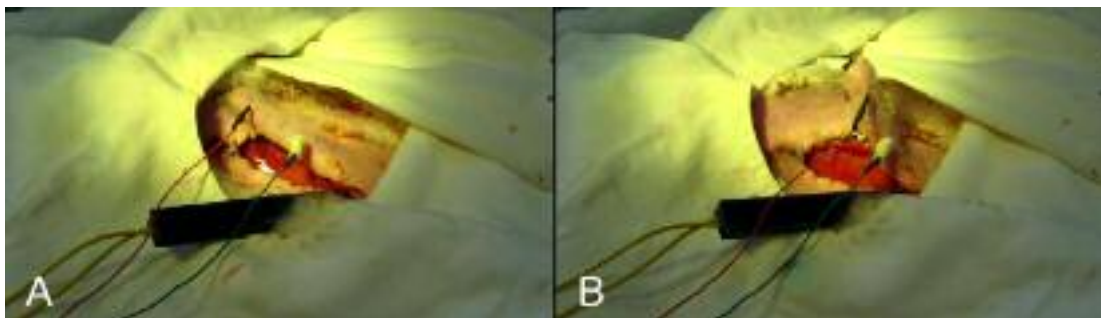
The CMAP of the anterior muscle bundle was recorded by the needle-shaped electrodes synchronously (Figure 5). The detailed images showed that the direction of the initial wave was upward (Figure 5B), unlike the downward wave

of any injured nerve potential, proving that the neuromuscular pathway was still intact after the surgery. Besides, we also found another series of spontaneous electric activities recorded next to the artificial-induced CMAP. The shape of these waves was similar to the CMAP but their peak potentials were lower (Figure 5A).

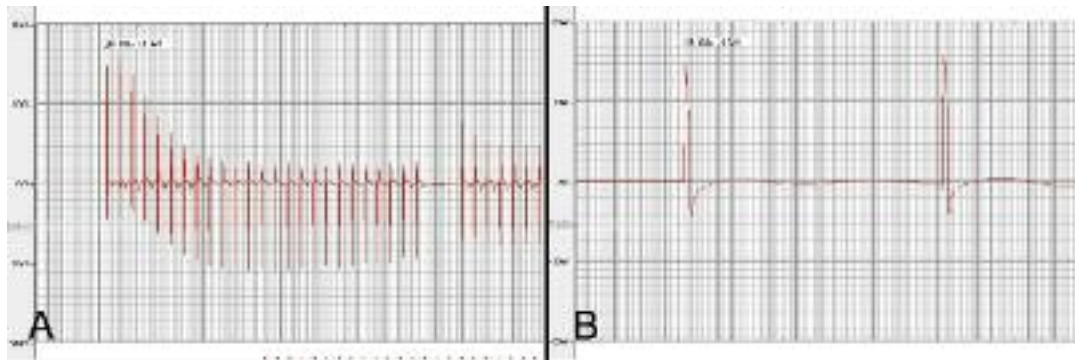
#### **Discussion**

The gracilis muscle, because of its reliable vascular supply, ease of harvest and its expendability, is commonly utilized by reconstructive surgeons in a variety of operations such as muscular flaps, myocutaneous flap for soft tissue coverage or functional reconstruction<sup>17,18</sup>. Recently, as the concept of neuromuscular compartmentalization has been gradually accepted by more doctors, many operations with segmental gracilis muscular or myocutaneous flap transfer have been reported.

In 1990, Wellisz et al<sup>14</sup> longitudinally split the gracilis into halves and used them for free-tissue coverage of two sites in a patient with bilateral calcaneal fractures and posttraumatic osteomyelitis. Schoeller et al<sup>19</sup> then transversely split the gracilis into two flaps for coverage of two separate defects in a patient with multi-fracture and crush injuries in the right foot. No matter the difference in splitting the muscle, they both achieved reduction of donor-site morbidity to a minimum and utilizing a single muscle to a maximum<sup>20</sup>. These operations also illustrated that the gracilis muscle could be harvested with a high degree of tailoring. Later on, Copeland et al<sup>21</sup>, Soper et al<sup>22,23</sup> and Kayikcioglu<sup>24</sup> designed the short gracilis myocutaneous flaps, which is supplied by the branch of obturator artery for vaginal, vulvo-



**Figure 4.** When there was titanic contractions in the anterior muscle bundle, the distal end of the reconstructed penis became “erected”, as is seen in Figure 4A and 4B.



**Figure 5.** The stimulation frequency was 25Hz and there was tetanic contractions in the anterior muscle bundle. The CMAP was recorded synchronously by the needle-shaped electrodes. A series of similar spontaneous electric activities could be found next to the artificially-induced CMAP (Figure 5A). When the scanning speed decreased from 40 ms/div to 2 ms/div, the upwards initial wave is shown clearly in a more detailed figure (Figure 5B).

vaginal or scrotal reconstruction. Cavadas et al<sup>15</sup> designed the segmental gracilis free flaps for traumatic defects based on secondary pedicles. The muscle was simply harvested according to the need of the size of recipient site.

