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*Original Article*

# Applying modified small bladder patch-to-bladder double-layer sutures to establish a rat renal transplantation model

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**Abstract:** Rationale: A modified, rat small bladder patch-to-bladder double-layer suture for urinary tract reconstruction was used to establish a rat kidney transplantation model, and the model was compared with the donor bladder patch-recipient bladder anastomosis method to determine its effect on the improved urinary tract reconstruction rat renal transplantation method. Methods: Rats were randomly divided into two groups. Group A: The donor, small bladder patch-recipient bladder mucosa seromuscular layer single-layer suture group (n=10). Group B: The donor bladder patch-recipient bladder double-layer suture group (n=11). The operation times and the urinary tract reconstruction complications were compared. Results: The urinary tract reconstruction times were 14.12±1.73 min in Group B and 10.16±1.19 min in Group A. The difference between the two groups was statistically significant ($P$<0.05). The incidence of urinary tract complications in Group A was 25%, and the incidence of urinary tract complications in Group B was 9.09%, and the difference between the two groups was statistically significant ($P$<0.05). Conclusion: The modified urinary tract reconstruction with small bladder-receptor bladder double-layer suture technique significantly reduces urinary tract complications compared with the traditional single-layer suture technique, but the urinary tract reconstruction time is longer than the traditional single-layer suture time. It can significantly reduce the possibility of urinary leakage and is a highly reliable reconstruction procedure.

**Keywords:** Rats, kidney transplantation, urinary tract reconstruction, double-layer sutures, bladder patches

## Introduction

The rat kidney transplantation model plays an important role in the study of transplantation immunity. It allows researchers to manipulate rejection, has the potential to study tolerance induction, and contributes to understanding its pathogenesis. At present, rat kidney transplantation models have been widely used to study immune tolerance, ischemia-reperfusion injuries, the development of new immunosuppressive agents, and the pathogenesis of acute and chronic rejection.

Functional renal transplantation requires the establishment of blood flow between the donor blood vessels and the recipient's systemic circulation by anastomosing the arterial and venous vessels and by draining the urinary tract to the recipient urinary tract using anastomotic techniques.

Fisher first described the renal transplantation model in rats in 1961 and published his results in 1965 [1]. In the past 50 years, with the progress in microsurgery, the anastomosis of small blood vessels is more than 95% and is able to penetrate the blood flow, which makes survival after kidney transplantation in rats routine.

The inner diameter of the ureter in rats is only 0.2-0.3 mm, and urinary tract reconstruction is one of the key techniques in the rat kidney transplantation model. Ureteral complications such as ureteral leakage, ureteral stricture, and ureteral necrosis may lead to hydronephrosis or the deterioration of renal function. The technique is similar to that used clinically, includ-

ing anastomosing the donor bladder patch containing the ureter to the recipient bladder, ureterovesical anastomosis, and end-to-end ureteral anastomosis.

Different urinary tract reconstruction methods can also significantly affect the short-term and long-term function of the transplanted kidney [2].

We used a small bladder patch-to-bladder double-layer suture technique for the urinary tract reconstruction to establish a rat renal transplantation model, and at the same time, we compared it with the traditional urinary tract reconstruction with the bladder patch anastomosis to the recipient bladder single-layer suture methods to clarify the effect of a modified urinary tract reconstruction method in rat kidney transplantation.

## Materials and methods

All protocols were in line with the *Guidelines for Animal Care and Use in Laboratories* revised by the National Institutes of Health in 1985.

### *Experimental animals*

The kidney transplantation was performed in Sprague Dawley rats at 8-10 weeks, 200-250 g body weight, purchased from Beijing Vital River Laboratory Animal Technology Company (Beijing, China). The rats were fed a standard diet and water ad libitum. The rats were kept in cages with 4 animals in each cage, and the pads were changed regularly. The feeding environment was set as a 12 h:12 h day and night cycle, with the indoor temperature set at (21±2)°C, and the indoor humidity set at (55±2)%.

