Improved Long Ureteral Reconstruction with Ileum By Longitudinal Clipping and Mucosal Stripping: an Animal Study

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Purpose: To investigate the possibility of bridging long ureteral defects by longitudinal clipping and mucosal stripping of the pedicled segment of ileum (CMSPI).

Materials and Methods: Ten beagle dogs (five males and females aged 2-3 years) were used to model a defect of the entire ureter. An ileal segment was selected, and half of the intestinal segment was longitudinally resected, without mesenteric resection. The intestinal mucosa was removed. Then, the ileum was sutured to form a tube connecting the renal pelvis to the bladder. A 5F ureter stent was inserted into the ileum and removed 4 weeks after surgery. Intravenous urography (IVU) was used to observe the reconstructed ureters at 6 and 12 weeks after the operation. Blood samples were collected before surgery and during each radiological examination to assess electrolyte and renal function. Five dogs were randomly euthanized after each IVU. After macroscopic analysis, hematoxylin-eosin (H&E) staining was performed to observe the microscopic changes in the reconstructed ureter.

Results: All dogs were in good condition after surgery. Changes in blood electrolyte and renal function after surgery were not significant. IVU demonstrated no ureteral obstruction or extravasation of the contrast agent; however, mild hydronephrosis were observed in three dogs. Macroscopic analysis indicated that the reconstructed ureter was intact without strictures. H&E showed that no mucosal structure was present on the luminal surface.

Conclusion: CMSPI is feasible for bridging long ureteral defects and has shown good efficacy in this preliminary study.

Keywords: ureteral defect; ureteral reconstruction; ureteral replacement; ileal ureter; reconstructive urology

INTRODUCTION

In view of the development of endourologic procedures, laparoscopic surgery, and radiation therapy, the incidence of iatrogenic ureteral injury, including long ureteral defect, has increased significantly in the past decades[1]. The treatment of severe ureteral defects is a challenge for urologists. Therefore, suitable and efficient treatment methods are crucial. Ureteral replacement is common when the ureteral defect is longer than 5 cm, especially in cases of full-length ureter defects. The main corrective methods are ileal ureter replacement, autotransplantation, and Boari tubularized bladder flap[2]. Ileal ureter replacement is more common than the other two techniques and is often used as the final solution after the failure of other methods[3,4].
Ureteral replacement with ileum to correct full-length ureteral defects was first described by Schoemaker in 1906 and was later popularized by Goodwin et al.[5] in 1956. Since then, this technique has improved and has become a useful alternative for reconstructive urology[6]. However, the extended use of this technique is limited by surgical complications, including urinary fistula, infection, obstruction, urine reflux, chronic pyelonephritis, electrolyte imbalance, and gradual decline in renal function[7,8]. Postoperative complications in the ileal ureter are attributable to the large ileum diameter and the presence of the intestinal mucosa. In the past studies which had focused on improvement of ileum ureter and only some complications were addressed[9]. To overcome these complications, the traditional technique was modified by using longitudinal clipping and mucosal stripping of the pedicled segment of ileum. This study investigated the feasibility and the initial outcomes of CMSPI for reconstructing the full-length ureter in an animal ureteral defect model.

MATERIALS AND METHODS

Experimental animals

Five female and five male healthy adult beagle dogs (aged 2-3 years; weight 10–15 kg) were obtained from the Animal Experiment Center of the Third Military Medical University, China. The level of microbial

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Table 1. Electrolyte and renal function before and after ureteral reconstruction (mmol/L)

Index[a]	Baseline (n=10)[b]	6 weeks (n=10)[c]	12 weeks (n=5)	F	*P*
K+	4.32 ± 0.72	4.21 ± 0.46	4.39 ± 0.67	0.162	0.851
Na+	145.25 ± 2.02	143.19 ± 5.16	144.88 ± 2.35	0.85	0.441
Cl-	109.87 ± 2.27	110.97 ± 4.17	111.72 ± 3.94	0.531	0.595
Ca+	2.44 ± 0.20	2.35 ± 0.14	2.43 ± 0.11	0.739	0.489
Ur	4.30 ± 1.06	4.10 ± 0.85	4.01± 0.80	0.195	0.824
Cr	82.20 ± 11.65	85.3 ± 12.38	82.00 ± 9.77	0.222	0.802