However, all the above-mentioned segmental gracilis flaps were only focused on soft tissue coverage and most of them had sacrificed the contractive function of the muscle. Yet Zuker et al<sup>25</sup> restored the facial animation of children with Mobius syndrome by segmental gracilis muscle transplantation. To reduce bulk, the anterior 60–75% of the circumference of the muscle was harvested and the neurovascular pedicle was protected at the midpoint of the segment. The substantial debulking and hemostasis control with cautery might cause denervation and devascularisation<sup>26</sup> of the muscle. Another risk was ischemia of flaps, which happened when the segment harvested was so small that few vessel branches had entered it or entered it but did not provide proper perfusion<sup>15</sup>. The success of these procedures depended on a precise knowledge of the neurovascular anatomy within the gracilis<sup>27</sup>. In order to minimize the damage to the survival and contractile function of the muscle segment, we need to split the gracilis according to the distribution of its intramuscular nerve and vessel branches.

To the best of our knowledge, despite the numerous studies about the neurovascular anatomy of the gracilis muscle, few studies in the literature have shown the intramuscular nerves and blood vessels displayed simultaneously on the same specimen<sup>26,28</sup>. We combined the colorful vascular perfusion with modified Sihler's intramuscular staining to show the intramuscular

nerve and vessel branches within the same muscle accurately and intuitively. Based on the clear relationship of intramuscular nerves and blood vessels<sup>29</sup> we, then, longitudinally split the rabbit gracilis muscle into two approximately equal halves, unlike some of the other previous operations which only partly split the muscle<sup>16</sup>. The results were satisfactory that this simple and safely longitudinally split method would not affect the survival and contractile function of the segment.

In designing the surface coverage of the reconstructed penis we referred to Yousif et al<sup>30</sup> which concluded that the musculocutaneous perforators of the proximal gracilis muscle tended to orient themselves in a transverse direction, also its different blood supplies were well communicated. Then, when we were splitting the gracilis, the skin above the posterior muscle bundle was raised but the connection between the anterior portion and skin was left untouched. The cutaneous flap far beyond the contour of anterior muscle bundle was then supplied by the transversely oriented perforators and survived well, no prosthesis was exposed. Thus, a different method of forming the gracilis myocutaneous flaps or tubes was also successfully shown in this study.

Within our animal models designed referring to Orticochea's operation<sup>7</sup>, the anterior gracilis muscle bundle was the functional unit and blood supply to the surface coverage, whereas the planted silastic support helped in maintaining the penile appearance and proportionate muscular tension. It also provided the fulcrum of rotation. When the dominant nerve was electrically stimulated with silver electrodes, the muscle bundle contracted, leading to an "erection" of the reconstructed penis.

The distal end could rise a to the highest position and maintain the maximum height for several seconds when the muscle occurred tetanic contractions, simulating the erectile action satisfactorily. And the CMAP was recorded synchronously by the standard circuit formed according to the requirements of Kimura<sup>13</sup>, which proved that the neuromuscular pathway was intact after the operations. As for the series of spontaneous electrical activities recorded next to CMAP, we considered that it might relate to the strong traction created by muscle bundle having tetanic contractions which pulled the dominant nerve ahead and upward and crushed by the electrodes with some kind of mechanical stimulation.

Moreover, we had observed and recorded several times that paroxysm dithering occurred in the reconstructed penis when an operated rabbit was moving its lower extremities. The swinging activities of the reconstructed penis was strong especially when rabbit's lower extremities was left hanging and its paws, knees or groin area was touched, this was also noticed even when the touch point was upon the un-operated contra-lateral lower extremity. These voluntary muscle contractions have provided us with more objective evidence than the results of induced experiments that the neuromuscular pathway remained intact after the surgery. Furthermore, patients who had psychological rejection of a penis with entirely artificial erectile launching mechanisms could prefer a mechanism as described in this study. Actually we could not verify the independence of the gracilis anterior muscle bundle's function much further by ordering the rabbits to contract particular muscle and control the intensity or range of contraction as would have been possible in a human subject. Nor could we activate the anterior muscle bundle selectively as in biofeedback guidance experiments as in humans<sup>31</sup>. However, according to the concept of neuromuscular compartmentalization<sup>32</sup>, the maintained function of the anterior muscle bundle corresponded to the compartmentalization of the intramuscular nerve branches suggested by our anatomical study. It confirmed the feasibility of our surgical method which longitudinally completely split gracilis into two segments.

When considering the anatomical differences between a human and a rabbit, the fact that restoration of a urethra and of sensory function also needs to be included in a typical penis reconstruction, our current model is far from completion, but it has, however, provided some valuable theoretical basis for the improvement of clinical

penis reconstruction surgery. The results have also enriched the anatomical and physiological studies on compartmentalization of the gracilis. Moreover, we hope that this report will be useful to surgeons undertaking operations which have similar requirements for the donor tissue flaps such as in facial reanimation or tongue reconstruction<sup>25,33</sup>.

## Conclusions

According to the distribution of intramuscular nerve and vessel branches, rabbit gracilis muscle can be divided into two segments: the anterior and posterior bundle. The model penis reconstructed with longitudinally split rabbit segmental gracilis myocutaneous flap was able to satisfactorily simulate the erectile action. It has met the requirements of both restoring erectile function and improving the aesthetic appearance of a reconstructed penis.

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## Conflict of Interest

The Authors declare that they have no conflict of interests.

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