

Comparison and Investigating the Effect of Using an Amniotic Membrane and Rectus Sheath to Repair Damage of the Tunica Albuginea in the Penis: An Animal Study on Rabbits

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Abstract

In this study, the rectus sheath and amniotic membrane were utilized to repair tunica albuginea damage in rabbits. Ten male New Zealand white rabbits were included in the study. Group 1 consisted of 5 rabbits in which the tunica albuginea injury was repaired using amniotic membrane from pregnant rabbits. Group 2 consisted of 5 rabbits in which their rectus sheath was used to repair the tunica albuginea injury. Our results showed that both the use of amniotic membrane and rectus sheath are effective in repairing tunica albuginea damage in rabbits. Based on our macroscopic and microscopic findings, there was no statistically significant difference between the effectiveness of amniotic membrane and rectus sheath in repairing tunica albuginea damage. Both the amniotic membrane and the rectus sheath were equally effective in repairing tunica albuginea damage.

Keywords: Amniotic membrane, Rectus sheath, Damage of the tunica albuginea, Penis

Introduction

In the past decade, significant progress has been made in andrological surgery, leading to better treatment for conditions like Peyronie's disease (PD), tumor resection, organ reconstruction, and penile augmentation. All surgical approaches for these conditions involve repairing or reconstructing the tunica albuginea (TA), which surrounds the corpora cavernosa penis [1,2]. Despite extensive research on using various biological materials like skin, muscle aponeurosis, human dura mater, tunica vaginalis, and

pericardium, engineered TA suitable for graft use has not yet been successfully developed [3,4]. Recent efforts using acellular matrices prepared from different tissues have also not produced the anticipated results in PD penile reconstruction [5,6].

The human penis is structurally and functionally unique. This characteristic, along with the distinctive biochemical, structural, and ultrastructural features of its extracellular matrix (ECM), differentiates it from the reproductive organs of other mammals and all other human organs. This makes it

challenging to find biocompatible materials [5,7]. Advances in microsurgery and transplantation immunology have made allogeneic tissue transfer the preferred method for managing patients with severe penile conditions. Clinical studies have documented composite tissue allograft transplantation [8,9].

The amniotic membrane has been used extensively to treat various diseases and injuries because it stimulates the immune system and helps repair damaged tissue. It has a long history of use in different parts of the body. This membrane provides anti-inflammatory, antimicrobial, and anti-scar properties, as well as low immune stimulation, promotes new blood vessel growth, and reduces oxidative stress to aid in tissue repair [10-13]. The first report of using amniotic membrane in urology dates back to 1982 when it was stabilized with glutaraldehyde for bladder reconstruction [14]. Unfortunately, there have not been proper studies in this field since then, despite the high potential for helping patients in urology [15,16]. On the other hand, although the rectus sheath is mentioned as a suitable graft for repairing tunica albuginea in various articles, limited studies have been conducted in this area [17].

This study reports on the role of the amniotic membrane and the rectus sheath in causing damage to the rabbit penis, as well as the transplantation of the human amniotic membrane and the rectus sheath. It also examines the histopathological changes of the samples after transplantation.

Materials and Methods

Animals

The study was conducted on rabbits using the tunica albuginea penile injury model. Rabbits were chosen for their penile similarity to humans, availability, and well-known

histology. After obtaining approval from the educational council and the specialized committee on ethics in working with laboratory animals of Jundishapur University of Medical Sciences, Ahvaz (IR.AJUMS.HGOLESTAN.REC.1402.134), ten male New Zealand white rabbits of similar age and weight (about 2 to 4 months old and weighing 2.5 to 3.5 kg) were included in the study. These rabbits were procured from Tehran Pasteur Institute. The rabbits were placed in the ward one week before the study began and were maintained under controlled conditions: air temperature between 16 and 22 degrees Celsius, air humidity between 30 and 70%, a 12-hour light cycle, a 12-hour darkness cycle, and access to food and drinking water.

Group 1: Five rabbits were used to repair the damage to the amniotic membrane of pregnant rabbits. Group 2: It consisted of five rabbits, and their rectus sheath was used to repair the injury.

Tissue damage

Anesthesia was administered with a face mask under the supervision of a veterinary anesthesiologist in the operating room. A nerve block of the penis was carried out using 2% prilocaine. A 0.5 x 0.5 cm incision was then carefully made in the tunica albuginea with a scalpel blade. After surgery, rabbits were given 15 mg/kg of acetaminophen, followed by the same dose administered as a suppository, effectively controlling pain for up to 6 hours (**Figure 1**).

