Renal Transplantation in a Unique Porcine Model: Step-by-Step

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ABSTRACT

Background: Kidney transplantation is the most common solid organ transplantation. The aim of our research is to describe technical and surgical aspects of a porcine model of heterotopic kidney allotransplantation, using up-to-date techniques, utilized nowadays during human kidney transplantation.

Methods: We performed a total of 20 heterotopic kidney transplantations on a porcine animal model. The manipulation time, cold ischemia time and surgery time were measured and analyzed over time.

Results: Over the course of the experiment manipulation time decreased by 32% from 28min to 19min, cold ischemia time decreased by 33% from 46min to 31min, and surgery time decreased by 28% from 180min to 130min. All animals had successful reperfusion of the grafts. We witnessed urination of all grafts before performing the ureteral anastomosis. Three grafts had an early graft failure due to an early arterial thrombosis; in the first two cases, the grafts had their artery anastomosed onto a thin external iliac artery, the third thrombosis occurred on a graft with complex arterial anatomy with implanted pole artery.

Conclusion: Our experimental model demonstrated that a pig laboratory model is a useful and valuable tool for surgical training. It can help to shorten the operation times and lower the complication rates. This specific model can also be extended to serve not only as a simple training tool for surgical techniques. Considering pig to human similarities in physiology, biochemistry, and immunology, it can also be used as a short- or long-term model in kidney transplantation.

KEYWORDS: Renal transplantation; Animal model; Porcine; Experimental study

INTRODUCTION

Kidney transplantation is a treatment of choice for patients with end-stage renal disease. When compared to a hemodialysis patient, kidney transplantation gives patients significantly improved quality of life and longer life expectancy [1, 2].

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Currently, kidney transplantation is the most common solid organ transplantation. We are witnessing an increase in the total number of transplant-centers performing renal transplantation. Furthermore, the total number of renal transplants increases each year [3]. Consequently, this widens the pool of surgeons performing the procedure.

Since the first kidney transplantation in 1954 by Bent *et al.* the surgical procedure underwent an evolution [4]. Although the details of the procedure itself vary between transplant centers, the basic surgical technique is now well established.

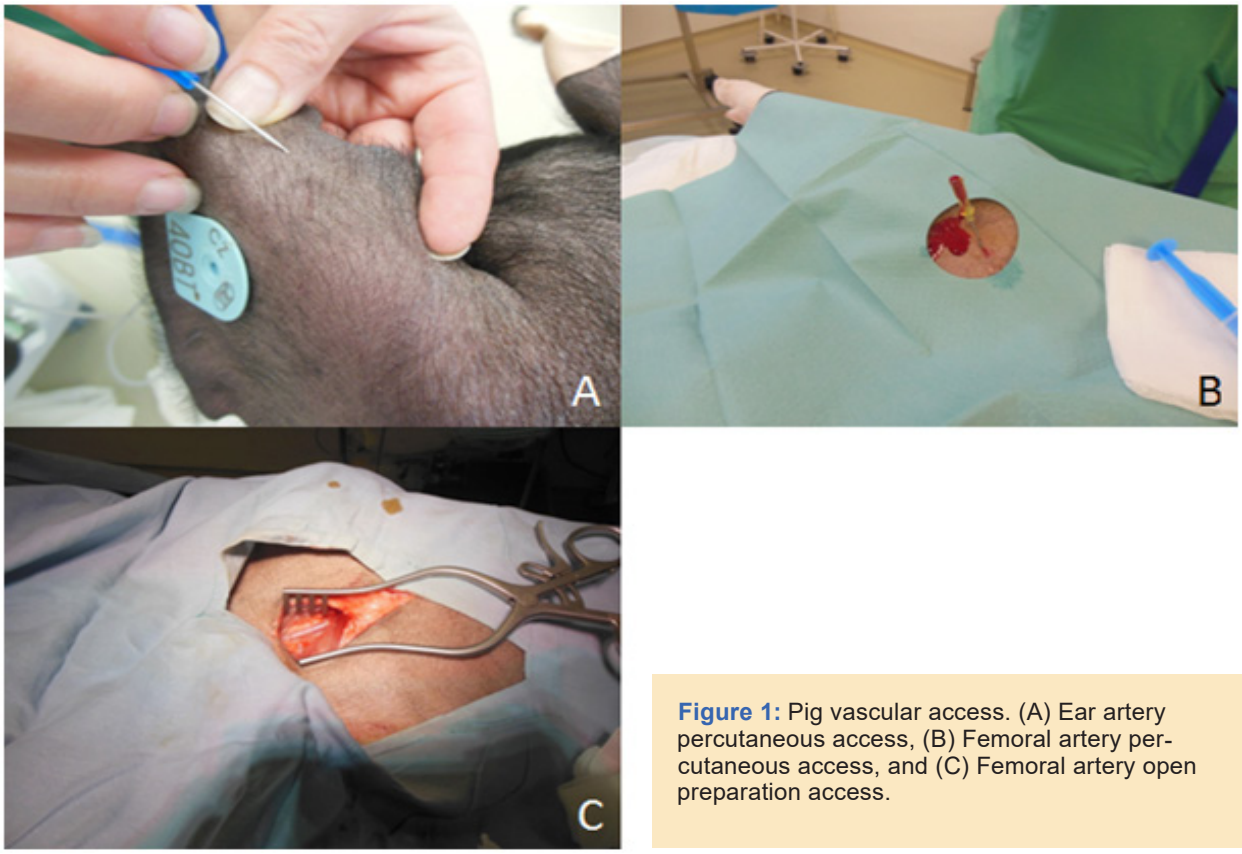

Figure 1: Pig vascular access. (A) Ear artery percutaneous access, (B) Femoral artery percutaneous access, and (C) Femoral artery open preparation access.

