Vein "Beltization" Technique Facilitates Venous Anastomoses for Laparoscopic Orthotopic Kidney Transplantation in a Pig Model

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ABSTRACT

Objective. This study was performed to investigate the safety and efficiency of the renal vein "beltization" technique for laparoscopic orthotopic kidney transplantation (LOKT) in a pig model.

Material and Methods. Kidney autotransplants were conducted, in which the renal vein beltization technique was employed, in 12 domestic pigs. In the operation, the right kidney was autotransplanted to the left kidney vessel trunks. Data of the operative time, venous anastomotic time, vein stenosis, blood loss, kidney graft function, and survival for the pigs were recorded. One week later, the transplanted autograft was removed for observation from the surviving animals while under anesthesia. A silicon tube cage was used to induce hypothermia during laparoscopic operation.

Results. All 12 LOKTs were finished. The mean warm ischemia time was 5.22 ± 1.33 minutes, and the mean cold ischemia time was 263.44 ± 50.82 minutes. The mean transplant operative time was 305.67 ± 30.88 minutes. The venous and arterial anastomotic times were 68.42 ± 8.51 minutes and 40.92 ± 9.83 minutes, respectively. After revascularization, an immediate and viable blood supply was seen in 7 grafts based on the appearance of a bright red color. Four pigs urinated immediately after the operation, and 3 produced urine on the next day; 4 pigs received life-supporting renal autografts for 7 days. Autopsy results showed 3 artery stenoses and 3 vein stenosis. Thrombi were found in all of these strictured vessels. The median survival time of the pigs was 5.55 days.

Conclusions. The renal vein beltization technique may facilitate laparoscopic pig venous anastomotic procedures and ensures the quality of reconstruction. It is helpful for venous anastomoses in LOKT.

LAPAROSCOPIC kidney transplantation (LKT) is being performed more frequently in the current era of minimally invasive surgery. Meraney et al was the first to conduct entirely laparoscopic renal autotransplantation [1], and they achieved this in 6 female pigs. The first clinical case of LKT was reported by Rosales et al [2], and this was followed by 4 procedures performed by Modi et al [3]. LKT has now become a well-accepted modality of treatment for end-stage renal disease patients by Modi et al at their treatment center [4,5]. Laparoscopic orthotopic kidney transplantation (LOKT) has also been attempted in a pig model [6–8]. These experimental and clinical studies show promising results of LKT.

However, LKT is challenging to transplant surgeons, and LOKT may be even more challenging to them. A large number of animal experiments should be conducted for

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learning and training courses. Pigs have many anatomical characteristics in common with humans, and they constitute one of the best large animal models for kidney transplant practice and research [9,10]. However, pigs have very thin renal arteries and weak and fragile renal veins. Therefore, during LKT in pigs, vascular anastomoses (especially venous anastomoses) are very technically demanding. Most techniques, devices, and instruments used for arterial and venous anastomoses in open surgery, such as the 4-quadrant technique, ring pin stapler, end-to-end sleeve-locked anastomosis technique, vascular closure stapler metallic clips, and vascular coupling system [11–15] are unsuitable for or cannot be used in laparoscopic surgery.

A new and simple technique for venous anastomoses in laparoscopic organ transplantation was developed and tested by our team. We herein describe the renal vein "beltization" technique, and we report the results of a study in which the technique was employed for LOKT in a pig model.

MATERIAL AND METHODS

Animal Preparation

The animal experiments in this study were approved by the animal ethics committee of Beijing Chaoyang Hospital. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The pigs (female, weight range, 35–45 kg) were transported to the animal experimental laboratory and acclimated for 2 days prior to the experiment. A standard diet was maintained. Fasting and water deprivation were enforced in all pigs 12 hours before the operation.

Study Design

LOKT, in which the renal vein beltization technique was employed, was performed in 12 domestic pigs. During LOKT, laparoscopic nephrectomy was first performed in the right kidney. Following left nephrectomy being performed, the right kidney with the renal vein belted was autotransplanted to the left kidney vessel trunks. A silicon tube cage (Patented in RP China, Patent No.: zl2014 2 0820782.9) was used to induce hypothermia during LOKT, as we previously reported [7]. Data of the operative time, venous anastomotic time, vein stenosis, blood loss, kidney graft function, and survival for the pigs were recorded. One week later, the transplanted autograft was removed from the surviving animals while under anesthesia for observation.

