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Penile enhancement with rectus muscle fascia and testicular tunica vaginalis grafts: an experimental animal study

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Abstract
Purpose To enhance rat penises experimentally with rectus muscle fascia (RMF) and testicular tunica vaginalis grafts (TVG).
Methods Twelve Wistar albino rats were distributed into two equal Groups, A and B. There were six rats in each group. RMF and TVG were used to enhance rat penises in Groups A and B, respectively. Circumferences of the penises were measured preoperatively and at three different times after the operation. Two, two and eight rats were killed 10 days, 1 month and 2 months after the operation, respectively, for histopathological examinations.
Results When we compared the measurements of preoperative and immediately postoperative circumferences, the mean increase was 23.4 ± 2.9 % in Group A and 19.9 ± 1.7 % in Group B. According to paired *t* test, the difference was significant ($p < 0001$), but the comparison between preoperative and postoperative first-month measurements was not found to be significant ($p > 0.05$). Histological examinations revealed an intensive inflammatory process at 10 days after the operation. Grafts were found to be totally absorbed in the first- and second-month examinations.
Conclusion In our study, implanted TVG and RMF could not survive because of insufficient vascularization and failure to maintain satisfactory surgical success. More studies are needed to increase the effectiveness of surgical techniques.

Keywords Animal model · Inflammation · Penile enhancement · Transplantation

Introduction

Penile length and thickness have been a cause of anxiety among men throughout history. Individuals with abnormal penile size may require surgical treatment, but sometimes men of normal penile size may also require help. Although arguable, there are two main underlying goals when men demand enhancement of their penis; one is to increase their self-confidence, and the other is improved satisfaction for their partners.

There are many defined surgical techniques for penile enhancement such as fat, silicon, hyaluronic acid gel injections, dermal fat grafts, allografts and venous grafts. The ultimate aim of these techniques is to have a symmetric and uniformly enhanced penis, but the long-term outcomes of existing surgical techniques have not been particularly successful [1]. The goal of our study was to enhance rat penises with RMF and TVG.

Materials and methods

Animals

Wistar albino (WA) rats were obtained from the Ministry of Agriculture, and this study was performed in the Experimental Animals Laboratory of the Ankara Research and

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Training Hospital. We used 12 adult WA rats with a mean weight of 260 g. Rats were housed under standard lighting and given food and water. The local ethical committee of the Ankara Research and Training Hospital approved all procedures.

Working schema

There were six rats in each group. RMF and TVG were used in Groups A and B, respectively. Circumferences of the penises were measured preoperatively and immediately after the operation. One, one and four rats in each group were killed 10 days, 1 month and 2 months after operation, respectively, for histopathological examinations

Working schema

Group A1: One rat. Penile thickness will be measured and after penectomy, and it will be killed on the tenth day, postoperatively.
Group A2: One rat. Penile thickness will be measured and after penectomy, and it will be killed at the end of the fourth week, postoperatively.
Group A3: Four rats. Penile thickness will be measured and after penectomy, and they will be killed at the end of the eighth week, postoperatively.
Group B1: One rat. Penile thickness will be measured and after penectomy, and it will be killed on the tenth day, postoperatively.
Group B2: One rat. Penile thickness will be measured and after penectomy, and it will be killed at the end of the fourth week, postoperatively.
Group B3: Four rats. Penile thickness will be measured and after penectomy, and they will be killed at the end of the eighth week, postoperatively.

Surgical procedure

A surgeon certified for animal experiments performed the surgeries for this study. Initially, ether (inhaler) + ketamine (intramuscular) 100 mg/kg was used for the standard anesthesia, and then, the rats were fixed to the surgical board with surgical tapes. After initializing the anesthesia, penile length and thickness were measured twice on the stretched flaccid penises and their mean value was noted.