It is essential to shorten the learning curve and to prepare young surgeons sufficiently. One of the possible tools is an animal training model [5-7].

In our experimental work, we propose a laboratory model of a porcine kidney autotransplantation procedure. This model can be effectively used as an instruction tool for young transplant surgeons. The model can also be used to mimic conditions similar to human transplantation. Unlike other experimental animal models, we are introducing a few novel approaches to imitate the conditions of the current human kidney transplantation technique.

MATERIALS AND METHODS

Animal Model

A porcine model was chosen for our study. Porcine model is well established for kidney transplantation [5, 8-13]. The distribution of internal organs is comparable to human anatomy, but there are a few distinctions we need to be aware of [13].

Unlike other laboratory animals', a porcine kidney has similar anatomy as a human kidney. Its parenchyma structure is multilobular, and it has a similar urinary and vascular system [14, 15]. The main difference can be found in the amount of perirenal fat, but that may be beneficial for safe and swift nephrectomy. Iliac artery anatomy is different. Unlike humans, the porcine external iliac arteries are usually separately branched from the distal abdominal aorta, having no common iliac artery. The external iliac artery is slimmer in pigs than in humans, because pigs are four-footed [5].

Though technically located retroperitoneally, the porcine urinary bladder expands widely into the abdominal cavity making the retroperitoneal approach limited. When considering a suitable location for ureterovesical anastomosis, it is ineffective to be approached retroperitoneally. The bladder wall is muscular and similar in width to a human [16].

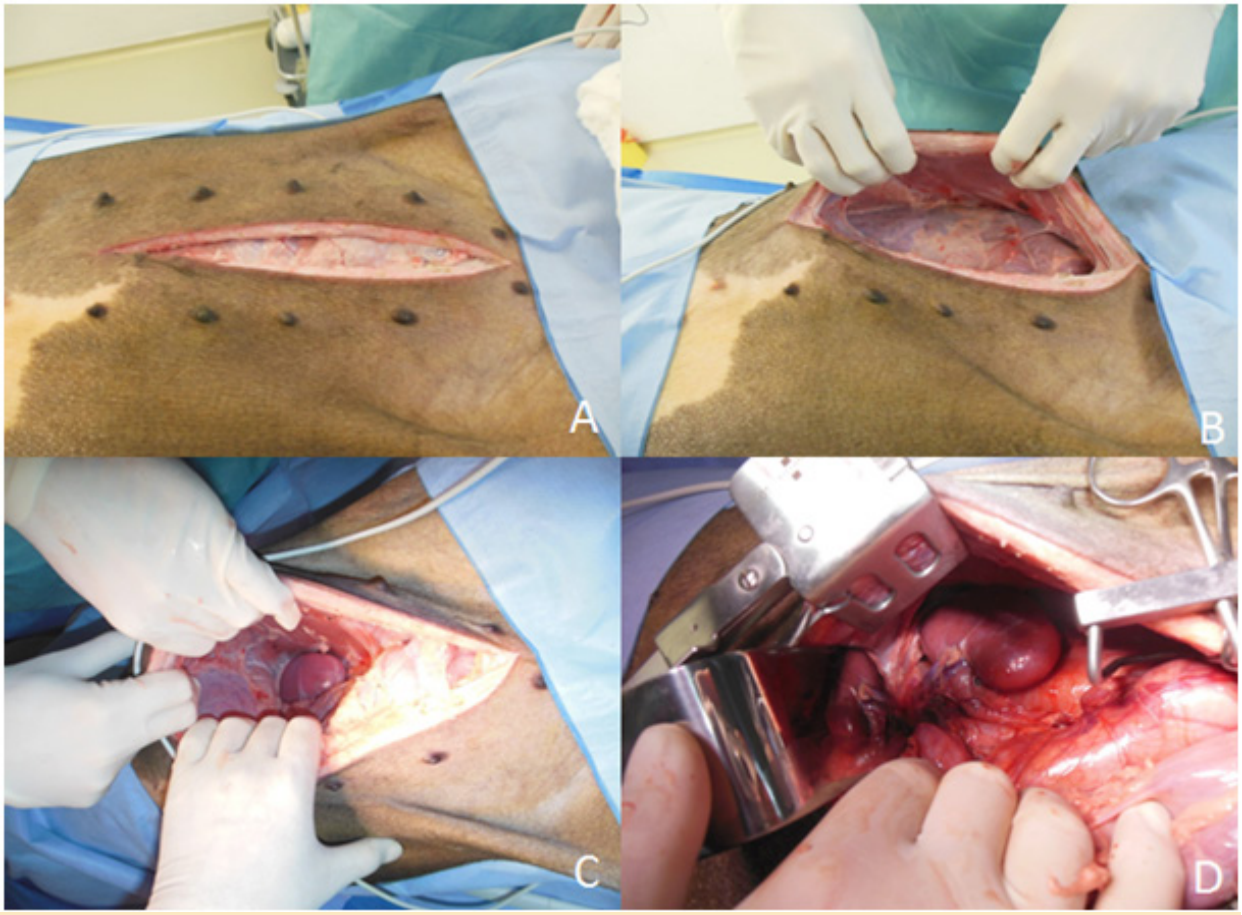

Figure 2: Extraperitoneal surgical access site. (A) Midline laparotomy, (B) Extraperitoneal preparation, (C) Extraperitoneal approach to retroperitoneum, and (D) Kidney preparation using extraperitoneal approach.