[a]Data are presented as mean ± SD
[b]Blood samples were collected before surgery.
[c]Blood samples collected at 6 weeks after surgery and five dogs were euthanized after intravenous urography.

control was standard. Surgeries were performed in the Surgical Laboratory of Zunyi Medical University. All dogs fasted for 24 hours before undergoing surgery. Five dogs were examined at 6 weeks postoperatively and five dogs at 12 weeks postoperatively.

Materials and equipments

Surgical bed, shadowless lamp, intestinal resection, and anastomosis bag (Surgical Laboratory of Zunyi Medical University); 3/0 absorbable sutures (Huawei Brand HY3901 20PCS); 20 mL disposable syringe and 22G disposable indwelling needle (0.9 × 25 mm; Condele); 5F double-J ureteral stent tube (Huaye Medical Instruments); metronidazole and ampicillin; specimen fixation (10% formaldehyde); digital radiography machine (Shimadzu Sonialvision G4); and automatic biochemical analyzer (BACKMAN AU5821).

Experimental methods

Preoperative preparation

The dogs were anesthetized with 3% pentobarbital sodium (30 mg/kg) intraperitoneally. Hair was removed using a sodium sulfide solution. The dogs were fixed supine. The depth of anesthesia was monitored at a respiratory frequency of 16-20 times/min. Femoral venous blood (5 mL) was collected to perform electrolyte and renal function assay.

Establishing the full-length ureteral defect model

A midline abdominal incision was performed. The abdominal cavity was explored to ensure the absence of apparent abnormalities. The left lateral peritoneum was dissected, and the renal pedicle was separated and clamped. After that, the left kidney was removed. Then, the right lateral peritoneum was incised. The entire ureter was separated and resected, and the bladder stump was sutured.

Ileum preparation and mucosal stripping

Starting at 10 cm proximal to the ileocecal junction, an ileal segment of approximately 10-15 cm in length was sectioned. The stumps of the primary ileum were end-to-end anastomosed with 1-0 silk suture in front of the selected ileum to restore ileal continuity, and the mesenteric openning was closed. Residues in the selected ileum were washed off with physiological saline.

Half of the ileum on the contralateral side of the mesentery was clamped with leather forceps and excised, and the remaining half ileum was washed with saline. A 5 mL syringe needle was inserted into the space between the mucosa and the submucosa along the edge. Physiological saline was injected into this space to separate the mucosal layer from the submucosal layer, and the mucosal layer was removed. Electrocoagulation was used to interrupt bleeding.

Ureteral reconstruction

The pedicled half clipping ileum was sutured into a tubular tract with 3/0 absorbable sutures to serve as the reconstructed ureter, and then a 5F double-J ureteral stent tube was inserted in the tube. The proximal end of the reconstructed ureter was anastomosed to the renal pelvis, and the distal end was anastomosed to the bladder to maintain the direction of intestinal peristalsis. The ureteral stent was fixed to the renal pelvis using 3/0 absorbable sutures to prevent dislocation.

The abdominal cavity was checked to confirm the absence of abnormalities. Ampicillin 0.5 g and metronidazole 50 mL were injected into the abdominal cavity. The abdominal incision was sutured layer-by-layer. After recovery from anesthesia, the dogs were fasted for 24 hours, but were given water, and then fed normally after enteral feeding.

