Dorsal tunica vaginalis graft plus onlay preputial island flap urethroplasty: Experimental study in rabbits

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Abstract  Objective: To assess the use of tunica vaginalis graft plus onlay preputial island flap in urethral reconstructive surgery in rabbits through histopathology.
Material and methods: We developed an experimental model of urethroplasty that resembles one-stage complex hypospadias surgery with divided urethral plate. The tunica vaginalis graft is dorsally placed to recreate the urethral plate and the internal preputial island flap is placed onlay to complete the urethroplasty. Sixteen animals were divided into four equal groups and sacrificed at 2, 4, 8 and 12 weeks after surgery, the penis being sent for histopathological evaluation.
Results: There were no deaths related to the procedure or wound breakdown; all rabbits voided spontaneously after surgery. Two urethrocutaneous fistulae were found. Microscopically, good tissue integration was observed, the tunica vaginalis mesothelium was gradually replaced by a more stratified epithelial lining, similar to the urothelial lining of the native urethra. The stratified squamous non-keratinized lining of the internal preputial island flap also changed into a thinner epithelial lining with only 4–5 cell layers. Two urethral diverticula were found.
Conclusion: A tunica vaginalis graft placed dorsally plus an onlay internal preputial island flap was shown to be a successful technique for urethroplasty in an animal model.

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Introduction

Severe hypospadias, comprising 20%–30% of cases, represents one of the most challenging problems in pediatric urology. A major issue in the management of such cases is preservation of the urethral plate. Although most current techniques are based on urethral plate preservation by its tubularization or as a base for an onlay procedure, in a few selected patients, especially those with severe chordee, it is almost impossible to preserve the urethral plate [1]. Hollowell et al. reported that it was necessary to divide the urethral plate in 10% of cases at an important hypospadiology center [2].

Most authors prefer two-stage procedures after sectioning the urethral plate. Macedo and Srougi published a new approach: using a buccal mucosal graft to rebuild the urethral plate and enable an onlay urethroplasty with a preputial island flap so as to make a neourethra in a one-stage procedure, the so-called three-in-one approach [3]. Subsequently, Calado et al. successfully used a tunica vaginalis graft dorsally placed in a rabbit urethroplasty model [4]. Our purpose was to combine these two principles and create an experimental animal model of one-stage hypospadias surgery after dividing the urethral plate that precludes the use of extra-genital tissue. The tunica vaginalis is used as a graft to rebuild the urethral plate associated to an onlay preputial island flap. The theoretical advantage of this concept is in keeping to a one-stage repair but replacing buccal mucosa with tunica vaginalis, and, therefore, working only in the genital area.

Despite much clinical information regarding preputial island flaps, no experimental studies on healing and tissue changes in this important urethral reconstructive surgery were found in the literature.

Materials and methods

We chose the rabbit because it has important features, including ease of manipulation and ready access to the urethra, and has been widely used as a model for male genital surgery. The experimental protocol was reviewed and approved by the Local Animal Research Committee. A total of 16 New Zealand white rabbits, aged approximately 8 weeks and weighing 2.5–3.0 kg, were acclimated at the Experimental Research Animal Surgery Department for 1 week before the procedures.

The rabbits were anesthetized intramuscularly with ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg), and local anesthetic (xylocaine) was used to perform a penile block. All animals were operated under sterile conditions and optical magnification (2.5×).

A 10-Fr urethral catheter was inserted and the penis was released by dividing the perineal skin web between the ventral aspect of the penis and the anus (Fig. 1A). Buck’s fascia was incised at the junction between the corpus spongiosum and the corpus cavernosum on each side; the urethra was then mobilized off the tunica albuginea (Fig. 1B). The corpus spongiosum was completely transversely sectioned resulting in two urethral stumps that, as in human urethral surgery, tend to retract making urethroplasty impossible (Fig. 1C). To overcome this problem, the urethral stumps were fixed to the underlying tunica albuginea, 0.7 cm apart, using 6.0 polydioxanone sutures (Fig. 1D).