We chose animals weighing approximately 40 kg as the kidney is comparable in size with human kidney.

A single surgeon performed all the autotransplantations. We performed a total of 20 heterotopic renal transplantations on laboratory pigs in two years. The animals were monitored for 48 hours after the surgery. During the monitoring, the animals were without anesthesia and without any physical restrictions. After the 48 hours, the animals were put under anesthesia and the transplanted organ was evaluated. The animals were terminated in full anesthesia.

Anaesthesia Protocol

a) Sedation: Intramuscular injection of Ketamine+Azaperone+Atropine.

b) Venous access [17]: Marginal ear vein – medial or lateral auricular vein.

c) Anaesthesia: Intravenous bolus infusion of Propofol+Fentanyl. It is sustained by continuous inhalation of Isoflurane + continuous intravenous infusion of Fentanyl.

d) Relaxation: Intravenous bolus of Pipecuronium Bromide.

e) Antibiotics: Amoxycilline (875mg/125mg) with an enzyme inhibitor.

f) Arterial access [17]:

- Ear arteries – Medial auricular arterial branch or Intermediate auricular arterial branch through percutaneous access by Seldinger technique (Fig 1A)

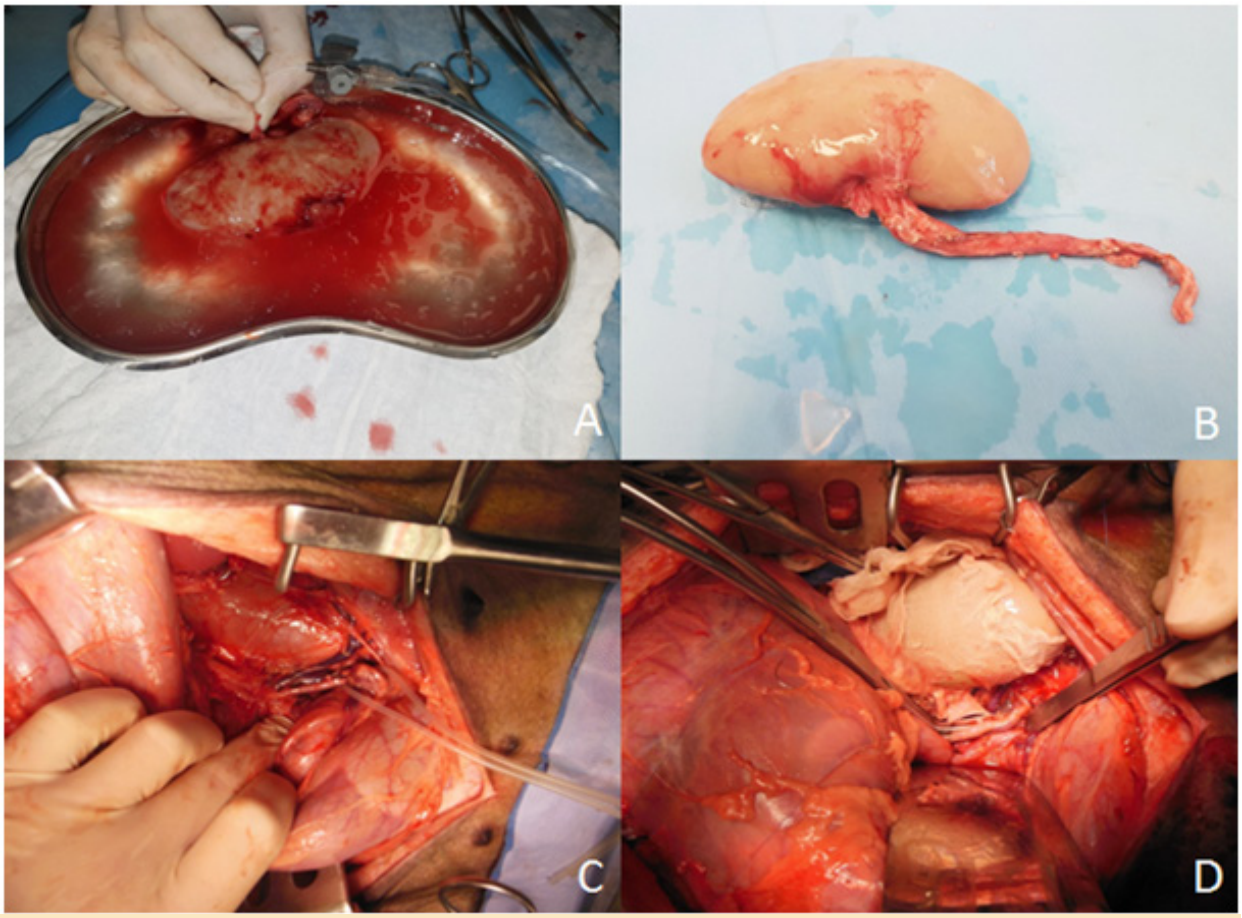

Figure 3: Renal graft perfusion and heterotopic position. (A) Conservation solution perfusion of the kidney graft, (B) Kidney graft after conservation solution perfusion, (C) Common iliac vessel preparation, snd (D) Kidney graft in situ prepared for vessel anastomosis.