Treatment and assessment

After preparing the amniotic grafts and rectus sheath, the repair was done using 0.6 and 0.7 Vicryl threads in a running stitch loop. Tetracycline ointment was applied to the repair site.



Figure 1. Damage caused to the tunica albuginea of the rabbit penis.

After delivery, the amniotic membrane was retrieved from the placenta of the female rabbit and separated from the chorion under sterile conditions. The membrane was then washed with a normal saline solution that contained antibiotics. The antibiotic composition included 50 mg/ml of penicillin, 100 mg/ml of gentamicin, 50 mg/ml of streptomycin, and 2.5 mg/ml of amphotericin B. After washing, the amniotic membrane was cut into suitable pieces and used immediately in the surgical procedure.

To prepare the rectus sheath, after incising the skin and subcutaneous tissue, the rectus sheath was sectioned into 1 x 1 cm pieces, detached from the surrounding tissues, and utilized as a graft.

After four weeks, the operation site was examined to evaluate postoperative bleeding, inflammation, induration around the wound, infection at the wound site, and possible death of the animal.

At the microscopic level, rabbits were euthanized using an anesthetic overdose method (sodium thiopental at a dose of 300 mg per kilogram intravenously) to obtain samples from the penile tissue in the repair site. The samples were fixed in formalin and transferred to the pathology laboratory.

After preparing suitable slides from the samples and performing hematoxylin-eosin staining, a pathologist examined the samples using a light microscope to assess the formation of necrosis and scarring in the operation site, the presence of inflammation, and the degree of typical restoration of epithelial tissue.

Statistical analysis

The data from the study was analyzed using Graph Pad Prism version 8 software. After checking for normality, we used parametric tests for comparing two groups in cases of normal data and non-parametric tests for non-normal data. We considered a significance level of less than 5 percent ($p < 0.05$).

Descriptive data were reported as numbers and percentages.

Results

The results revealed that in the group treated with amniotic membrane, 2 cases of mild bleeding occurred after surgery, which resolved on their own without any intervention. In the group treated with the rectus sheath, no bleeding was observed after surgery. Two cases of inflammation and induration around the wound were reported in the amniotic membrane group, and one case in the rectus sheath group. There were no instances of infection at the wound site in any of the groups studied. One animal in the amniotic membrane group did not survive (**Table 1**). There was no significant difference in the parameters mentioned between the two groups.

During the histological examination, normal re-epithelialization was observed in three cases of the amniotic membrane group (rabbits No. 1, 2, and 3) and three cases in the rectus sheath group (rabbit's No. 1, 2, and 3). The thickness of the epithelial layer was less than usual (close to normal) in one case from each group (No. 1 in the amniotic membrane group and No. 2 in the rectus sheath group). Incomplete re-epithelialization was reported in one case of the amniotic membrane group (No. 4) and two instances of the rectus sheath group (No. 4 and 5). In rabbit's No. 4 in both groups, incomplete re-epithelialization, inflammation, and fibrosis were observed; however, in rabbit's No. 5 in the rectus sheath group, only mild fibrosis was seen without any inflammation. Mild inflammation was observed in two cases (No. 1 and 4) of the amniotic membrane group and one (No. 4) of the rectus sheath group. No severe inflammation was observed in either group. Additionally, mild fibrosis was noted in two cases (No. 1 and 4) of the amniotic membrane group and three cases (No. 2, 4, and 5) of the rectus sheath group. However, there were no cases of moderate or severe fibrosis in either group. There was no significant difference in the parameters mentioned between the two groups (**Table 2** and **Figure 2**).

Table 1. Basic information.

	Amniotic Membrane Group	Rectus Sheath Group	p
Bleeding after surgery	1 (20)	0 (0)	>0.05
Inflammation and induration	2 (40)	1 (20)	>0.05
Infection	0 (0)	0 (0)	>0.05
Animal death	1 (20)	0 (0)	>0.05

Table 2. Appearance characteristics of wounds in the studied groups based on frequency and percentage.