Renal Vein Beltization Technique

The renal vein in pigs is a very soft, weak, and fragile tissue. The end of the renal vein is often not in an open state after it is removed from the animal's body. The anterior and posterior walls can be difficult to distinguish during laparoscopic surgery. In the present study, we used one 5-0 or 6-0 Prolene (Ethicon, Somerville, NJ, United States) thread to suture the renal vein wall circumferentially at 2–3 mm away from the margin with a 2-mm interval between each stitch. After the last suture, both ends of the proline thread were gently pulled, and a knot was made against the expanded vessel forceps. This permitted the circumferential circle to widen to its maximal size while preventing a purse-string effect at the edge of the renal vein (Figs 1, 2). If the renal vein was very thin, spatulation of the end of the renal vein was performed before beltization. If the renal vein was very short, it was extended 2–3 cm by angioplasty with an alcohol-processed pig corpse vessel. In these cases, beltization was performed in the alcohol-processed vessel.

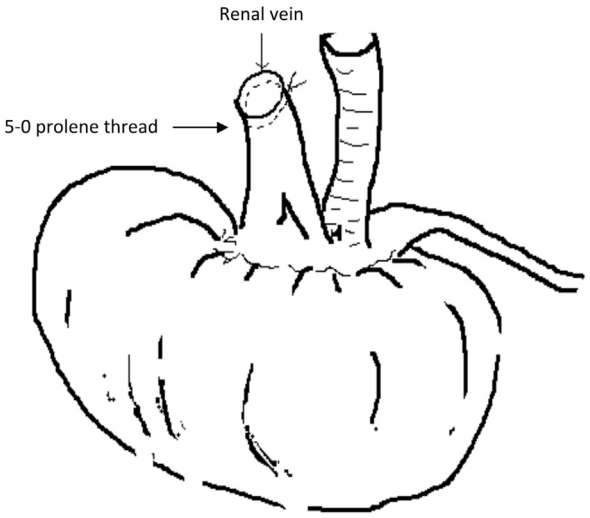

Fig 1. Illustration of the beltization technique: a 5-0 Prolene thread was used to suture the vein wall circumferentially along its margin with a 2-mm interval between each stitch.

Surgical Technique

Under general anesthesia, the pig was first positioned in a left lateral decubitus position for performing right nephrectomy by open surgery. A midline incision (7–8 cm) was made. The ureter was clipped distally and divided. The renal artery and vein were isolated and divided near the aorta and vena cava. Then the right kidney was manually removed. The kidney was perfused with perfusion solution at 0–4°C and at a pressure of approximately 80 cm H_2O.

The pig was repositioned in a right lateral decubitus posture. Then, the left native kidney was removed through the same wound. This nephrectomy was carefully performed with precise dissection of the renal artery and vein inside the renal hilum to preserve the greatest renal vessel length possible. The retroperitoneal space was enlarged by blunt dissection with the surgeon's hand. The graft with the cooling device was brought into the operative field through the incision and properly positioned for anastomosis. The vessels anastomotic technique used in the present study was the same as that described in our previous studies [6,7]. The renal vein and artery were anastomosed to the native renal vein and artery, respectively, with 5-0 Prolene sutures. Spatulation of the ends of the native renal vein and artery was performed as necessary to ensure that the size of the opening matched the diameter of the donor kidney's vein and artery. Venous reconstruction was performed first. For the venous anastomoses, the stitch interval was maintained at no more than 2 mm. Finally, the anastomosis was finished at the same point at that it was begun (Fig 3). The first and last stitches were ligated together using the same tail of the suture.

We also utilized the "quiet needle holder" technique during vessel anastomosis. This technique meant that we held the needle holder as one would hold forceps and did not button up any tine clips on the needle holder during the vessel anastomosis. 2.8-mm laparoscopic instruments were used for the vessel anastomoses.