### *Experimental grouping*

The rats were randomly divided into two groups: Group A (Single-layer suture group): The donor bladder patch anastomosis to the recipient bladder group (n=14). Group B (Double-layer suture group): The donor small bladder patch-recipient bladder mucosa seromuscular layer double-layer suture group (n=14).

### *Establishment of the rat kidney transplantation model*

*Anesthesia:* All rats were fasted for 12 hours without water. Then the rats were placed in a plexiglass anesthesia induction box, and the anesthesia induction box was connected to the anesthesia machine through a threaded tube circuit. The oxygen and isoflurane volatilization tank was opened, and oxygen (2 L/min) and isoflurane were input into the anesthesia induction box, and the concentration of isoflurane was set to 3% for the induction of anesthesia [3]. We monitored the respiratory frequency of the rats to prevent accidental death from the excessive use of isoflurane.

When the righting reflex of each rat disappeared and no longer responded to the pain stimulation, the rat was removed, the nose of the rat was put into the mask, and the concentration of isoflurane was adjusted to 2% to maintain anesthesia. The shaving device performs epilation, and the epilation range was from the lower edge of the xiphoid to the upper edge of the pubic symphysis, including the upper third of the lower limbs. We exposed the entire abdominal skin as much as possible. The rats were moved to a 30 cm × 30 cm rat test bench, covered by a sterilized surgical mat towel, and then we placed each rat in a supine position.

### *The establishment of the animal models*

*Donor nephrectomy:* After the anesthesia was achieved, iodophor was used to disinfect and towels were spread around. A large abdominal "+" incision was used. We removed the surgical instruments from the aseptic bag, cut the skin along the middle of the abdomen, cut the muscle layer along the white line of the abdomen, wrapped the intestines with wet gauze, and pushed the intestines to the right as much as possible. Next we adjusted the operating microscope to 16 times, aimed it at the operation area, carefully separated the abdominal aorta and inferior vena cava, and exposed the left kidney and left ureter. The adrenal veins and spermatic vessels were cut off using electrocoagulation, and then we went along the renal vessels and dissociated it with the initial segment in a positive direction. We dissociated the whole length of the left ureter to the bladder and cut the bladder patch with a diameter of about 3 mm at the place where the left ureter was injected into the bladder. We blocked the abdominal aorta and inferior vena cava, cut a small opening in the inferior vena cava to serve as the outflow tract of the renal lavage fluid, placed a needle in the blocked abdominal

aorta, and lavaged the left kidney with a heparin solution (125 U/Ml) at 4°C until the kidney became yellowish and the effluent was clear. We then cut the root of the left renal artery and vein, removed the donor kidney, soaked it in heparin Ringer's solution at 4°C and stored it in a refrigerator at 4°C.

*Recipient surgery*

*Vascular anastomosis:* After anesthesia was achieved, iodine was used to disinfect, and a towel was laid down. We used the rectus abdominis longitudinal incision method to cut the skin from the lower edge of the xiphoid process to the upper edge of the pubic symphysis along the middle of the abdomen, and then we cut the muscle layer along the abdominal white line, opened the skin and muscle layers, wrapped the intestinal tube with wet gauze, and pushed the intestinal tube to the right as far as possible so that the abdominal cavity could be completely exposed. We adjusted the operating microscope to 10 times, aimed it at the operation area, carefully separated the abdominal aorta and inferior vena cava, opened the posterior peritoneum to expose the left kidney and ureter. The adrenal veins and spermatic vessels were cut off using electrocoagulation, and then we carefully dissociated the initial segment in a positive direction, and the renal arteries and veins were separated with microscopic forceps. The upper segment of the ureter was separated along the psoas major muscle, then the ureters were double ligated using a 0# silk suture and then they were severed in the middle of the ligation line.