The skin and dartos of the left hemiscrotum were incised to deliver the left testis in its tunica vaginalis covering. The tunica was incised longitudinally along the anterior surface and freed from its testicular attachment. The graft was obtained by sharp dissection with fine scissors (Fig. 2A) in order to avoid cremasteric fibers that might contract when grafted. The donor site was closed with a 5.0 running catgut suture.

The tunica vaginalis graft was dorsally placed over the corpora cavernosa in the gap between the two urethral stumps using 6.0 polydioxanone sutures (Fig. 2B), resembling the creation of a new urethral plate. The graft was placed in such a way that the tunica vaginalis mesothelial lining would face the neourethra lumen. An internal preputial island flap was used to cover the anterolateral aspect of the neourethra, since the posterior aspect had been covered by the tunica vaginalis graft. The flap was harvested similarly to current hypospadias surgeries, the only difference being that it was obtained from the prepuce ventral aspect (Fig. 2C and B). Once mobilized, the flap was rotated 180 degrees and anastomosed in an onlay fashion to the urethral stumps and tunica vaginalis graft. The skin was closed by recreating the web between the penis and the anus and the urethral catheter was removed immediately after the procedure. No stents or dressings were used.

The 16 animals were divided into four equal groups and sacrificed at 2, 4, 8 and 12 weeks after surgery. The entire penis was excised and fixed at 10% buffered formalin for 24 h, followed by paraffin wax embedding. Sequential sections of the penis from the glans to the base were obtained. Paraffin sections were stained with hematoxylin and eosin (H&E) as well as Masson’s trichrome.

Results

There were no deaths related to the procedure but one animal was sacrificed on postoperative day 3 due to local infection; the remaining animals were sacrificed on the date scheduled. All rabbits voided spontaneously after surgery and the macroscopic appearance of the penis was normal. Two urethrocysteustaneous fistulae were found (one in the 8-week group and the other in the 12-week group), resulting in a 25% major complication rate.

After sacrifice, the urethra was easily calibrated using a 10-Fr urethral catheter indicating that no strictures were formed. In order to guide the reader in interpretation of the histological sections, we made a scheme of the anatomical structures on the neourethra (Fig. 3).

Two weeks after surgery, microscopic examination showed significant polymorphonuclear cell infiltration representing an acute inflammatory reaction and an already moderate infiltration of lymphocytes and macrophages. Later the acute inflammation signs were less intense but did not disappear, and chronic inflammation signs were moderate even 12 weeks after the procedure, as shown in Table 1.

The mesothelial lining of the tunica vaginalis was gradually replaced by a more stratified epithelial lining, similar
to the native urethra urothelial lining. In the 2-week group the mesothelial lining, that corresponds to the last cell layer (Fig. 4), can be appreciated showing a good graft take; by 12 postoperative weeks it consisted of three or four cell layers of transitional cell-like epithelium.

After 2 weeks (Fig. 5) the non-keratinized stratified squamous lining of the prepuce island flap maintained its normal appearance; by 8 weeks, this epithelium showed signs of incomplete squamous metaplasia characterized by a columnar appearance of the outer cells. By 12 weeks (Fig. 6), this lining that originally consisted of many cell layers had its thickness dramatically reduced to four or five cell layers, thus resembling the original urothelial lining. In two animals of the 12-week group, the island flap that should theoretically be made only of internal prepuce had a segment of external prepuce, probably due to the learning curve of the procedure. The external foreskin was divided to access the urethra. (B) Urethra mobilized. (C) Urethral stumps after division of the spongiosum. (D) Corpora cavernosa in the gap between the urethral stumps.