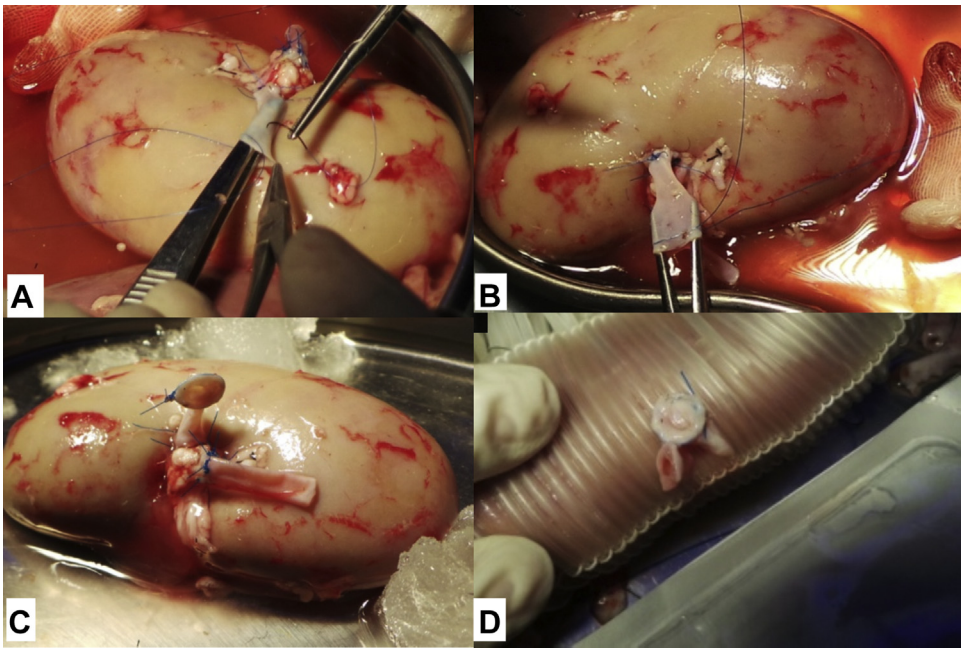

Fig 2. (**A**–**C**) Beltization of the renal vein, expanding the vessel wall and making the opening of the vein open, clear and endurable for anastomosis. (**D**) The kidney with beltization of the renal vein was placed into the cooling device with the blood vessels on the outside of the device for transplantation.