A non-invasive vascular clamp was used to clamp the renal artery and renal vein near the beginning of the renal vessel, and the left kidney was resected completely with microscissors. The recipient vascular lumen was flushed with a 4°C heparin solution (125 U/mL). Next we removed the donor kidney from the 4°C refrigerator and wrapped the kidney with a wet cotton pad with some ice shavings on the surface. We put the kidney into the left renal fossa and added ice shavings regularly during the operation. We adjusted the operating microscope to 16 times, aimed it at the operation area, and then renal vein reconstruction was used to carry out the end-to-end continuous anastomosis. We fixed a needle at each end of the donor renal vein and the recipient renal vein, clamped the fixation line to maintain a certain tension of the anastomosis, and sutured the vein continuously with a 10-0 vascular suture. The renal artery was anastomosed using end-to-end intermittent anastomosis and a 10-0 vascular suture was used to suture the positive and negative sides of the anastomosis, and then sardine forceps were used to lift it gently on both sides to maintain a certain tension in the middle suture. The reverse anastomosis was stitched intermittently, and then the front anastomosis was stitched intermittently. After the anastomosis, we took out the 37°C normal saline from the constant temperature bath box and rewarmed the rat quickly. We also opened the venous clamp and observed whether there was blood leakage in the vascular anastomosis. After ensuring that there was no blood leakage in the anastomosis, we opened the blood flow.

*Ureteral anastomosis:* Group A (Single-layer suture group, n=14): donor bladder patch-recipient bladder single-layer anastomosis was performed.

We carefully dissociated the recipient bladder, selected the low blood supply area of the left wall of the bladder as the anastomotic area, and opened the whole layer of the bladder wall. The recipient bladder was opened, and the donor bladder patch was intermittently sutured with a 7-0 vascular suture. A total of 8 to 10 stitches were sutured. After the suture was finished, normal saline was injected into the bladder with a syringe to check for exudation. If there was a clear exudation point, we stitched it again.

Group B (Double-layer suture group, n=14): Double-layer anastomosis of the donor bladder patch and the recipient bladder was performed.

We trimmed the donor bladder patch to about 3 mm in diameter (see **Figure 1A**). We carefully dissociated the recipient bladder, selected the low blood supply area on the left side of the bladder as the anastomosis area, opened the seromuscular layer of the recipient bladder, cut a small opening about 3 mm long, exposed the pale mucous layer (see **Figure 1B**), put a little pressure on the bladder with a cotton swab, separated the mucous layer from the seromuscular layer so that part of the mucous

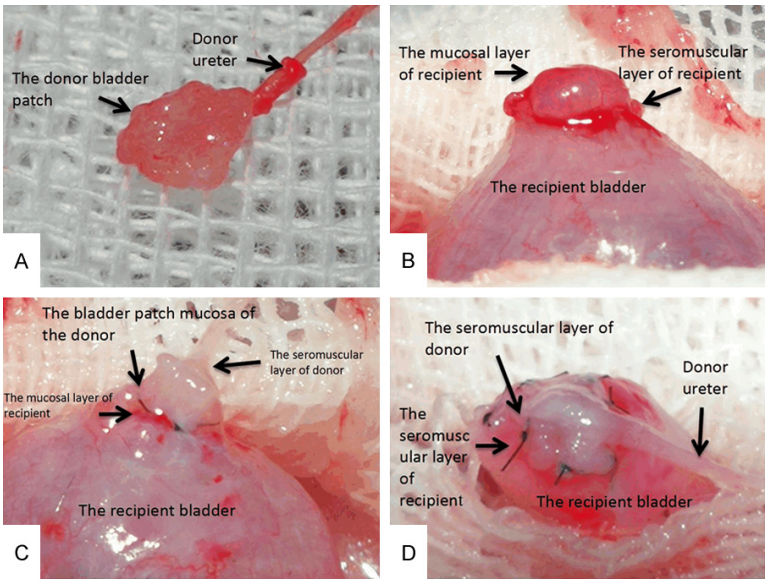


**Figure 1.** Double-layer anastomosis of donor bladder patch and recipient bladder. A. Trim the donor bladder patch to about 3mm in diameter; B. Open the sarcomuscular layer of the recipient bladderr to expose the mucosal layer of recipient (black arrow); C. The donor ureter and recipient bladder mucosa were sutured continuously, and the black continuous suture line below the sarcomuscular layer was seen (black arrow). D. Interstitial Intermittent suture of the seromuscular layer of the donor ureter and the recipient bladder can be seen on the surface of the seromuscular layer (black arrow).