- Femoral artery – percutaneous access by Seldinger technique (Fig 1B)
- Femoral artery – open preparation and approach (Fig 1C)

g) Monitoring: Continuous arterial pressure, ECG, Saturation (tongue, lip)

h) Termination: bolus Thiopental (1-2g) + KCl 7,45% solution (40-50ml) i.v.

Surgical Protocol

Approach and Nephrectomy

Lower midline laparotomy (15-25cm) is performed spreading from umbilicus to the suprapubic abdominal area (Fig 2A). The fascia is dissected, approaching the preperitoneal fat, located just over the peritoneum. The peritoneum itself is left intact. The dissection continues extraperitoneally to the left side of the animal (Fig 2B). The retroperitoneal fat is displaced laterally, leading straight to the left kidney in the cranial part of the cavity (Fig 2C). Caudally, the ureter is lifted with the peritoneum, approaching the aorta and left common iliac vessels. The kidney is released from the adhesions approaching the hilum (Fig 2D). The pelvic-ureteral transition is separated caudally. The ureter is separated from the peritoneum. It is cut at the level of aorto-iliac branching; the distal part ligated; the proximal part is left open. The renal artery is identified. The left renal vein and left renal artery are separated and prepared for clamping at the level of the aorta.

The heparin (150 units/kg) is administered. The 4°C saline solution and Custodiol solution (CUSTODIOL® CE N - Dr Franz Köhler Chemie GMBH) infusion are prepared. The

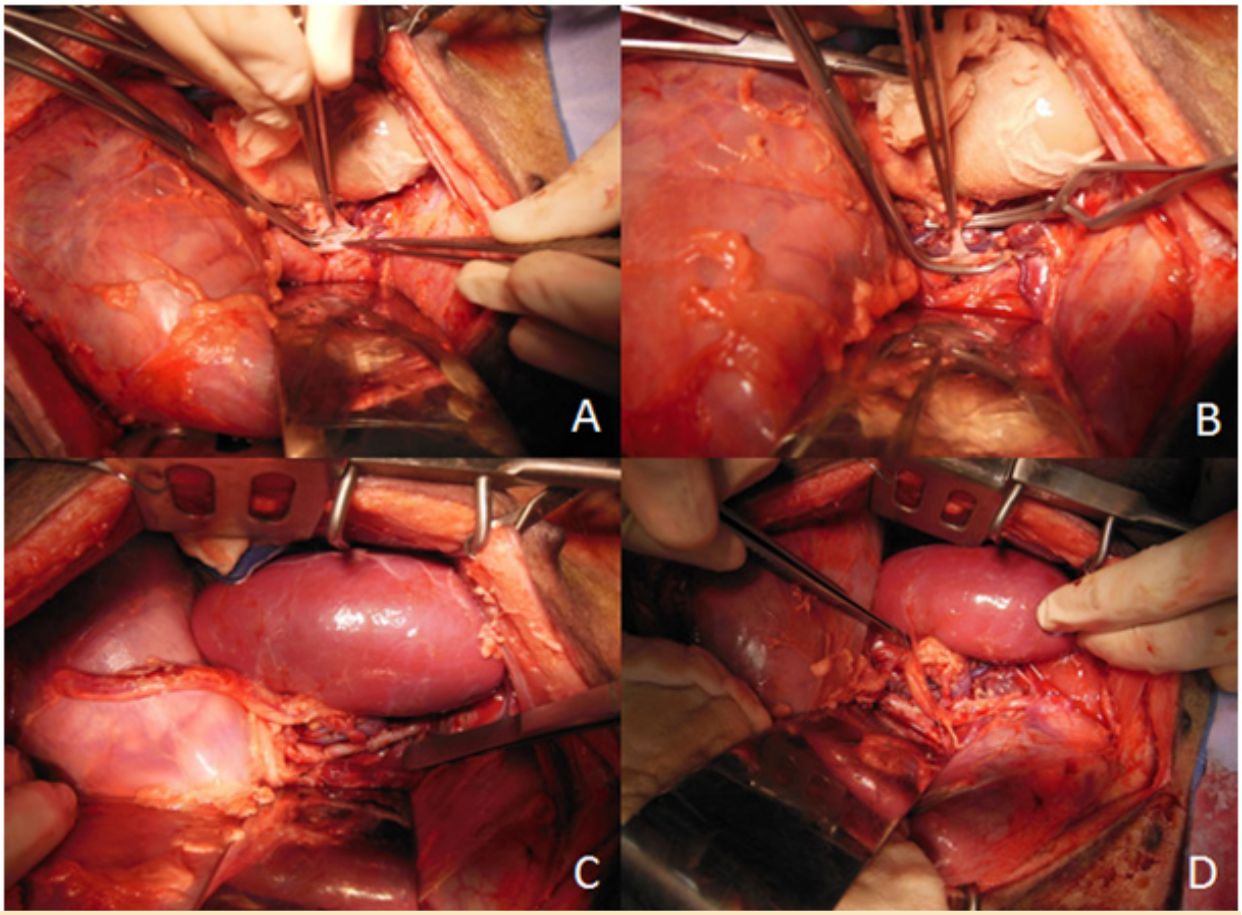

Figure 4: Renal graft vascular anastomosis and reperfusion. (A) EtS graft renal vein - common iliac vein anastomosis, (B) EtS graft renal artery - common iliac artery anastomosis, (C) Graft reperfusion, and (D) Kidney graft position before wound closure.