Postoperative measurements

The dogs' mental status, food intake, defecation, and emiction were assessed after surgery. A small abdominal incision of approximately 3.0 cm was made at 4 weeks after the operation. The distal end of the double-J stent was located in the bladder and the whole stent was pulled. The bladder wall and abdominal incision were sutured separately. Two weeks after removing the double-J stent, all dogs were anesthetized, and venous blood (5 mL) was collected again for analyzing postoperative electrolyte and renal function. Five dogs were randomly selected for digital radiography (DR) of the kidney, ureter, and bladder (KUB). Then intravenous urography (IVU) was performed after injecting iohexol (25 mL) into the lingual vein to perform intravenous urography (IVU). After that, all five dogs were euthanized to assess the morphology of the peritoneum, kidney, and the reconstructed ureter, and obtain tissue specimens. Venous blood (5 mL) was collected from the other dogs at12 weeks after surgery, and KUB+IVU was performed. After morphological analysis, kidney specimens and the reconstructed ureters were fixed in formalin.

This study was performed with the approval of the Institutional Animal Care and Use Committee of Zunyi Medical University. All experimental procedures were conducted according to local guidelines on the ethical use of animals and the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised in 1996). This study did not involve human participants.

Statistical Analysis

Quantitative data were expressed as means ± standard deviations. The repeated measure ANOVA was used for making comparisons between two groups. Data

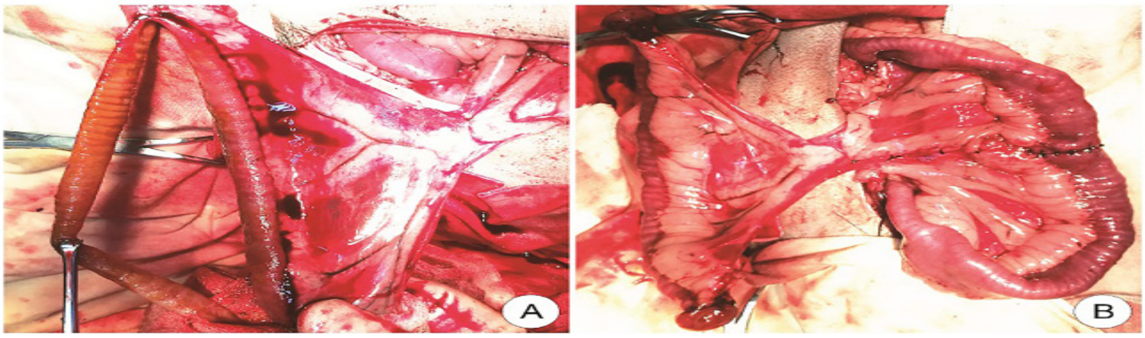

Figure 1. Ileum clipping and removal of the ileal mucosa. **(A)** Half of the ileum on the contralateral side of the mesentery was removed, and the other half of the ileum on the mesenteric side was preserved. **(B)** A tubular duct was formed after mucosal stripping, and the original ileal stumps were sutured to restore intestinal continuity.

were analyzed using SPSS software (SPSS, Inc., Chicago, Illinois) version 17.0. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Establishment of the model

The intraperitoneal injection of 3% pentobarbital sodium (30 mg/kg) achieved a satisfactory anesthetic effect. All surgical procedures were performed by the same urological surgeon using standard protocols (**Figure 1A**). After removing the mucosa from the ileum, the submucosa was dense and smooth and strongly attached to the muscle layer. No significant bleeding occurred. Physiological saline (20 mL) was injected into the reconstructed ureter to check for leakage. The diameter of the reconstructed ureter was approximately four times that of the original ureter. Intraoperative blood loss was 40-60 mL (average of 50 mL), and operative time was 100-160 min (average of 130 min).

Blood analysis

There were no significant differences in the electrolyte and renal functions of the ten dogs between before and after surgery, and no significant decrease in function between week 6 and week 12 after the operation (**Table 1**).

KUB and IVU

KUB and IVU were performed at 6 and 12 weeks after the surgery (**Figure 2**). On the IVU, the kidney was filled with the contrast agent in 5 minutes, the ureter and bladder were filled in 10 minutes. The ureters presented no obstruction or extravasation of the contrast agent. Hydronephrosis did not occur in the dogs euthanized at 6 weeks after surgery, However, mild hydronephrosis were observed in three dogs that survived at 12 weeks after surgery.