layer bulged from the incision of the seromuscular layer, and cut an incision of about 3 mm in diameter on the protruding mucosa. The bladder patch mucosa of the donor and the bladder mucosa of the recipient were sutured about 6-8 stitches continuously with a 10-0 vascular suture (see **Figure 1C**). Then the seromuscular layers of the donor and the recipient were anastomosed along about 8-10 stitches intermittently with a 7-0 vascular suture (see **Figure 1D**). After the suture was finished, we used a syringe to inject normal saline into the bladder to check whether there was any exudation or not. If there was a clear exudation point, we stitched it again.

*Closing the incision*

The capsule fat of the transplanted kidney was fixed on the posterior peritoneum using 5-0 absorbable suture, and the abdominal cavity was examined for any obvious blood osmosis. The abdominal cavity was washed with warm normal saline three times. Aseptic gauze absorbed the water, and the intestinal tube was sequentially returned to the abdominal cavity (to ensure that the lumen was free from distortion and injury).

*Signs of successful renal transplantation*

After the end of vascular suture and the restoration of the blood flow, the renal artery filled rapidly and pulsed forcefully. After observing it for 3-5 minutes, we saw no obvious blood leakage, distortion, or stenosis at the suture site. The color of the transplanted kidney was bright red, the blood supply of the ureter was abundant, the spontaneous peristalsis of the ureter was good, a clear urine outflow could be seen, the color of the bladder patch was normal, and there was no obvious urine leakage in the bladder anastomosis. After they woke up, the rats were able to walk freely, drink water on their own, and have a good state of mind. On the second day after the transplantation, they were able to take the initiative to eat and drink, their eyes were wide open, their spirits were better, and they could move about freely. Survival for more than 7 days after operation indicated that the model was successful.

*Comparison of the operation times between two groups of rats*

The duration of the urinary tract reconstruction in each rat was recorded from the time when the recipient bladder was fully dissociated to the time when the bladder was completely sutured. The warm ischemia time and the total operation time of each rat were recorded in detail.

*Comparison of postoperative urinary tract complications between two groups of rats*

The gingiva, fur, and activity of the rats were examined every day after each operation, and the daily food intake and water consumption were recorded. When a recipient died, an autopsy was performed immediately to determine the cause. Two weeks after the transplantation, the rats were sacrificed humanely, and they all were dissected to observe the occur-

**Table 1.** Comparison of operation time between Single-layer and Double-layer suture Groups

| Group | n | Total operation time (min) | Hot ischemia time (min) | Urinary tract reconstruction time (min) |
|---|---|---|---|---|
| Single-layer suture Group | 10 | 70.34±2.77 | 23.27±2.15 | 10.16±1.19 |
| Double-layer suture Group | 11 | 76.81±3.22* | 23.88±2.23 | 14.12±1.73* |

*$P$<0.05 Compared with the Single-layer suture Group.

**Table 2.** Occurrence of postoperative urinary tract complications in two Groups of rats

| Group | n | Urinary leakage | ureteral obstruction | Urinary tract complications |
|---|---|---|---|---|
| Single-layer suture Group | 10 | 16.67% | 8.33% | 25.00% |
| Double-layer suture Group | 11 | 0* | 9.09% | 9.09%* |

*$P$<0.05 Compared with the single-layer suture Group .

rence of any urine leakage and any ureteral obstructions and infections.

*Statistical analysis*

The results were assessed using t-tests of two independent samples to compare the mean difference between the two groups, with $P$<0.05 considered indicative of significant differences. The data are expressed as the mean ± standard error of the mean (SEM).