**Discussion**

A great variety of tissues used as flaps or grafts have been tried, both experimentally and clinically, for urethral repair, mainly in proximal hypospadias. The most important grafts are penile or preputial skin graft, hairless-skin graft, bladder mucosal graft, tunica vaginalis graft, buccal mucosal graft, peritoneal graft, intestinal submucosa graft and vein graft. The most used donor sites for flaps are foreskin, tunica vaginalis and dartos. The use of many different tissues indicates that the ideal graft or flap material has not been identified yet. In the present study we evaluated the combination of a tunica vaginalis graft and internal preputial island flap.

Tunica vaginalis is a readily available tissue that has several advantages, including close proximity to the penis, ease of use and high vascularity; however, it may not be available in patients who underwent previous orchidopexy or herniorrhaphy [6]. When the patient has an associated undescended testicle, an additional inguinotomy may be necessary to harvest the tunica vaginalis and perform the orchidopexy. The main uses in penile surgery are related to urethral reconstructive surgery and repair of the corpora

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**Figure 1** Experimental procedure. (A) Skin web divided to access the urethra. (B) Urethra mobilized. (C) Urethral stumps after division of the spongiosum. (D) Corpora cavernosa in the gap between the urethral stumps.
Figure 2  Experimental procedure (cont.). (A) Tunica vaginalis graft harvested. (B) Tunica vaginalis graft placed dorsally in the gap between the urethral stumps. (C) Internal preputial island flap mobilized. (D) Island flap placed onlay anatomosed to the urethral stumps and the tunica vaginalis graft.

Figure 3  Transverse section of a rabbit penis and correspondent schema of the neourethra components: blue/corpora, orange/dorsal tunica vaginalis, yellow/preputial flap, brown/peri-urethral glands.
cavernosa in Peyronie’s disease [7] and children with chordee [8].

In 1967, Ariyoshi [9] reported the first use of tunica vaginalis for urethroplasty in an experimental study. Theodorescu et al. [10] compared the use of tunica vaginalis as a tube and an onlay, and showed that the optimal use of this tissue is as an onlay, because all tubes underwent contracture probably secondary to the presence of the cremasteric muscle. The tunica vaginalis was also studied as a ventral [11] or dorsal [4] graft in experimental urethroplasty models and both present good results. Clinically, the use of tunica vaginalis for urethroplasties was evaluated in two studies with different results. Joseph and Perez [12] found no advantage to its use as an onlay flap in proximal urethral repair due to a high stenosis rate. Snow and Cartwright [13] found it useful mainly in difficult cases, as long as there is no external exposure. The most established clinical use of tunica vaginalis in urethral surgery is to prevent or treat urethrocutaneous fistulae [14].

Regarding the use of tunica vaginalis there are many experimental studies and few clinical studies. However, as to preputial island flaps there are many clinical studies and no experimental models. The island flaps were first designed to be used as tubes [15] but, in 1987, Elder et al. [16] made a very important modification when placing them in an onlay fashion. The concept of preserving the urethral plate made this change a very popular and reliable tool in urethral surgery, and the current general consensus is that onlays lead to fewer complications than tubes. Based on the reliability of onlays, we believe that in severe hypospadias surgery, in which the urethral plate must be divided, it is possible to obtain good results through grafts in rebuilding the urethral plate, enabling the use of onlay flaps in a one-step procedure. Hence, we developed an experimental model to make a histological evaluation of the association between a graft to rebuild the urethral plate and an onlay island flap.

The replacement of the mesothelial lining of the tunica vaginalis by a stratified epithelium similar to native urethral epithelium as seen in this study is very similar to what has