	Amniotic Membrane Group	Rectus Sheath Group
Normal re-epithelialization	3 (75)	3 (60)
Incomplete re-epithelialization	1 (25)	2 (40)

Mild inflammation	2 (50)	0 (0)
Moderate inflammation	0 (0)	1 (20)
Severe inflammation	0 (0)	0 (0)
Mild fibrosis	50 (50)	3 (60)
Moderate fibrosis	0 (0)	0 (0)
Severe fibrosis	0 (0)	0 (0)

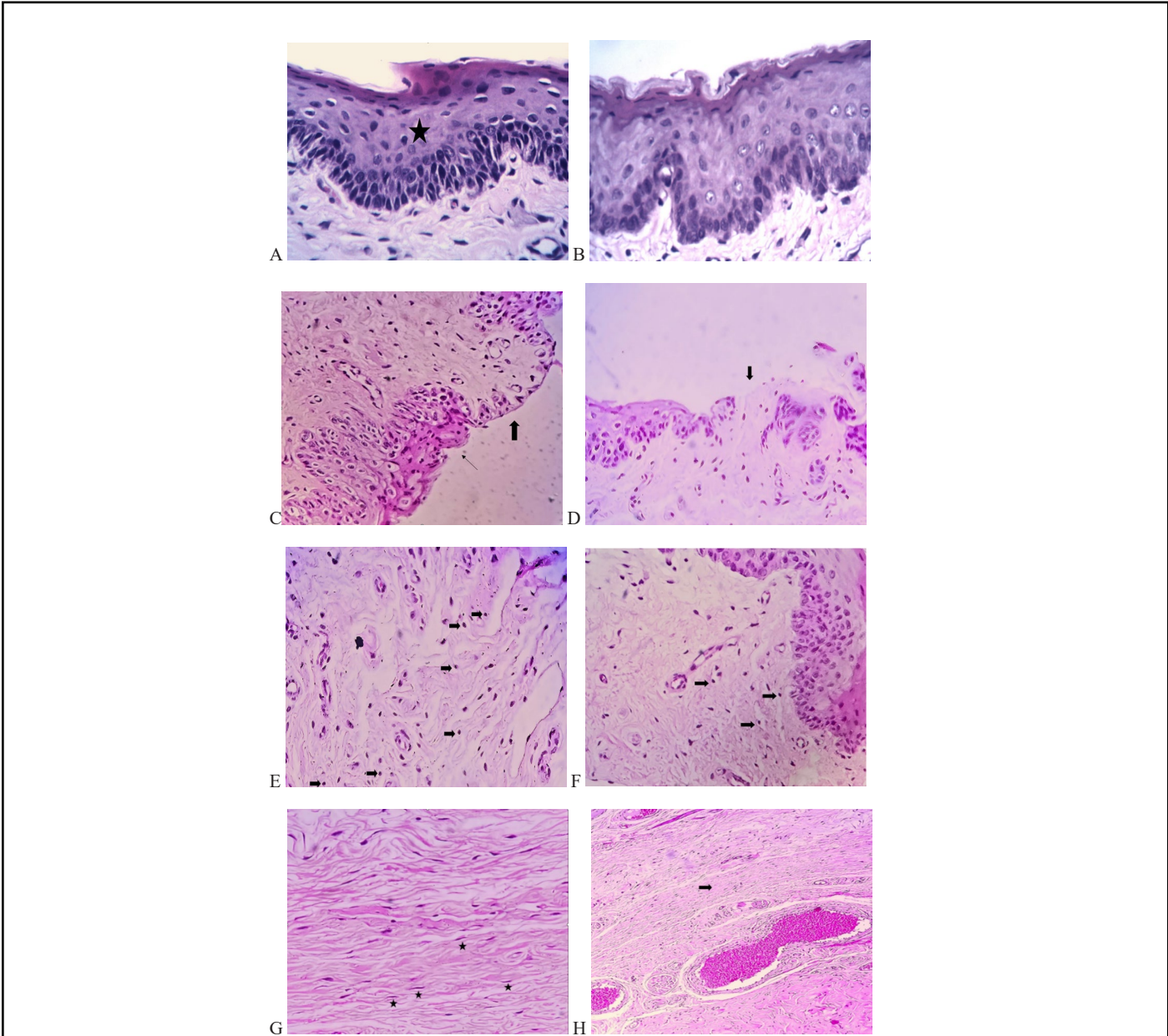


Figure 2. Pathological findings (hematoxylin and eosin staining). **(A)** Complete reepithelialization in the rectus sheath group. **(B)** Complete reepithelialization in the amniotic membrane group. **(C)** Incomplete reepithelialization in the rectus sheath group. **(D)** Defective reepithelialization in the amniotic membrane group. **(E)** Lamina propria around the corpus cavernosum in the rectus sheath group. Note the moderate number of chronic inflammatory cells (arrows) in the lamina propria. **(F)** Lamina propria around the corpus cavernosum in the amniotic membrane group. Note the moderate number of chronic inflammatory cells (arrows) in the lamina propria. **(G)** Fibrosis and fibroblast cells in the rectus sheath group. **(H)** Fibrosis and fibroblast cells in the amniotic membrane group.