The rats were randomized into two groups; Group A and Group B. Each group had six rats. In Group A, suitably sized RMF grafts were dissected after a median abdominal incision of each rat after local cleaning with povidone iodine. In Group B, suitably sized TVG was

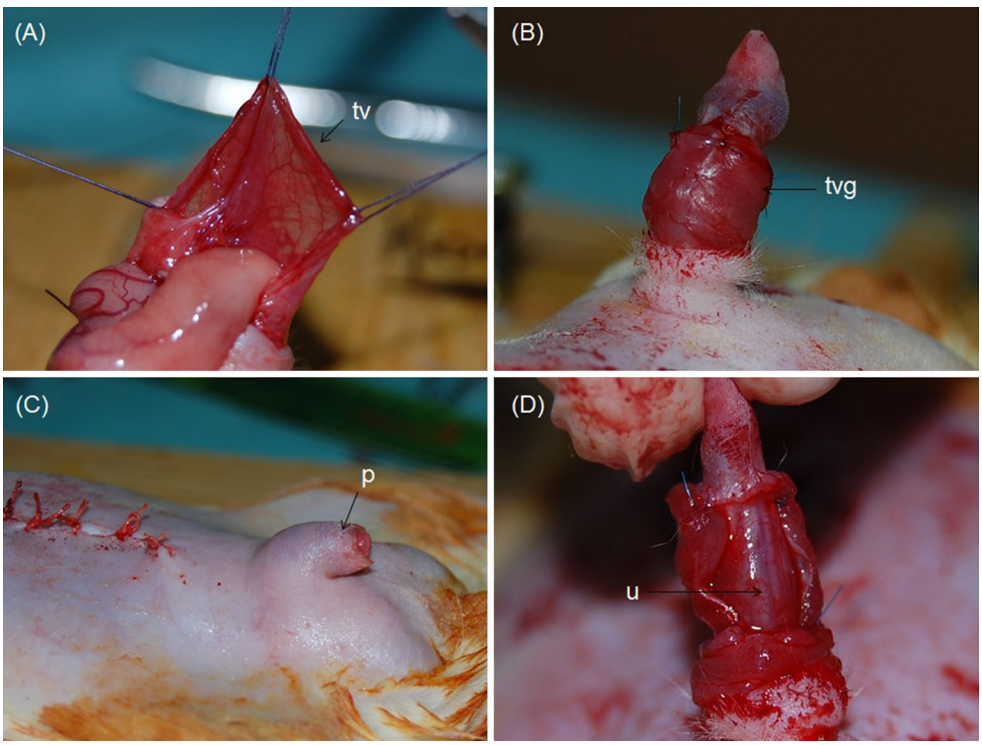

Fig. 1 Dissection and wrapping of TVG (**a**, **b**, **d**) and rat penis after enhancement with RMF (c). **a** Harvesting TVG from testicular tunica vaginalis (tv). **b** Wrapped appearance of corpus cavernosum with TVG (tvg). **c** Rat penis (p) after enhancement with RMF. **d** Preserved urethra (u) after wrapping corpus cavernosum with TVG

Table 1 Group A and B, preoperative and postoperative comparison of penile thicknesses

	Mean (mm) ± std		*p* value	
	Group A	Group B	Group A	Group B
Preop–postop 0	(10.3 ± 0.8)–(12.7 ± 0.9)	(11.3 ± 0.8)–(13.5 ± 0.5)	$p < 0.001$	$p < 0.001$
Preop–postop day 10th	(10.3 ± 0.8)–(12.3 ± 0.8)	(11.3 ± 0.8)–(13.3 ± 0.8)	$p < 0.001$	$p < 0.001$
Preop–postop 1st month	(10.3 ± 0.8)–(10.2 ± 0.8)	(11.3 ± 0.8)–(11.2 ± 0.8)	$p = 0.374$	$p = 0.374$
Preop–postop 2nd month	(10.3 ± 0.8)–(10 ± 0.8)	(11.3 ± 0.8)–(11 ± 0.8)	$p = 0.391$	$p = 0.391$

dissected after a median incision to the scrotum of each rat (Fig. 1a).