## Results

*The situation after the rat kidney transplantations*

28 renal transplants were performed in the rats under the same experimental conditions. Sprague Dawley (SD) rats were used as donors and recipients. 2 rats in the single-layer suture group bled to due to hemorrhagic shock and died after the fluid replacement. 2 recipients in the double-suture group died of intra-abdominal bleeding on days 2 and 3 after their operations. Two rats in the single-layer suture group died of abdominal infections caused by urinary leakage and died on days 4 and 5 respectively, and one rat in the double-layer suture group died of venous thrombosis on day 2 after its operation. The incisions of the other rats recovered well, there was no blood oozing or any infections, and they all survived until the end of the experiment. Finally, the 10 rats in the single-layer suture group were followed up to the end of the experiment, and the 11 rats in the double-layer suture group were followed up to the end of the experiment.

*Comparison of urinary tract reconstruction times between the two groups of rats*

The total operation times of the single-layer suture group (Group A) was 70.34±2.77 minutes, and in the double-layer suture group (Group B) the operation times were 76.81±3.22 minutes, which was significantly longer than of the times in Group A ($P$<0.05), and the warm ischemia times of the two groups were 23.27±2.15 min and 23.88±2.23 min, respectively, and there was no significant difference between the two groups. The time of urinary tract reconstruction in Group A was 10.16±1.19 minutes, and it was 14.12±1.73 minutes in Group B, which was significantly longer than it was in Group A ($P$<0.05), as shown in **Table 1**.

*Comparison of postoperative urinary tract complications between the two groups of rats*

The incidence of postoperative urinary leakage in the single-layer suture group (Group A) was 16.67%, and that in the double-suture group (Group B) it was 0, which is significantly lower than it was in Group A ($P$<0.05). The incidences of ureteral obstruction in the two groups were 8.33% and 9.09% respectively, and there was no significant difference. The total incidence of urinary complications in Group A was 25.00%. The total incidence of urinary complications in Group B was 9.09%, which was significantly lower than it was in Group A ($P$<0.05), as shown in **Table 2**.

## Discussion

Although the degree of transplant rejection is different between humans and animals, animal models are of great value for the study of transplant rejection. The remarkable advantage of the animal model is that it can produce damage equivalent to that of human allograft rejections on a shorter time scale. Although the

rat kidney transplantation model is technically challenging, the pathogenesis of chronic rejection after rat kidney transplantation is similar to that after human kidney transplantation, and the pathological characteristics of chronic rejection are similar to those that occur several years after a human kidney transplant. The experimental model of renal transplantation in rats plays an important role in understanding transplantation immunology, ischemia-reperfusion injuries, acute and chronic rejection, and the effects of immunosuppressants on the short-term and long-term outcomes after renal transplantation.

Reconstruction of the urinary tract is one of the key technologies for rat kidney transplantation models. Because the ureter has a very narrow caliber, it can easily cause complications such as urine leakage, ureteral necrosis, and ureteral stenosis, which may further lead to hydronephrosis or renal function deterioration. The technique used is similar to that used clinically, including anastomosis of the patch of the donor bladder containing the ureter to the recipient bladder, and the ureter is sutured directly to the bladder and end-to-end suture of the ureter. Different surgical methods of urinary tract reconstruction may also lead to different kidney transplantation outcomes [2]. The inner diameter of the rat ureter is only 0.2 mm~0.3 mm, and Wagner listed the rat kidney transplantation model as a second grade difficult operation (the difficulty of the operation was divided according to the third grade) [4].

With the development of microsurgical technology, the patency rate of small vascular anastomosis has reached 95%, and the rats' survival rate after renal transplantations has been greatly improved. The initial reconstruction of the urinary tract in the rat model of renal transplantation was made using ureterostomy, but the inner diameter of the ureter was small, so it was extremely prone to stricture and infections at the ureteral skin stoma, which was soon eliminated [5]. Since then, the ureteral catheterization method was adopted: intermittently suture both ends of the ureter and place a silicone stent in it [6]. Because the diameter of the stent required is so small, it is extremely difficult to obtain, and it is easy to have internal stent displacement, blood clot obstruction, ureteral wall perforation, leading to ureteral obstruction and hydronephrosis, and the suture is prone to ischemia and then it can lead to necrosis and urine leakage, which cannot be used on a large scale [7].

