

## Appraisal of the porcine kidney autotransplantation model

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## 1. ABSTRACT

Animal models are extensively used for transplantation related research, especially kidney transplantation. Porcine autotransplantation models are considered to be favorable regarding translatability to the human setting. The key determinants for translatability of the model are discussed, comprising animal age, development, anatomy, anesthesia and surgical protocols, and perioperative care. With the detailed discussion of these determinants and the pitfalls in diagnosing animal discomfort, an attempt is made to provide a uniform porcine kidney autotransplantation model with tools to improve currently used models.

## 2. INTRODUCTION

The waiting list for a kidney transplant has exceeded 11,000 registrations in the Eurotransplant and 93,353 in the United Nations Organ-Sharing network, emphasizing that kidney grafts are in high demand. Use of marginal kidney grafts such as from non heart-beating donors (NHBD) can alleviate organ shortage but incorporates relatively high rates of delayed graft function (DGF) and primary non-function (PNF). (1-3) This outcome is mainly exerted by the prolonged ischemic times in combination with reperfusion (ischemia/reperfusion injury, IRI), experienced by the NHBD kidney graft. (1-3) A clinically relevant, reproducible animal kidney

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transplantation model is imperative to investigate and improve intervention and preservation strategies to unlock the full clinical potential of NHBD organs. (4,5) An autotransplantation model is highly suitable to isolate the effects of the intervention or preservation method from the possible effects of graft rejection.

Crucial for animal models is the translatability to the human setting in which trans-species extrapolation of small animal models (e.g. mice, rats) to humans can be precarious. (6) Nonetheless, substantial research has been performed in these models, despite the differences in renal (micro-) anatomy, related technical difficulties, young age of animals (reflecting infants), circadian rhythm-related issues, dosage/metabolism conversions, and differences in pharmacokinetics/pharmacodynamics. (4,5,7-10) Most of these issues can be diminished by using the porcine model (see discussion section) for research relevant to the clinical transplantation setting.

We therefore present herein, current insights in the application of anesthetics, surgical techniques, and perioperative care for a porcine kidney autotransplantation model. Our goal is to provide a standard model of high reproducibility, employing the advantages of a large animal model, i.e. low cost, high availability, comparable anatomy and physiology to the human setting. Special attention is paid to the anesthesiological, surgical and postoperative aspects of this model.

### 3. PORCINE CHARACTERISTICS AND PRE-OPERATIVE CARE

Several factors influence translatability and graft outcome of the porcine autotransplantation model, including age, sex, housing, stress, and feeding.

#### 3.1. Age and Sex

Renal development in pigs differs from humans, where human kidneys are anatomically and functionally mature at gestational age, porcine kidneys are mature at 2 to 3 months (20-30kg) of age. (11,12) Thus, to translate porcine organ function to the human, adult setting, age should at least be above 3 months. Furthermore, at 7 to 8 months (100 -120kg) of age pigs reach puberty during which androgens can influence IRI injury by inhibiting non-androgen receptor-mediated processes. (13) This inhibition leads to increased inflammation and functional kidney injury. (14) Secondly, while a midline abdominal incision can be used for transperitoneal access to the retroperitoneum (see chapter 4), the male pigs midline urethral position hampers the latter and poses unnecessary infectious risks. Age and sex related endocrine factors should be considered in experimental designs while young piglets (20-30kg) are developmentally equal to human infants regarding functional characteristics and anatomy of the kidney. (12) Therefore, we pose an optimal age with female pigs for the autotransplantation model of 5 months (50kg), representing comparable kidney function to adult humans.

#### 3.2. Housing & Stress

Pigs are social and curious animals and need to be housed in enclosures that allow social interaction and stimulation of natural behavior (rooting) to reduce induction of stress. Putative floor areas per pig minimally encompass 1.08 m<sup>2</sup>, 1.35 m<sup>2</sup>, or 2.16 m<sup>2</sup> for 25, 50, and 100 kg pigs, respectively. (15) A reduction in recommended floor area per animal will result in poor growth due to an increase in stress levels. (16,17) Stress has detrimental effects on animal well-being and transplantation outcome. A chronic stress response can be induced by transportation from supplier to animal facility and repetitive, yet minor threatening approaches to the pig. (18,19) The stressed animal tends to dehydrate, lose weight, and undergo immunological and metabolic changes. (20,21) Reperfusion dynamics after graft implantation can be altered by these influences, underscoring the need for proper animal management. (18) A pre-pathological state of the cardiovascular and exocrine system, gastrointestinal tract, and adrenal medulla can be evoked by the chronic stress syndrome, or synonymously, wasted pig syndrome. (22) Changes like thymus atrophy and enlarged adrenal glands are correlated with the pre-pathological state. (23) Cortisol levels however, are proven inaccurate as stress indicator in a porcine model. Stressed pigs showed decreased plasma cortisol levels and plasma cortisol binding capacity. (22) While cortisol levels are typically indicative for the amount of stress, it is proposed that wasting (characterized by an aberrant feeding pattern and non-nutritive behavior) is used as an early warning sign for stress. (22) Shifts in dietary behavior therefore underscores the essence of a well-designed acclimatization and fasting protocol, where acclimatization should be 5-7 days to minimize stress. (21) Surveillance of individual behavior patterns during the acclimatization period can aid judgment of post-operative discomfort and accustom the animal to the caretaker/researcher.