Before grafting, circumcision was performed and the penises were degloved. Then the grafts from rectus muscle fascia were implanted in the rats' penises in Group A, and the grafts from testicular tunica vaginalis were implanted in the rats' penises in Group B (Fig. 1b, c). The grafts were wrapped on to the tunica albuginea of the corpus cavernosum with a crescent shape except for the urethral surface and fixed with 6/0 polydioxanone. The suturation was on both sides of the urethra. Dorsal penile veins and the urethra were all preserved (Fig. 1d).

Evaluating penile thickness

After the penile enhancement surgery, thickness of the flaccid penises was measured immediately after surgery, on the tenth day and at the end of the fourth and eighth weeks, postoperatively. Penile thicknesses were measured twice on two different parts of the penis shaft, and the average measurements were noted. A length of surgical suture was used for circumferential measurement, and then, the suture's length was measured on a surgical ruler. In addition, penectomy was performed after measuring penile thickness on the stretched flaccid penis of one rat in each group on the tenth day, on one rat in each group at the end of the fourth week and on four rats in each group at the end of the eighth week, postoperatively for histopathological examination. After the penectomy, rats were killed with an overdose of ketamine.

Histopathological examination

A pathologist conducted histopathological examinations of the grafts after penectomy on the tenth day and at the end of the fourth and eighth weeks, postoperatively. After penectomy, specimens were fixed in a 10 % formalin solution. Afterwards, fixed penises were paraffinized and cut into 5-μm slices with a microtome blade. Hematoxylin–eosin staining was performed, and the slices were examined under light microscopy. Histopathological examination was performed at the Department of Pathology, Ankara Training and Research Hospital.

Statistical analysis

SPSS 15.0 for Windows (Statistical Program for Social Sciences) program was used for the statistical analysis. Shapiro–Wilk test was used to evaluate the disturbance of normality. Repeated-measures test was used for variance analysis. Preoperative and postoperative comparisons were done with paired *t* test for each group. Statistically significant *p* value was $p < 0.05$.

Results

Penile thicknesses

The list of the circumferential measurements of the penises before surgery, immediately after surgery, on the tenth day and at the end of the fourth and eighth weeks postoperatively is shown in Table 1.

According to the Shapiro–Wilk test, measurements made in the experimental models were distributed normally.

When postoperative measurements were compared with early preoperative dimensions, there was an average increase of 2.42 mm (mean 23.4 ± 2.9 %) in the RMF group and an average increase of 2.25 mm (mean 19.9 ± 1.7 %) in the TVG group ($p < 0001$). However, it was recorded that the increases in penile thickness declined over time. An increase of 2 mm (mean 19 %) and 2 mm (mean 17 %) in RMF and TVG groups was found in day 10 measurements, respectively ($p < 0.001$) (Fig. 2).

Paired *t* test was used for the preoperative and postoperative comparison of each group.

For Group A, there was a statistically significant difference between the preoperative measurements and postop day zero measurements ($p < 0.001$). There was also a statistically significant difference between preoperative measurements and tenth day measurements after surgery ($p < 0.001$). However, there was no statistically significant difference between preoperative measurements and those at the end of the fourth and eighth weeks, postoperatively ($p = 0.374$ and $p = 0.391$, respectively) (Table 1). The penile thickness measurements after the first and second months were the same (Fig. 2).