The end-to-end anastomoses of the donor and recipient ureters with 5/0 gut lines and a direct anastomosis between the donor ureter and the recipient ureter with the assistance of temporary internal stents were successful [8]. However, the formation and infection of urinary calculi run the inherent risk of having non-absorbable stents [9].

French [10] and White [11] performed direct end-to-end anastomoses of donor and recipient ureters. This technique is based on the preliminary experience of renal transplantation in 34 rats, of which 32 were successful. Most of the donor ureters were located at the lower pole of the flat left kidney. The external diameter of the ureter in rats is only 0.3 mm~0.5 mm, so the end-to-end technique needs to be carried out more carefully, for the inherent risk of ureteral necrosis can lead to urinary extravasation, anastomotic stricture, and thrombus obstruction leading to hydronephrosis and graft loss.

In addition, some scholars use an oblique incision of the donor ureteropelvic junction to anastomose with the recipient bladder, instead of cutting the ureter in the middle between the kidney and the bladder, obliquely cutting the ureter close to the donor ureteropelvic junction. This technique has tripled the diameter of ureteral anastomosis. Compared with the traditional end-to-end ureterostomy, the oblique incision of the donor ureteropelvic junction greatly reduces the incidence of anastomotic stricture. Using a traditional end-to-end suture, the incidence of ureteral stricture was 12.5% (3/24), while the use of a donor ureteropelvic junction with a recipient bladder completely avoided this complication (0/45). In addition, the risk of ureteral injury is reduced when there is no need to dissociate the distal ureter [12].

In the uretero-bladder anastomosis, the tip of the artery bending forceps was inserted into the bladder or the 21# needle punctured the recipient bladder for direct implantation of the donor ureter into the recipient bladder. The donor ureter was dragged into the recipient

bladder, and the outer wall and tissue of the donor ureter were sutured to the outer wall of the recipient bladder. The donor ureter was then retracted into the bladder and the puncture hole of the donor bladder was sutured. Postoperative hematuria comes from the blood vessels adjacent to the ureter, and the blood vessels at the distal end of the ureter should be ligated. In another technique, the end of the donor ureter penetrates into the bladder and is fixed to the recipient bladder wall with a 6-stroke absorbable suture [1]. Alternatively, the anastomotic ureter and bladder mucosa were anastomosed with 3-4 stitches with a 10-0 nylon suture, and the bladder wall around the anastomosis was sutured with a purse suture to bury the anastomosis [13]. Some domestic scholars have adopted the urinary tract reconstruction by donor ureter-recipient bladder seromuscular tunnel method, and the incidence of urinary tract complications is about 12%.

The anastomosis of the ureter with bladder patch to the bladder of transplanted kidney was first described by Silber [14]. This operation is also widely used in the renal transplantation model. However, this method also has the following shortcomings: (1) the free donor rat ureteral bladder is time-consuming and laborious, and it is easy to damage the ureteral blood supply in the process of dissociation. (2) ureteral distortion and obstruction can easily occur after the operation. (3) it is possible to suture the opening of the ureter by mistake in the process of suturing it.

The anastomosis between the donor bladder patch attached to the ureter and the recipient bladder is simple and easy, so there is no need for ureteral stents [15]. Full-layer end-to-end anastomosis was performed with a 6-0 suture. An excessively large donor bladder patch can easily cause ischemic necrosis, so the removal of a donor bladder patch should be as small as possible. Donor bladder bleeding during the nephrectomy confirms the complete blood supply of the ureter. Although this technique can lead to ischemic necrosis of the donor bladder patch, urine leakage, and the risk of death, this technique is easier to implement [16].

In order to reduce the difficulty and complications of renal transplantation in rats, many scholars have optimized and improved urinary tract reconstruction. In this study, the modified donor small bladder patch was used for anastomosis, and the mucosal layer and seromuscular layer were sutured continuously, and a stable effect was obtained. There were no local infections of the bladder anastomosis, no urine leakage, and no empyema after the renal transplantation in 11 rats.