#### 3.3. Feeding and fasting

Rapid, dietary adjustment-related sequela can induce a pro-inflammatory state. A calorie-adjusted diet from the pig-supplier can be provided to alleviate the effects on intestinal villi, organ weights, and cytokine expression. (24) Due to high caloric demand of 8,410 kcal/day for a 50kg pig, pre-operative fasting should not evoke a physical response to starvation. (25,26) A 6-8 hour fast of solid food is sufficient to empty the upper gastrointestinal tract owing to short intestinal transport times. In case a complete empty stomach and small intestine is desired, 12 hours fasting will suffice. Additionally, oro-esophageal gastric suction is an option. For emptying the spiral colon, a 28 hours fast whether combined with enemas or not, is advised. Application of enemas, however, requires animal training to avoid stress. (21,27) Flavored sweet drinks, provided during fasting, help prevent hypoglycemia. Furthermore, water should never be withheld while drinking is linked to eating and vice-versa. (26)

In summary, 50kg female pigs are favorable for the autotransplantation model, minimizing androgenic influences and employing anatomically and functionally

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mature kidneys in a pre-pubertal state. The related age of 5 months provides a 1-2 months period on the lower- and higher –age range to prevent age-related influences. Acclimatization should encompass 5-7 days in housing that is stimulating natural behavior and is stress-free, whereas pre-operative fasting should be minimized and accompanied by sweet flavored drinks to prevent hypoglycemia.

### 4. ANESTHESIA

Anesthesia potentially has profound effects upon hemodynamics and the outcome of renal transplantation underscoring the need for a uniform protocol. The currently applied anesthesia protocols in porcine studies (Table 1) decrease comparability through anesthesia-exerted effects upon transplantation outcome. (28-30) It is therefore imperative that a more uniform protocol is put in place to reduce animal discomfort, facilitate animal handling, and minimize influential effects on hemodynamics, inflammation, and kidney graft.

#### 4.1. Premedication

Premedication aims to relieve anxiety, stabilize autonomous reflexes, facilitate animal handling, and reduce the dose of general anesthetics needed during induction and maintenance of anesthesia, ideally without cardiovascular influences. Combinations of intramuscular (IM) or intravenous (IV) benzodiazepines with dissociative anesthetics (e.g. ketamine, tiletamine) or azaperone (butyrophenon derivative) combined with a dissociative agent provide cardiovascular stable premedication. (28) Atropine (an anticholinergic agent) IM is often added to blunt vagal airway reflexes as broncho-secretion and salivation to aid intubation. Undesirable in this experimental setting are alpha 2-agonists (e.g. xylazine, medetomidine) due to potent cardiovascular side-effects and reduction in sympathetic tone. (29) However, a low-dose of these drugs (40mcg/kg) in combination with low-dose midazolam (0.2mg/kg) showed minimal side-effects. (30) Alpha 1-antagonists block norepinephrine-release at renal terminal nerve endings in rats thereby preventing acute ischemic renal failure. (31) Consequently, low alpha 2-selectivity (i.e. xylazine with relatively low alpha 2/alpha 1-selectivity) exerts relatively high alpha 1-stimulation, potentially leading to ischemic renal effects. (31)

#### 4.2. Induction

Continuous IV-access throughout the surgical procedure is facilitated via an auricular IV-catheter or central venous catheter for sustainable access. Induction of general anesthesia can be achieved using inhalants or injectables, where halothane, as a volatile inhalation anesthetic, sensitizes the myocardium and induces anesthesia slowly due to a relatively high blood/gas partition coefficient. (32) Furthermore, isoflurane and sevoflurane, while being capable of relatively fast induction of anesthesia without sensitizing the myocardium, have shown to execute anti-inflammatory and anti-necrotic effects in-vitro in proximal tubular cells. (33,34) Injectable induction comprises hypnotics (e.g. thiopental, propofol), dissociatives (e.g. ketamine, tiletamine), and opioids (e.g.

sufentanyl). After adequate reduction of swallowing reflexes and jaw muscle tension, orotracheal intubation still is arduous because of typical anatomical characteristics of the porcine oropharynx. These comprise a long soft palate, pharyngeal diverticulum, angular, caudoventrally positioned, slit-like vocal cords, and sigmoidal laryngeal passage. (35) Furthermore, high susceptibility for laryngospasms and hypoxia makes careful pre-oxygenation prior to intubation crucial. (35)