renal artery is clamped as close to the aorta as possible. This should be marked as a beginning of warm ischemia time. The renal vein is clamped at the same level as the artery. The vessels are cut, and the kidney is removed from the animal.

Kidney Perfusion and BackTable

The kidney is placed in the saline solution. The artery is immediately cannulated, and the perfusion of the organ is performed (Fig 3A) using 250-500ml Custodiol solution. The perfusion is ended when the fluid flowing out of the vein is clear, and the parenchyma color turns grey. The start of the perfusion should be marked as the beginning of "cold ischemia" time. The artery is checked for damage, preventing the intimal dissection. The vein is shortened to match the length of the artery (Fig 3B).

Kidney Autotransplantation

The left external iliac artery is dissected from the external iliac vein. The vessels are dissected from surrounding tissue, mainly lymphatic vessels and lymph nodes. The blood vessels are dissected for at least 5cm in order to make them a suitable site for anastomosis (Fig 3C). The vein is clamped using vascular clamp and venotomy is performed. The width of the venotomy should fit the diameter of the renal vein. The venotomy is rinsed with a heparin solution. The kidney is removed from the saline solution and inserted into retroperitoneum with hilum facing the external iliac vessels (Fig 3D). This should be marked as a beginning of the "manipulation time". An end-to-side anastomosis between renal vein and the external iliac vein is performed with running suture using 6.0 polypropylene suture (Fig 4A). Then, the renal vein is clamped with a bulldog clamp to avoid premature retrograde

Table 1: Basic experimental characteristic.

		Median	Standard Deviation (SD)
N	20		
Sex (M:F)	0:20		
Weight (kg)		37.5	±1.88
ST (minutes)		153	±22.14
CIT (minutes)		37	±10.02
MT (minutes)		23.5	±3.83
Custodiol perfusion (mL)		250	±71.59

Abbreviations: N, number of procedures; M, male; F, female; ST, surgery time; CIT, cold ischemia time; MT, manipulation time

reperfusion of the graft. The graft is held up against the peritoneum in case of standard position. In the case of upside-down position, the graft is not relocated. The artery is clamped using vascular clamp and arteriotomy is performed on the lateral side of the artery. The width of the arteriotomy should fit the width of the renal artery. The arteriotomy is rinsed with a heparin solution. An end-to-side anastomosis between the renal artery and the external iliac artery is performed with running suture using 6.0 polypropylene suture (Fig 4B). The clamps are released, and the kidney graft is re-perfused with blood (Fig 4C). This marks the end of "cold ischemia" time and "manipulation time". The graft is externally warmed with a 30-50°C saline solution. The urinary bladder is identified within the peritoneum. The location for the urinary anastomosis is selected dorsally. The bladder and overlying peritoneum are fixated with polyglactin 3-0 suture and pulled ventrally. A small hole is prepared below the fixating suture. The urine is sucked out of the bladder. The ureter is shortened, and the opening is widened using a longitudinal cut. At the base of the cut, the suture is established. The anastomosis is performed between the ureter and the bladder - full-thickness ureteroneocystostomy. Before the final stitch is performed, the throughput of the anastomosis is verified using a dissector. The graft is positioned in the retroperitoneum (Fig 4D). The pulsation of the artery is checked. In the case of arterial spasm, the Papaverinhydrochlorid solution (Paveron N © - Linden Arzneimittel-Vertrieb-GmbH) is considered. The free outflow of the vein is verified. The implantation of drainage is considered according to the amount of bleeding or lymphatic secretion. The fascia is closed using a polyester 1-0 suture. The skin is closed using a monofilament 3-0 nylon suture.

Ethical Consideration

This study was reviewed and approved by the Ministry of Health of the Czech Republic – num. 29/2016.

All legal requirements for this animal laboratory experiment have been met and the study design was approved by the ethics committee. The Institute for Clinical and Experimental Medicine, Prague, Czech Republic (IKEM), is in possession of an active license, which enables it to take part in experimental work on laboratory animals. All transplantations and procedures performed on laboratory animals were carried out in an operating theatre under the care of a trained anesthesiologist.