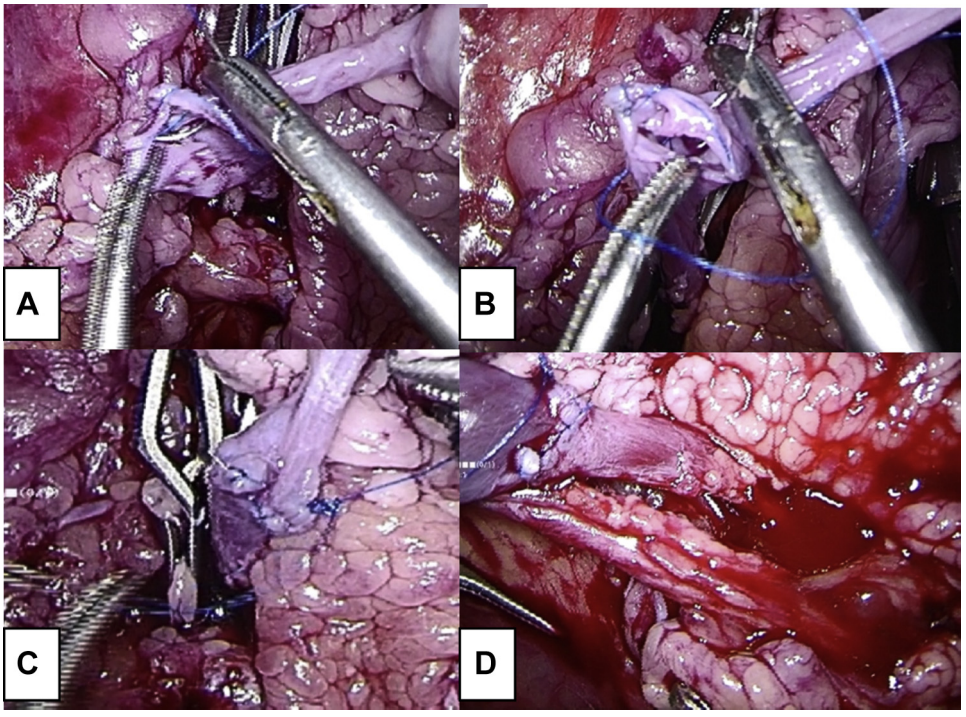

Fig 3. (**A**–**D**) During the operation, both the posterior and anterior walls of the belted renal vein were clearly seen. The renal wall margin was strengthened by beltization with Prolene sutures, providing the tissue a higher tolerance to endure the anastomotic manipulation. The beltization stitch interval acted as a parameter for the anastomotic stitch interval and yielded better anastomotic quality.

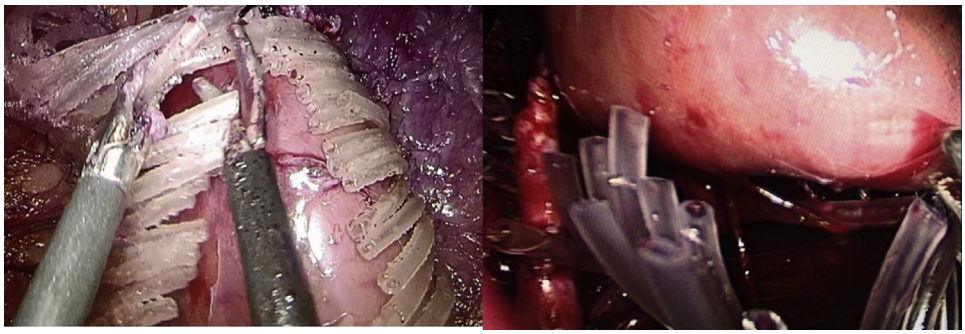

Fig 4. The appearance of the renal autograft after reperfusion.

After reperfusion, the cooling device was cut and removed, and the wound was finally closed. Endostenting of the end of the ureter-ocutaneostomy was performed to ensure complete diversion of urine.

RESULTS

All 12 LOKT experimental operations were finished. The mean warm ischemia time was 5.22 ± 1.33 minutes, and the mean cold ischemia time was 263.44 ± 50.82 minutes. The mean transplant operative time was 305.67 ± 30.88 minutes. The venous and arterial anastomotic times were 68.42 ± 8.51 minutes and 40.92 ± 9.83 minutes, respectively. After revascularization, an immediate and viable blood supply was seen in 7 grafts based on the appearance of a bright red color (Fig 4). Four pigs immediately urinated after the operation, and 3 produced urine the next day; however, 2 of these 3 stopped urinating later, one at 2 days later, and one at 4 days later. Four pigs received life-supporting renal autografts for 7 days. Eight pigs with anuria or oliguria showed signs of impending death on postoperative days 1, 1, 2, 2, 3, 5, 6, and 6 and underwent exploration under general anesthesia before euthanasia. Of them, one pig died of autopsy-proven colic and intestinal necrosis on postoperative day 6, and one pig suffered wound infection. Animals that survived were euthanized after exploratory surgery on day 7 after transplantation. Autopsy results showed 3 artery stenoses and 3 vein stenoses. Thrombi were found in all of these strictured vessels. Histopathologic examination of the autografts demonstrated 6 pigs with acute tubular necrosis. The median survival time for the pigs was 5.55 days. These data and results of the operation are detailed in Table 1.