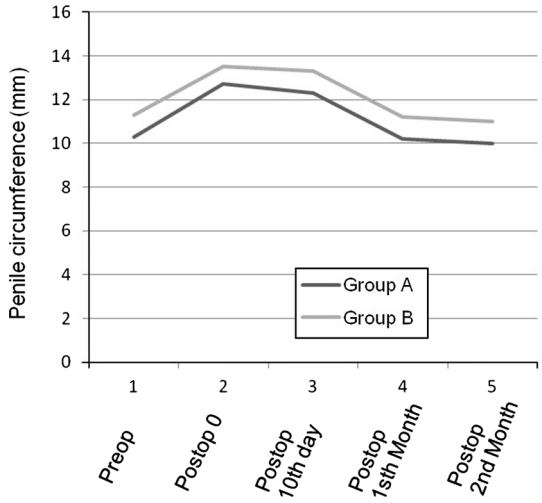

Fig. 2 Penile thickness differentiations in time for both Group A and Group B

For Group B, there was a statistically significant difference between the preoperative measurements and post-op day zero measurements ($p < 0.001$). There was also a statistically significant difference between preoperative measurements and the tenth day measurements after surgery ($p < 0.001$). However, there was no statistically significant difference between preoperative measurements and those at the end of the fourth and eighth weeks, postoperatively ($p = 0.374$ and 0.391, respectively) (Table 1). The penile thickness measurements at the end of the first and second months were the same (Fig. 2).

Histopathological results

Hematoxylin–eosin staining revealed an intense inflammatory reaction and fibrosis around the tunica vaginalis and rectus fascia grafts on the tenth-day examination (Fig. 3a). The grafts were totally resorbed, and there were some signs of suture materials microscopically in the examination at the end of the first month (Fig. 3b). It was revealed in the histopathological examination that the grafts were totally resorbed in each group at the end of second month.

Discussion

After years of surgical experience, even basic issues such as patient profile and the identity of those subgroups of males who would benefit from penile augmentation surgery are unclear [2]. Moreover, valid indications for performing such procedures, selection of the most suitable procedure and designation of the outcome measures are all open issues. The absence of universally acceptable parameters for normal penile length and girth further complicates these issues.

Both RMF and TVG can be used for different surgeries. RMF grafts are used for female stress urinary incontinence surgeries. In a study, Athanasopoulos et al. [3] treated 264 female patients for stress urinary incontinence using the autologous fascia rectus sling. They reported that 224 (85 %) patients were successfully treated and satisfied. The mean follow-up time was 27.8 months. Also, fascia rectus can be used for abdominal sacrocolpopexy [4]. Tunica

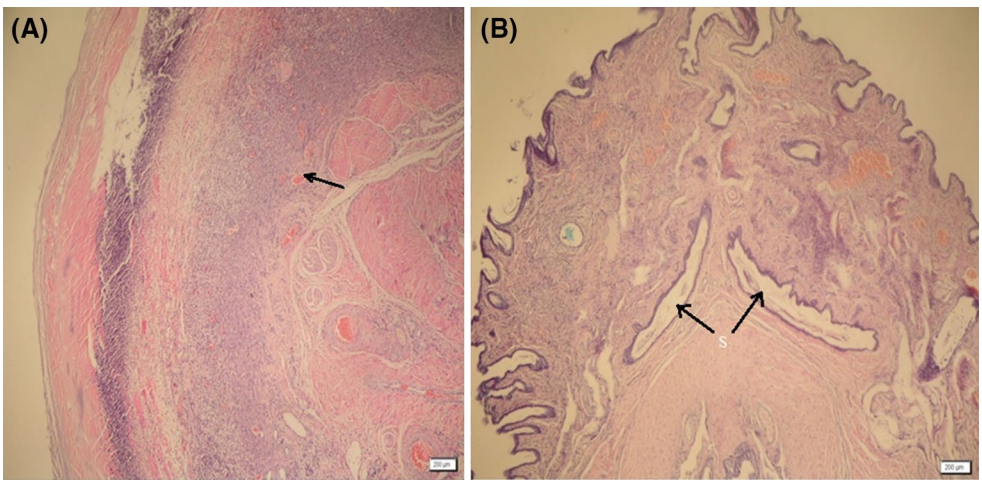

Fig. 3 Histological appearances of rat penises after graft implantation. Hematoxylin–eosin staining. **a** Histological appearance of intense inflammatory reaction (i) at the day of tenth examination. **b** Histological appearance of the rat penises at the end of first month, no evidence of graft material except suture materials(s). (**a**, **b**) *Bar* 200 μm

vaginalis grafts are described in urethroplasty for urethral strictures, severe chordee reconstructions and urethral fistula repairs [5–7]. In our study, we used RMF grafts and TVG to enhance rat penises experimentally.