Table 3. Histopathological findings.				
	NO	re-epithelialization	Inflammation	Fibrosis
Amniotic Membrane Group	1	0	1	1
	2	0	0	0
	3	0	0	0
	4	1	1	1
Rectus Sheath Group	1	0	0	0
	2	0	1	1
	3	0	0	0
	4	1	1	1
	5	1	1	1

Discussion

PD affects nearly 10% of men and is characterized by TA fibrosis, plaque formation, and potential calcification, leading to painful erections and penile curvature. This condition can complicate sexual intercourse, often requiring reconstructive surgery in its chronic stage. Surgical options include shortening the convex TA, lengthening the concave side with grafts, or penile implants. The unique structure of the human penis complicates grafting with synthetic or heterologous materials, often resulting in inflammatory responses and complications. Acellular matrices are potential candidates for genitourinary reconstruction, while autologous TA grafts show promise but are limited by cost and inconsistent outcomes. The quest for ideal TA materials continues [18-23].

In this study, the researchers investigated the impact of the amniotic membrane and rectus sheath on genital damage in rabbits. It was found that the healing of the wound was better in the rectus sheath and amniotic membrane groups, as indicated by the improved formation of the skin epithelium layer and reduced inflammation. However, there was no significant difference in the parameters mentioned between the two groups.

The tunica albuginea may suffer damage due to PD, physical injury, or aging. This can lead to issues like penile curvature, painful erections, and difficulties with intercourse, typically requiring surgical intervention. Some researchers suggest using autologous sheaths, the submucosa of the small intestine [24,25], the pericardium [26], muscle fascia [27], or porcine bladder for repairing the tunica albuginea.

The use of an amniotic membrane is considered an innovative method for treating PD, penile cancer, and congenital genital abnormalities. Salehipour *et al.* [28], evaluated the effect of human amniotic membrane transplantation on the tunica albuginea defect of the dog's penis. Ten healthy male dogs were selected for the study. A 3 x 2 cm diamond cut was made on

the tunica albuginea and the squamous epithelium covering it was removed. The amniotic membrane was folded twice and grafted on the defect. After eight weeks, artificial erection was performed for five dogs, and another five dogs underwent the procedure after 12 weeks. Artificial erections were successful in all dogs, and no infections or complications were observed. Histopathological examination showed complete re-epithelialization with squamous epithelium and collagen fiber deposition [28]. Dysplasia was not observed. Despite the high potential of the amniotic membrane for therapeutic urology, concerns remain about the lack of a standard protocol for its preparation and storage, its heterogeneity, as well as its fragility and low tensile strength [16].

In a study conducted by Craatz *et al.* [2005], the researchers investigated the outcomes of surgery and the incidence of complications in 12 patients with PD who underwent grafting with a rectus sheet to fix penile curvature. The results of the study indicated successful correction of penile deviation in 10 patients, while two patients still had remaining deviations of 15° and 35°. Additionally, six patients experienced minor post-surgery complications that did not necessitate further surgical intervention. The study suggested that the rectus sheath possesses morphological characteristics similar to the tunica albuginea, making it an ideal autologous graft [17].

Conclusion

The amniotic membrane and rectus sheet can be used as effective methods for treating tunica albuginea. These constructs are safe, affordable, biodegradable, and readily available, and can be used to treat Peyronie's disease, penile cancer, congenital penile deformities, and penile reconstructive surgery. However, the amniotic membrane has limitations for bedside use. This membrane is thin, has low resistance to pressure, lacks good plasticity, is difficult to suture, and may be absorbed by the tissue before complete repair of the damaged area. In general, our results have shown that the use of a rectus sheet in tunica albuginea

injury has better capabilities for macroscopic wound repair in terms of the formation of the skin epithelium layer and the disappearance of apparent inflammation compared to the amniotic membrane. More comprehensive studies are needed to investigate the impact of amniotic membrane or rectus sheath in tunica albuginea injury.

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Abbreviations

TA: Tunica Albuginea; PD: Peyronie's Disease.

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Conflicts of Interest

The authors declare no relevant conflicts of interest.

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