Postoperative macroscopic observation of the reconstructed ureters

The reconstructed ureters were resected and observed macroscopically (**Figure 3**). There was no significant change in renal volume. The shape of the reconstructed ureters was regular and cylindrical in all dogs. No fistula or scar nodule was observed in the ureters. There was no abnormal hyperplasia or mucous secretion. The ureteral diameter was approximately 10 mm. The shape of the bladder was normal. Mild stenosis at the renal pelvis anastomotic site with hydronephrosis was observed in one dog survived at 12 weeks after surgery.

Hematoxylin-eosin (H&E) staining and microscopic observation

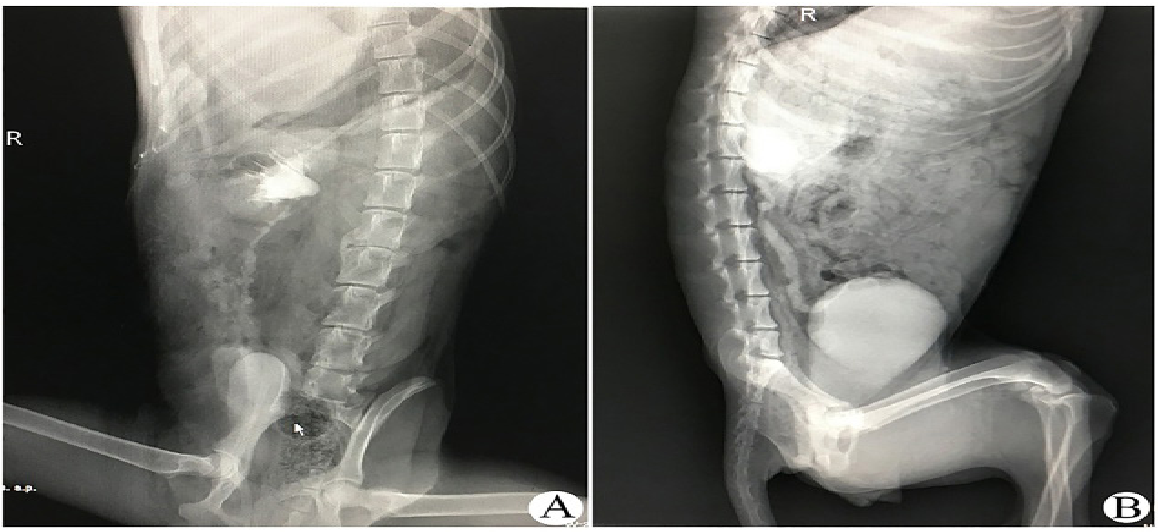

Figure 2. Representative images of intravenous urography (IVU) after surgery. (A) IVU image at 6 weeks postoperatively. (B) IVU image at 12 weeks postoperatively.