DISCUSSION

There is a current trend toward the use of laparoscopy for kidney transplantation because of its advantages of reduced postoperative pain, lower analgesic requirement, better cosmesis, and lower complication rates. However, its technical complexity and high mortality rates have hindered its clinical use and even its experimental practice. This procedure is in its infancy, and further animal experiments are required before it can be widely used clinically. Vascular anastomosis is a central technique for solid organ transplantation and even more for LKT in the pig. A few vascular anastomosis techniques that were developed for open surgery, such as 2-point anastomosis [16] and the one suture-one knot technique [17] are now used for LKT. However, venous walls in the pig are thin, elastic, fragile, and easily ruptured or lacerated by suture needles, and the venous opening is difficult to visualize during anastomotic suturing. We designed the herein described beltization technique to overcome these difficulties. Our study shows that this technique is safe and can reduce the anastomotic and ischemic times of grafting while providing good patency. The following advantages of the technique should be noted: (1) because the "belted" vessel margin is open, this technique avoids the possibility of the posterior and anterior wall being caught up with each other; (2) the vulnerable vessel wall margin is strengthened by the Prolene suture, and beltization provides the tissue a higher tolerance to endure anastomotic manipulation, decreasing the rate of vessel wall laceration; and (3) excess elasticity of the vessel wall can be decreased at its margin, and the beltization stitch interval can act as a parameter for the anastomotic stitch interval providing a uniform and firm stitch (Fig 3). In addition, the beltization technique is simple, fast, and easy to learn and use. It could be reasonably used for end-to-side anastomoses as well. It could also be used in dogs and other animals and for both laparoscopic- and robot-assisted LKT for training practice. It could be even used in open transplantations. This technique is not only for kidney transplants but also for pancreas transplants. In our initial animal experimental study on pancreatic transplantation, the right kidney of the recipient was first removed. Then, the pancreatic vein, approached with beltization technique, was anastomosed end-to-end to the native renal vein, and all 3 pancreatic grafts had autopsy-proven reliable vein anastomoses [18].

Our study utilized an animal survival model to observe the immediate function of the autograft and autopsy or exploration to inspect vascular anastomosis patency. The limitations of this study are that: the number of animals used in the experiment was small, the observation period was only 1 week, there are no vessel growth and microscopic studies on vascular anastomosis with adequately long observation periods, and its applicability is limited to only animal experiments.

Table 1. The Observation Data of Laparoscopic Orthotopic Kidney Transplantation for the Graft and Recipient Pigs

	Angioplasty		Ischemia Time (min)		Anastomotic Time (min)					Anastomoses			
Case No.	Artery	Vein	Cold	Warm	KV	KA	Operation Time (min)	Blood Loss (mL)	Blood Supply	KV	KA	Serum Creatinine	Survival Time at 7 Days (μmol/L) (Median, Days)
1			240	4	75	40	335	100	Purple	S			1
2	Extended with pig corpse artery		215	3.5	65	50	305	80	Pink				3
3			245	7	54	35	320	50	Pale		S		1
4		Extended with pig corpse vein	220	8	85	45	275	30	Pale	S			2
5			235	5	65	35	320	50	Pink			486	7
6			196	6	70	55	235	80	Pale		S		2
7	Extended with pig corpse artery		210	5	64	30	280	50	Pink				6
8			300	4	75	50	335	30	Pink			325	7
9		Extended with pig corpse vein	285	6	75	38	330	100	Purple	S	S		6
10			350	5	66	55	285	60	Pink			550	7
11		Extended with pig corpse vein	280	5.5	70	28	318	50	Pink				5
12			295	4	57	30	330	40	Pink			221	7
Mean ± SD			263.44 ± 50.82	5.22 ± 1.33	68.42 ± 8.51	40.92 ± 9.83	305.67 ± 30.88	60.00 ± 24.49				395.50 ± 149	5.55

Abbreviations: KA, kidney artery; KV, kidney vein; S, stricture.

CONCLUSION

In the mini invasive surgery era, LKT is increasingly preferable. Therefore, there is great need for experimental LKT in large animals by transplant surgeons. Basic medical research in organ transplantation needs a high-quality animal model. Our study indicated the beltization technique facilitates laparoscopic pig venous anastomotic procedures and ensures the quality of reconstruction. The use of this new technique to quickly perform procedures such as anastomoses during LOKT may reduce the cold ischemia time and yield better postoperative results. The beltization technique for venous anastomoses in transplant operations with a pig model should be recommended.

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