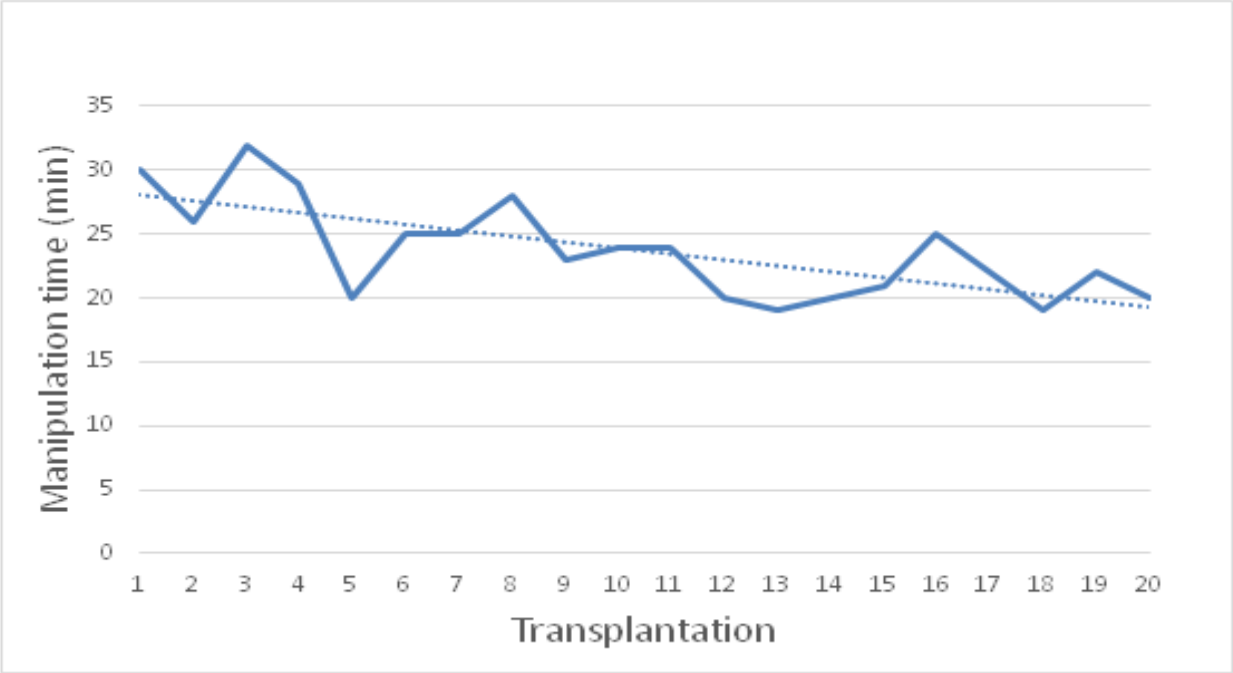

Figure 5: Changes of the manipulation time depending on the number of transplantations. Thread line shows diminution of the average time.

RESULTS

The experimental animal model procedure of heterotopic renal transplantation was performed on 20 pigs between August 2017 and January 2019 (Table 1).

Over the course of the experiment, the thread line showed manipulation time decrease by 32% from 28min to 19min, cold ischemia time decrease by 33% from 46min to 31min, and surgery time decrease by 28% from 180min to 130min (Fig 5-7).

No animal had to be sacrificed before the end of the experiment. No animal died during the surgery or within the postoperative period. We did not experience any hemodynamic complications during the procedure. All animals had successful reperfusion of the grafts. We witnessed urination of all grafts before performing the ureteral anastomosis.

Three grafts had an early graft failure due to an early arterial thrombosis; in the first two cases, the grafts had their artery anastomosed onto a thin external iliac artery. We modified the technique of the transplantation afterwards. The third partial-thrombosis occurred on a graft with complex arterial anatomy with implanted pole artery.

Due to intraabdominal and renal anatomy, pigs seem to the best available laboratory species for kidney transplantation model.

DISCUSSION

Animal laboratory models are expensive, but valuable tools for young surgeons to learn proper techniques and to further train their skills. Even though they are not widely used, they are highly effective in shortening the learning curve, shortening surgery time and mastering the specifics in surgery techniques [5-7].

Choosing the right animal model is very important to adopt similar technical skills as used in human surgery [10]. Due to intraabdominal and renal anatomy, pigs seem to the best available laboratory species for kidney transplantation model [8, 14]. The explored intraabdominal, and kidney anatomy is almost identical to human.