<table>
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<tr>
<th></th>
<th>2 Weeks</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
</tr>
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<tbody>
<tr>
<td>Acute inflammation</td>
<td>4+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>2+</td>
<td>3-4+</td>
<td>2-3+</td>
<td>2+</td>
</tr>
<tr>
<td>Lining of the tunica vaginalis graft</td>
<td>No modifications</td>
<td>Beginning of stratification</td>
<td>Similar to 4-week group</td>
<td>3-4 Cell layers</td>
</tr>
<tr>
<td>Lining of the internal preputial island flap</td>
<td>No modifications</td>
<td>Incomplete squamous metaplasia</td>
<td>Similar to 4-week group</td>
<td>Marked reduction in thickness</td>
</tr>
<tr>
<td>Complications</td>
<td>1 Urethrocutaneous fistula</td>
<td>1 Urethrocutaneous fistula</td>
<td>1 Urethral diverticulum</td>
<td>1 Urethral diverticulum</td>
</tr>
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Inflammation score range: 0+: not present, 1+: minimal, 2+: mild, 3+: moderate, and 4+: extensive.
been reported in other publications. However, could this just be overgrowth of the urothelium on top of the tunica albuginea? In this study, that did not seem to happen; initially, the tunica vaginalis can be observed in the 2-week group showing good graft take, then analysis of the slides at 4, 8 and 12 weeks reveals a gradual change in the mesothelium per se. Moreover, an interesting fact is that this tissue showed the same behavior when used in very distinct applications in rabbit experimental models. In 1987, Talja et al. [11] used the tunica vaginalis as a ventral graft; in 1988, Khoury et al. [17] used it as a tubularized flap; in 1998, Theodorescu et al. [10], as an onlay flap and tube flap; and finally, in 2005, Calado et al. [4], as a dorsal graft. All these studies showed the same change in tunica vaginalis to resembling the native urothelium, which may be an inherent characteristic of this tissue when applied to the urethra.

Contraction is a possible concern in the clinical use of the tunica vaginalis as a dorsal graft in hypospadias surgery, especially when covering a long defect. This is a question that can only be answered with clinical experience, although the present experimental data show a good take and integration of the tunica vaginalis graft without contraction.

The behavior of the stratified squamous non-keratinized lining of the internal preputial island flap, which in our study showed a significant reduction in thickness, cannot be directly compared with any other publication because, to our knowledge, this is the first study to experimentally assess this surgical technique. However, in 1987, Talja et al. [11] suggested the opposite by demonstrating that the preputial graft did not undergo modifications and kept the same number of cell layers in a rabbit model. These authors did not specify whether the preputial graft was keratinized or not. Leger [18] performed endoscopic biopsies after a series of cutaneous urethroplasties and noted that the epidermis still showed a keratin Malpighian layer, often dense and with hyperacanthosis, hair and sebaceous glands. This study is not directly comparable to ours since the internal preputial lining was non-keratinized. Nevertheless, as previously described, in two animals of the 12-week group, the preputial island flap had a segment of keratinized epithelium and the slides showed similarities to what Leger noted. Thus, we can raise another question: are there any differences between keratinized and non-keratinized island flaps for urethroplasties?

The 25% complication rate (two fistulae and two diverticula) shows a similar pattern to the clinical series of 22 patients for the homologous procedure [3], which used buccal mucosa instead of tunica vaginalis as a new urethral plate; however, this experimental model has an important difference from real hypospadias surgery that can promote better healing for the neourethra. In severe hypospadias, there is often significant skin deficiency and the ventral surface of the penis has to be covered by skin flaps which do not have the same thickness and vascularity. In this model, the ventral aspect of the penis is backed up by the perineal peri-anal area when the skin web between these structures is reconstructed.

We believe that this experimental work will support the use of tunica vaginalis instead of buccal mucosa, in a strategy to create a new urethral plate to allow onlay repairs in one-stage surgical repair of complex hypospadias requiring urethral plate division. Using only tissue from the genital region, this approach theoretically reduces surgical morbidity.

Conclusions

The association of tunica vaginalis graft as a new urethral plate and an internal preputial island flap in an onlay fashion can be a successful urethral substitute in this animal model. Good tissue integration and an acceptable complication rate were demonstrated by histological evaluation. The mesothelial lining of the tunica vaginalis was replaced by a more stratified epithelium and the lining of the internal preputial island flap had its thickness reduced, to resemble the native urothelium.

References

applications and further experience with the onlay island flap urethroplasty. J Urol 1990;143:98–100 [discussion 100–1].