The advantages of the modified double-layer suture of the small bladder patch-bladder for urinary tract reconstruction are as follows: (1) The continuous suture of the mucosal layer and the seromuscular layer can effectively reduce the occurrence of urinary leakage and reduce the incidence of systemic infections such as local infections and renal empyema. (2) Vesicoureteral reflux does not easily occur. (3) The recipient bladder mucosa and the seromuscular layer are separated during the operation, which makes the bladder mucosa protrude from the bladder surface, and the anatomy is clear, so it is beneficial to the operation. (4) There are few urinary tract complications after the operation, which reduces the decline in renal allograft function caused by the urinary tract complications, and this is conducive to the long-term survival of the rats after their operations, and it has obvious advantages in the study of animal survival for a long time. Although there are higher requirements for microsurgical techniques and microscopic equipment, and the operation time is longer than that of traditional single-layer suture, urinary tract reconstruction with modified double-layer sutures of small bladder patch-bladder can effectively reduce the incidence of complications. It can still be used as a model with strong repeatability.

*Summary*

In this study, we successfully established a modified rat renal transplantation model with a double-layer suture of the donor small bladder patch-recipient bladder mucous layers and seromuscular layers.

The operating time is longer than the traditional single-layer suture operating time.

It can effectively reduce urinary tract complications.

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## Disclosure of conflict of interest

None.

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## References

[1] Fisher B and Sun L. Microvascular surgical techniques in research: special reference to renal transplantation in the rat. Surgery 1965; 58: 904-914.

[2] Kouwenhoven EA, Bruin RW, Heemann UW, Marquet RL and Ijzermans JN. Ureteroneocystostomy contributes to late functional and morphological changes in rat kidney transplants. J Urol 2001; 165: 1700-1704.

[3] Criado AB and Gómez e Segura IA. Reduction of isoflurane MAC by fentanyl or remifentanil in rats. Vet Anaesth Analg 2003; 30: 250-256.

[4] Wagner E. The rat as experimental model for organ transplantation: technique of rat kidney transplantation. Contrib Nephrol 1980; 19: 167-175.

[5] Nathan P, Gonzalez EE, Fowler R Jr, Pescovitz H and Miller BF. A method for transplantation of the rabbit kidney. Proc Soc Exp Biol Med 1961; 107: 51-55.

[6] Pietsch AP, Nett PC, Klar E, Sollinger HW and Hullett DA. A new modified technique of ureteroureterostomy in rat kidney transplantation. Transplant Proc 2005; 37: 189-191.

[7] Daniller A, Buchholz R and Chase RA. Renal transplantation in rats with the use of microsurgical techniques: a new method. Surgery 1968; 63: 956-961.

[8] Carmignani G, Farina FP, De Stefani S and Maffezzini M. A new technique for end-to-end ureterostomy in the rat, using an indwelling reabsorbable stent. Microsurgery 1983; 4: 229-232.

[9] Soma T, Lerut E, Billiau A, Waer M, Goebels J, Koshiba T, Uemoto S and Pirenne J. An easy and reproducible model of kidney transplantation in rats. Transplant Proc 2009; 41: 3422-3424.

[10] French ME and Batchelor JR. Immunological enhancement of rat kidney grafts. Lancet 1969; 2: 1103-1106.

[11] White E, Hildemann WH and Mullen Y. Chronic kidney allograft reactions in rats. Transplantation 1969; 8: 602-617.

[12] Gu YL, Dahmen U, Dirsch O and Broelsch CE. Improved renal transplantation in the rat with a nonsplinted ureteroureterostomy. Microsurgery 2002; 22: 204-210.

[13] Karatzas T, Santiago S, Xanthos T, de Faria W, Gandia C and Kostakis A. An easy and safe model of kidney transplantation in rats. Microsurgery 2007; 27: 668-672.

[14] Silber SJ and Crudop J. Kidney transplantation in inbred rats. Am J Surg 1973; 125: 551-553.

[15] Tinbergen WJ. The effects of some immunosuppressive agents on kidney graft survival in rats. Transplantation 1968; 6: 203-207.

[16] Han WR, Murray-Segal LJ and Mottram PL. Modified technique for kidney transplantation in mice. Microsurgery 1999; 19: 272-274.