The relatively rare published data have shown that penile enhancement techniques result in a 2.5-cm enhancement of penile circumference. The significance of these "successful" results is overshadowed by the fact that they do not directly correlate with patient satisfaction [8]. In a study, Yacobi et al. [9] reported penile thickening by injection of liquid silicon in 324 patients. In that study, the authors injected large volumes of silicone between penile skin and corpus cavernosum dorsally and laterally. It was reported that the average penile circumference was 9.5 cm preoperatively and 12.1 cm postoperatively. There was an average 27 % increase in penile circumference. Augmentation phalloplasty for penile enlargement, in which the corpora cavernosa is enlarged by bilateral venous graft implantation, was first described by Austoni et al. [10]. In Austoni's report, none of the 39 men who underwent this procedure had any postoperative complications. At the 9-month follow-up, penile diameter had increased by 1.2–2.1 cm. In another study, the changes in size and consistency of a dermal fat graft for penile girth enhancement were documented [11]. At 1 week after transplantation, the consistency of the dermal graft was still soft, with 70–90 % of fat preserved, but at 8 weeks most of the fat had been replaced by fibrotic tissue.

In our study, there was a mean increase of 2.42 mm (mean 23.4 ± 2.9 %) ($p < 0.001$) and 2.25 mm (mean 19 ± 1.7 %) ($p < 0.001$) in penile circumference measurements, in RMF and TVG groups, respectively, in the early postoperative evaluation. Increase in the circumference declined in time and no statistically significant difference between preoperative measurements and 8-week measurements postoperatively were recorded in either group ($p = 0.374$). Thus, the grafts in both groups failed to survive. In general, we can find no long-term results of penile enhancement surgeries in the literature and so we do not know whether the early postoperative success rates in the published articles were permanent or not.

There are too many serious complications reported after penile enhancement surgeries. In a study with 84 patients, authors reported the use of a biodegradable scaffold seeded with autologous fibroblasts that was formatted into a tube and wrapped around the degloved penis [12]. In this study, they reported infection, penile skin pressure necrosis and seroma in ten patients. There are also complications reported after subcutaneous fat injections for penile enhancement. After large amounts of fat injection, deformities such as curvature or asymmetry of the penis as well as the formation of nodules of liquefied, necrotic or calcified fat may occur [13]. In our study, we encountered no complications such as penile skin necrosis or penile shape distortion.

In our study, histopathological examinations revealed an intense inflammatory reaction and fibrosis around the grafts and vascularizations were insufficient for survival. Eventually, they were resorbed on the implantation site. For the acceptance of the grafts, both the graft's vascular structure and the acceptor site's vascularity must be suitable for neovascularization. In an animal study, autogenous tunica vaginalis grafts were used for augmentation phalloplasty [14]. They augmented dog corpus cavernosums by implanting tunica vaginalis grafts bilaterally after a longitudinal incision. They found mild inflammatory reaction and fibrosis around the grafts after surgery. In another study, porcine small intestinal submucosa (SIS) was used for penile enhancement in a rat model [15]. After bilateral longitudinal I-shaped incision to the tunica albuginea, they dissected the plane between tunica albuginea and corpus cavernosum and then patched the incision with SIS. At 2 months after surgery, penile circumference of the SIS group was significantly larger and histological study revealed minimal fibrosis.

There are no internationally accepted standards for penile girth, and this makes the surgery decision confusing. Many surgical techniques for penile enhancement have been described for years. However, it is unclear which technique has the best results and, more importantly, which patient groups can benefit from these surgeries.

Conclusion

In our study, implanted RMF and TVG could not survive because of insufficient vascularization and failure to maintain satisfactory surgical success. It seems that studies with pediculated grafts to enhance the penis are likely to succeed. Until more data on these issues are available, penile augmentation should be performed only when a penile prosthesis is implanted or when reconstruction of the penis is required for sexual function [2]. Our study may provide an experimental basis for surgical techniques of penile enhancement.

Conflict of interest The authors have no conflicting financial interests.

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