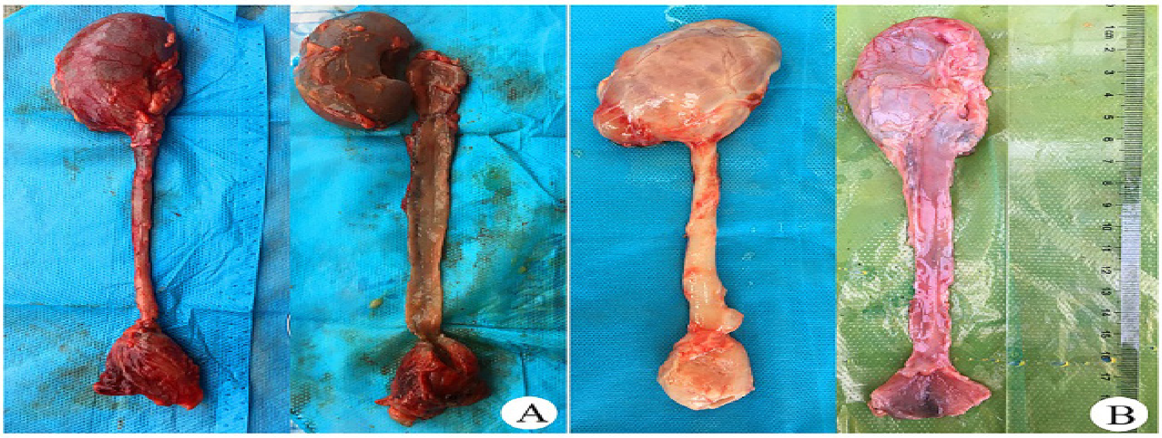

Figure 3. Morphological observation of ureter specimens. Morphology at 6 weeks **(A)** and 12 weeks **(B)** after surgery. The images showed that the reconstructed ureter presented a constant diameter without forming scar nodules and fistulas. The surface of the duct wall was smooth without stenosis. Mild hydronephrosis occurred in one specimen with a smooth inner surface of the reconstructed ureter.

The standard ileal segment and the reconstructed ureter specimens were collected and stained with H&E. The structure of the standard ileal wall was intact, and there were several mucous glands. The submucosal and mucosal layers were intact and clear (**Figure 4A**). In contrast, the reconstructed ureter had no mucosal layer, whereas the submucosal and muscle layers were intact. A small amount of inflammatory cell infiltration was observed (**Figure 4B**).

DISCUSSION

Long ureteral defect is a challenging condition for urologists when an end-to-end anastomosis is not feasible. Currently available methods including Boari flap, ileal ureter, and renal autotransplantation[2,10]. In clinical practice, ileal substitution is the most used procedure for ureteral defects; nevertheless, urologists have raised concerns about postoperative complications[7,8]. The aim of improving ileum ureter replacement is to transport urine from the kidneys to the bladder in the proper lumen with low pressure and decrease electrolyte reabsorption and mucus secretion[11]. These objectives can be achieved using several structures, including the appendix, greater omentum, and venous ureters; however, these applications are restricted because of the complexity of these procedures and related postoperative complications[12]. Based on the traditional method of ileal ureter we constructed CMSPI of beagle dogs to substitute the full-length ureter and assess the feasibility and efficacy of this technique. After ureteral reconstruction, all evaluated dogs survived and were in good condition. The filling time of the kidneys and ureter-bladder was adequate, without strictures or leakage of the contrast agent in the urinary tract. The electrolyte and renal function of the dogs after surgery was statistically similar to that at baseline, indicating a satisfactory outcome.

One of the strategies used to improve the traditional method of ileal ureter replacement is the Yang-Monti procedure, which reduces the pressure in the substituted ureter and the diameter of the ureteral lumen. Yang [13] described for the first time the transversal tubularization of the ileum for treating bladder carcinoma. Monti et al.[14] later described the same technique to form an efferent duct for urinary diversion in dogs, and Esmat et al.[15] applied the Yang-Monti principle to reconfigure ileal segments for ureter substitution. However, a retrospective study found that the major complication was infection caused by integrated intestinal mucosa[16]. Anatomical defects also need to be considered, and the