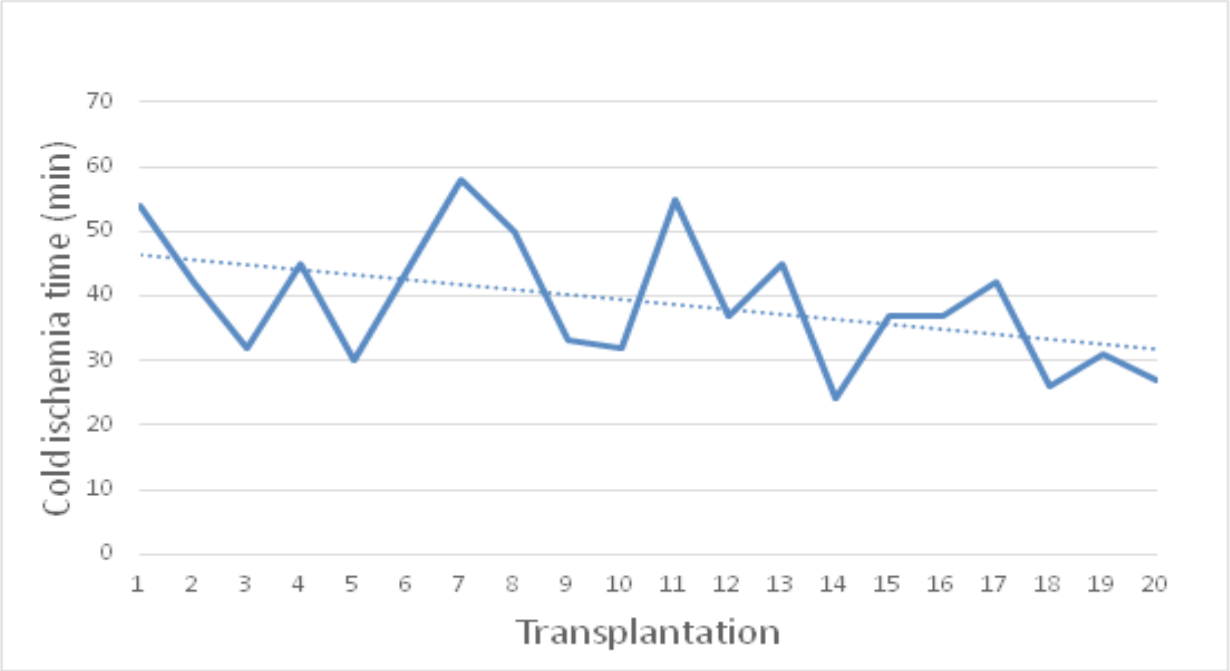

Figure 6: Changes of the cold ischemia time depending on the number of transplantations. Thread line shows diminution of the average time.

Furthermore, this model can be extended to serve not only as a simple learning tool for surgical techniques. Considering pig to human similarities in physiology, biochemistry, and immunology, it can also be used as a short- or long-term model in kidney transplantation [5, 9, 15]. In such a case, we recommend performing contralateral nephrectomy to mimic renal failure.

We introduced a few novelties in pig kidney transplantation; The extraperitoneal approach to the kidney and external iliac blood vessels proved to be effective and safe, easy to perform and maintain [11,12]. Without the need to keep the intraabdominal organs from the way of the surgery, the only concern is to retract the peritoneum medially enough to reach the blood vessels, eliminating the need for abdominal packing. Even using the midline laparotomy this proved to be comfortable and easily manageable in two surgeons. When aiming for good long-term results after transplantation, the peritoneum serves as a fixation for the graft, minimizing perirenal space and thus preventing the possibility of graft migration and vessel kinking and rotation [5]. We strongly suggest using this approach, as it can be adapted in both orthotopic and heterotopic (to the sub-renal fossa and the iliac fossa as well) renal transplantation in a pig model.

Due to the location of the laparotomy, we advise choosing female pigs for the transplantation courses.

To mimic the human transplantation conditions, we chose and recommend a heterotopic position of the graft placing it to the iliac fossa [5]. In most published studies this position is not used. This may be due to the anatomical challenges in anastomosis creation on the vessels. In most studies, the renal vessels are anastomosed to renal vessel stumps [11], or to the abdominal aorta and inferior vena cava in their proximity [12, 13]. This is only possible in orthotopic position of the graft, not when placing the graft to the iliac fossa [5].

In the literature, some studies describe a higher rate of vascular thrombosis, when performing the arterial anastomosis on the external iliac artery in the heterotopic position of the graft [5, 8]. This may be because of two reasons:

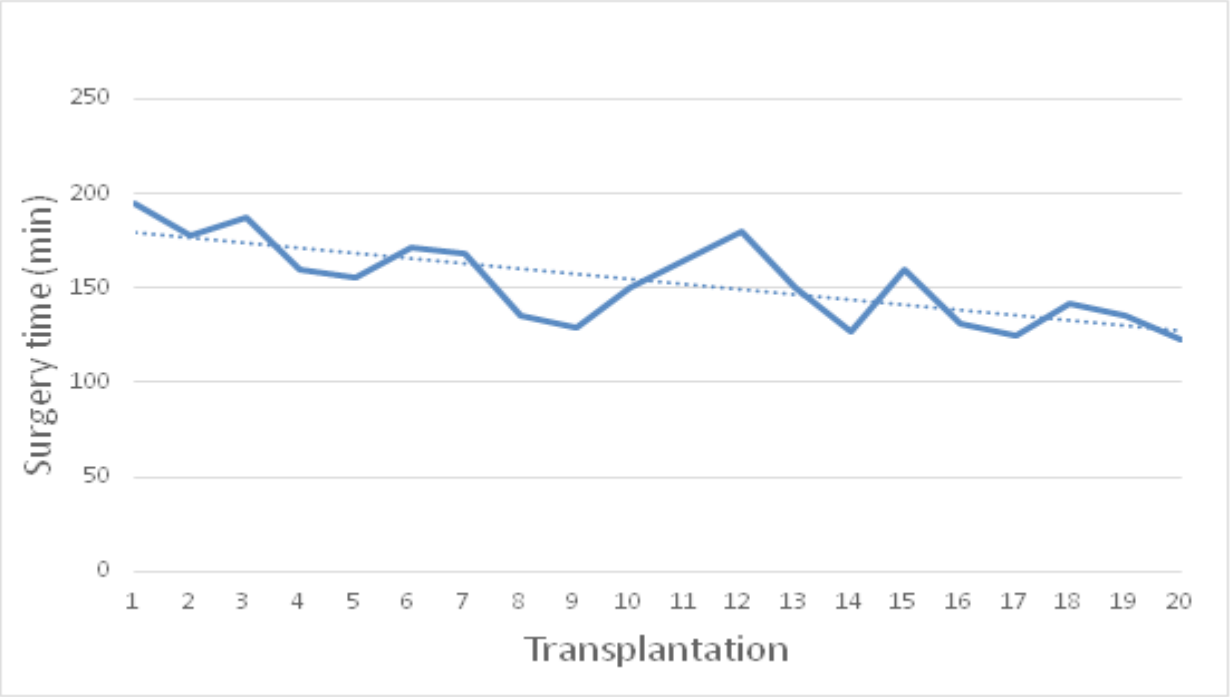

Figure 7: Changes of the surgery time depending on the number of transplantations. Thread line shows diminution of the average time.

1. The heterotopic transplantation may cause a little rotation of the graft because of smaller space in the iliac fossa. We did not find this significant. Though the longitudinal axis of the graft was usually not parallel to the aorta, we did not experience any kinking of the vessels. Additionally, using the retroperitoneal approach, the graft is fixated in the retroperitoneum, preventing further rotation or migration of the graft ventrally or laterally. The cranial migration though can still occur.

2. The external iliac artery of a pig is slimmer than one on humans – offering a lower arterial blood flow for the graft. We found this to be a significant problem in two of our transplantations. In case of dangerously thin external iliac artery, the anastomosis can be performed on the distal aorta just proximally from the external iliac artery origin. This is beneficial due to an increase in arterial blood flow through the anastomosis, while still allowing us to perform the anastomosis of the renal vein on the proximal external iliac vein.

As described, the arterial anastomosis on the external iliac artery is considered to have inferior results when compared to the anastomosis on the aorta [5, 13]. We found that to be true. When using this model solely for training purposes, we suggest using the iliac artery for your anastomosis.

On the other hand, when aiming for proper long-term graft function in the heterotopic position of the graft, we propose an anastomosis to the suprailiac region of the abdominal aorta. In both cases, the proximal part of the external iliac vein can be used for venous anastomosis. Furthermore, in both cases we did avoid clamping the entire abdominal aorta and inferior vena cava, preventing hemodynamic complications during the procedure. We believe this approach will prove comparable to anastomosing the vessels to abdominal aorta and inferior vena cava, as described in other studies, aiming for good long-term results.

We found the arterial spasm to be a severe problem during the pig renal transplantation. This concerns both the renal artery and the external iliac artery. We strongly advise use of antispasmodic drugs, such as local applica-

tion of Papaverine, to prevent difficulties when performing arterial anastomosis or during the graft reperfusion.

We find the upside-down position of the graft beneficial in case of ipsilateral autotransplantation. In pigs, the proximal external iliac artery is usually located just ventrally from the vein. In the upside-down position, the renal vein is naturally facing the dorsally located iliac vein, and the renal artery is facing the iliac artery. Furthermore, after the vein anastomosis, there is no need to relocate the graft. On the other side, this position differs from human surgery. The classic position can also be used, but in that case, the anastomosed graft blood vessels would have to cross at some point (the renal vein being originally located ventrally from the aorta), risking the kinking of the vessels around each other.

To avoid the upside-down position, keeping all the benefits of the graft position, we suggest performing the transplantation to the contralateral iliac fossa – mimicking the standard position in human first kidney transplantation. This can be easily achieved through the same midline laparotomy.

When aiming for long tern results, the upside-down position should be considered in case of ipsilateral autotransplantation. The only significant difference in the pig model is the approach to the urinary bladder, located mainly intraabdominally – proving a little obstacle in the iliac position of the graft. The extraperitoneal approach is non-beneficial, when in need of cranial cystostomy (like the Lich-Gregoir or Politano-Leadbetter anastomosis technique) [16].

Ureteroneocystostomy is one of the key elements to master in kidney transplant surgery. In the previous complex models, this type of anastomosis is rarely used (because of orthotopic position of the graft). This is a main reason a heterotopic transplantation should be considered. The long-term results of the uretero-ureterostomy are comparable with ureteroneocystostomy [16]. The quality of the urinary anastomosis is crucial to the proper long-term graft function and the patient's quality of life, and as such, it should be part of a learning process. The urinary stent should be considered when aiming for long-term performance of the graft.

As for the limitations of the study, the low number of animals may be the biggest issue. If aiming for significant statistical data, the amount of data would have to be higher.

In general, the porcine model of renal transplantation is a valuable tool for experimental and clinical surgeons. As described above, vast similarities exist between the porcine and the human anatomy and physiology, which allows fast translation of acquired skills into clinical practice.

In conclusion, the goal of this paper was to describe technical and surgical aspects of a porcine model of heterotopic kidney allotransplantation, using up-to-date techniques, utilized nowadays during human kidney transplantation. Our experimental model demonstrated that a pig laboratory model is a useful and valuable tool for surgical training. It can help to shorten the operation times and lower the complication rates. This specific model can also be extended to serve not only as a simple training tool for surgical techniques. Considering pig to human similarities in physiology, biochemistry, and immunology, it can also be used as a short or long term model in kidney transplantation. In such a case, we recommend performing contralateral nephrectomy to mimic renal failure.

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CONFLICTS OF INTEREST: None declared.

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