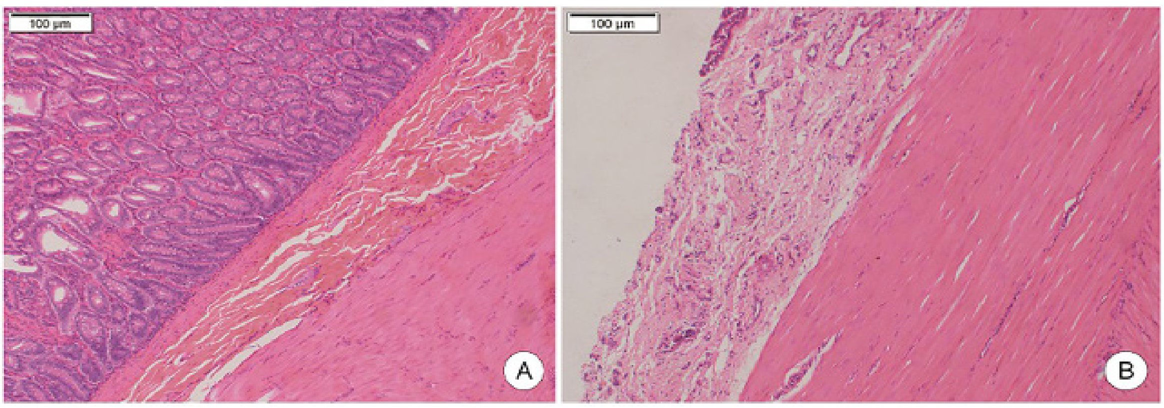

Figure 4. Morphological analysis of a normal ileum and the reconstructed ureter wall sections by hematoxylin-eosin staining (×100). **(A)** The structure of the ileal wall was intact, the mucosal and submucosal layers were clear, and there were several mucous glands in the mucosal layer. **(B)** The reconstructed ureter did not present a mucosa, but the submucosa and muscle layers were intact. A small amount of inflammatory cell infiltration was found in the interstitium.

retubularization process makes the muscularis move mode of the small intestine uniquely, the inconsistent and variable tension along the duct wall could create lax areas. Pouches and leakage could result directly from the formation of a false passage[17].

In the modified technique presented in this study, longitudinal clipping can maintain the movement of the muscularis propria and decrease the contractive force of the ileum, reducing the internal pressure of the renal pelvis and ureter. Moreover, the intestinal mucosa was completely removed to eliminate its absorptive and secretory functions. After surgery, all dogs survived for 6 weeks, whereas the last euthanized dogs survived for 12 weeks. IVU images confirmed the satisfactory patency of the reconstructed urinary tract. Macroscopic observation showed that the inner surface of the constructed ureter was intact and smooth, without strictures or leakage of urine throughout the urinary tract. Nevertheless, mild pyelectasis occurred in three of five dogs that survived for 12 weeks postoperatively and one of them had minor stenosis at the renal pelvis anastomotic site. Given that cystostomy and the anti-reflux technique were not performed in all the dogs, we believe that reflux hydronephrosis was possibly the primary cause of pyelectasis. The inhibition of intestinal secretion makes the use of the anti-reflux technique feasible in this procedure and ensures that the tube is not blocked by secretions. The effects of anti-reflux techniques on hydronephrosis will be investigated in a follow-up study.

Ureteral tissue engineering may serve as an alternative approach for decreasing absorption and secretion in the intestinal mucosa. Most studies investigating ureteral defects used extracellular matrix(ECM) and synthetic materials; however, the disadvantage of these materials is lack of flexibility and alloimmunization[18]. The use of the submucosa of the small intestine (SIS) without cellular pre-seeding to construct a tube could solve these problems and expedite the regeneration of the urothelium and smooth muscle[19]; however, this technique is limited to treating ureteral defects of less than 5 cm, and fibrosis occurs in some cases[20,21]. The complication rates of ureteral tissue engineering can reach 25%[22]. The main cause of fibrosis is the lack of effective blood supply. In the procedure described in this study, we removed the ileal mucosa to allow the submucosa to become the inner layer of the reconstructed ureter, and preserved the integrity of the muscularis propria layer and blood supply. During the 12-week study period, there was no sign of mucosal regeneration, and a satisfactory result was achieved without strictures or fibrosis of the submucosal and muscularis layers, which indicates the potential use of the ileal submucosa to replace the ureteral lining.

Our study focused on investigating the feasibility of longitudinal clipping and mucosa stripping of the pedicled segment of ileum to reconstruct the entire ureter in a beagle dog model. The results showed that CMSPI was a novel, feasible, and promising technique for bridging long ureteral defects. The study limitations were the absence of a control group, the small sample size, and the short-term follow-up. Moreover, further studies are necessary to assess the effect of anti-reflux techniques and whether the urothelium is regenerated in the inner layer of the reconstructed ureter after a longer period. However, this preliminary study is useful to urologists involved in urinary reconstruction.

CONCLUSIONS

CMSPI is feasible for replacing the entire ureter and showed good efficacy in this preliminary study. Furthermore, this procedure reduces the complications attributable to the large diameter of the ileal tract and mucosa. These initial findings may be helpful to urologists. The anti-reflux technique and whether the urothelium is regenerated will be addressed in the follow-up study.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Burks FN, Santucci RA. Management of iatrogenic ureteral injury. Ther Adv Urol. 2014; 6:115-124.
2. Li Y, Li C, Yang S, Song C, Liao W, Xiong Y. Reconstructing full-length ureteral defects using a spiral bladder muscle flap with vascular pedicles. Urology. 2014; 83:1199-1204.
3. Engel O, Rink M, Fisch M. Management of iatrogenic ureteral injury and techniques for ureteral reconstruction. Curr Opin Urol. 2015; 25:331-335.
4. Shekarriz B, Lu H, Duh Q, Freise CE, Stoller ML. Laparoscopic nephrectomy and autotransplantation for severe iatrogenic ureteral injuries. Urology. 2001; 58:540-543.
5. Goodwin WE, Winter CC, Turner RD. Replacement of the ureter by small intestine: clinical application and results of the ileal ureter. J Urol. 1959; 81:406-418.
6. Kim A, Nam W, Song SH, Jeong IG, Hong B. Use of the Ileum for Ureteral Stricture and Obstruction in Bilateral, Unilateral, and Single-kidney Cases. Urology. 2018; 111:203-207.
7. Kocot A, Kalogirou C, Vergho D, Riedmiller H. Long-term results of ileal ureteric replacement: a-25-year single-center experience. BJU Int. 2017; 120:273–279.
8. Zhong W, Hong P, Ding G, et al. Technical considerations and outcomes for ileal ureter replacement: a retrospective study in China. BMC Surgery. 2019; 19:9.
9. Ordorica R, Wiegand LR, Webster JC, Lockhart JL. Ureteral replacement and onlay repair with reconfigured intestinal segments. J Urol. 2014; 191:1301–1306.
10. Png JC, Chapple CR. Principles of ureteric reconstruction. Curr Opin Urol. 2000; 10:207-212.
11. Takeuchi M, Masumori N, Tsukamoto T. Ureteral reconstruction with bowel segments:

experience with eight patients in a single institute. Korean J Urol. 2014; 55:742-749.

12. Gild P, Kluth LA, Vetterlein MW, Engel O, Chun FKH, Fisch M. Adult iatrogenic ureteral injury and stricture–incidence and treatment strategies. Asian J Urol. 2018; 5: 101–106.
13. Yang WH. Yang needle tunneling technique in creating antireflux and continent mechanisms. J Urol. 1993; 150:830-834.
14. Monti PR1, Lara RC, Dutra MA. New techniques for construction of efferent conduits based on the Mitrofanoff principle. Urology. 1997; 49:112-115.
15. Esmat M, Abdelaal A, Mostafa D. Application of Yang-Monti principle in ileal ureter substitution: is it a beneficial modification? Int Braz J Urol. 2012; 38:779-785.
16. Bao JS, He Q, Li Y, Shi W, Wu G, Yue Z. Yang-Monti principle in bridging long ureteral defects: cases report and a systemic review. Urol J. 2017; 14:4055–4061.
17. Narayanaswamy B, Wilcox DT, Cuckow PM, et al. The Yang-Monti ileovesicostomy: a problematic channel?[J]. BJU Int. 2015; 87:861-865.
18. Zou Q, Fu Q. Tissue engineering for urinary tract reconstruction and repair: Progress and prospect in China. Asian J Urol. 2018; 5:57–68.
19. Smith TG 3rd, Gettman M, Lindberg G, et al. Ureteral replacement using porcine small intestine submucosa in a porcine model. Urology. 2002; 60:931-934.
20. El-Assmy A, Hafez AT, El-Sherbiny MT, et al. Use of single layer small intestinal submucosa for long segment ureteral replacement: a pilot study. J Urol. 2004; 171:1939–1942.
21. Paul K. J. D. de Jonge, Vasileios Simaioforidis, Paul J. Geutjes, et al. Recent advances in ureteral tissue engineering. Curr Urol Rep. 2015; 16:465.
22. Elliott SP, McAninch JW. Ureteral injuries: external and iatrogenic. Urol Clin North Am. 2006; 33:55-66.