#### 4.2. Maintenance

Maintenance of anesthesia by inhalation and/or IV anesthetics via the ear vein or central venous catheter potentially exerts side-effects during prolonged exposure. Halothane, for instance, combines the aforementioned sensitization of the myocardium with possible induction of malignant hyperthermia. (36) Low-flow technique sevoflurane anesthesia can cause renal toxicity by forming compound A (fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether) as degradation product by contact with carbon dioxide absorbents with a strong base. (37) IV-protocols are applied using opioids, dissociative agents, or benzodiazepines and muscle relaxants (Table 2). Propofol has even greater anti-inflammatory and protective effects compared to sevoflurane. (38) Ropivacaine, as epidural anesthetic, enhances the sensory block without compromising cardiovascular stability in isoflurane anesthetized pigs. (39) While hemodynamic side-effects can be circumvented by well designed anesthesia protocols, the depth of anesthesia should be carefully monitored and supported (e.g. fluid-, inotropic-, and vasopressor therapy) to safeguard a proper renal perfusion pressure of 40 to 60mmHg. (40)

#### 4.3. Recovery

For smooth and rapid postoperative recovery, correction of core body temperature, acid-base disturbances, and replenishment of energy stores is advised. Preferably, the correction is performed in combination with short-acting anesthetics or antagonizing agents. Furthermore, sedatives can prevent anxiety and stress in the acute postoperative phase for which purpose low dose selective alpha-2-agonists (e.g. medetomidine) can be used. (31) At this stage of the procedure; a stress-free recovery outweighs the potential cardiovascular side-effects of the sedative.

#### 4.4. Recommendations

A robust protocol should be based on maintaining anesthetic depth, securing vital parameters and animal comfort while minimizing influential effects and side-effects. Based upon literature and our experience, the following protocol is suggested for graft retrieval or implantation.

The use of a mixture of ketamine, midazolam, and atropine IM in the neck as premedication followed by a brief administration of isoflurane to allow positioning of an auricular cannula and orotracheal tube, proved rapid and without side-effects. Maintenance of anesthesia with sufentanyl, ketamine, midazolam, pancuronium, or analogs

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**Table 1.** Anesthesia type, dose, and postoperative management in large animal models described in literature.

Premedication	Induction	Ventilation route	Maintenance	Fluids	Diuretics	Analgesia	AB PO <sup>1</sup>	Reference
IM <sup>2</sup> : 0.5mg atropine, 2mg/kg azaperone, 15mg/kg ketamine	IV <sup>3</sup> : 10.5mg/kg eunarcon, 2-4mg/kg propofol 1%, 0.0785mg fentanyl	Orotracheal, volume-controlled	Continuous 200-220mg/h propofol, bolus 0.0785mg fentanyl intermittent	Isotonic saline 20-25mL/kg/hr	Use avoided	50mg/kg metamizole PO <sup>4</sup> , 0.8mg temgesic 3dd	Unmentioned	Zacherl (24)
Nasal 0.2mg/kg midazolam	8% sevoflurane	Unmentioned	50% O <sub>2</sub> , 50% NO <sub>2</sub> , 4% sevoflurane, 10mg/kg atropine end OR <sup>5</sup>	Unmentioned	Unmentioned	Unmentioned	Unmentioned	Baumert (70)
IM: 5-6mg/kg stresnil	IV: 5-8mg/kg propofol, IM: 0.05mg/kg atropine, 0.01mg/kg buprenorphine	Endotracheal	1-5% isoflurane, 2-4L/min O <sub>2</sub>	Hartmann solution 50mL/kg/24hr up to 48 hours PO	IV: 0.5g/kg mannitol	SC <sup>6</sup> : 30mL 0.5% bupivacaine, IV: 5µg/kg buprenorphine 3-4 times daily for up to 72 hours	750mg cefuroxime 2dd up to 48 hours	Nicholson (71)
10mg/kg tiletamine, 7.5mg/kg zolazepam, 10µg/kg fentanyl	Unmentioned	Unmentioned	Halothan, O <sub>2</sub>	Unmentioned	Unmentioned	Unmentioned	Unmentioned	Badet (72)
Unmentioned	Unmentioned	Unmentioned	Ketamine, fentanyl, pancuronium, O <sub>2</sub> , NO	20% Glucose, 0.9% NaCl PO when no oral intake after day 1	IV: 40mg furosemide after surgery	SC <sup>6</sup> : 50mg tramadol daily after surgery, 50mg tramadol daily PO	500mg ampicillin daily PO	Treckman (73)
IM: 10mg/kg ketamine, 2mg/kg xylazine, 10µg/kg atropine	IV: 0.3mg/kg midazolam, 0.15mg/kg pancuronium, 10µg/kg fentanyl	Endotracheal	1-5% isoflurane, 2-4L/min O <sub>2</sub>	2L 0.9% NaCl PO first 24 hours	100mL 50% glucose after reperfusion	Acetylsalicylic acid 1g daily	IV: 500mg ampicillin daily	Maathuis (74)
Unmentioned	2-5% halothane, 8L/min O <sub>2</sub> , IM: 0.6mg atropine, IV: 10-20mg diazepam	Intubation	0.5-1% halothane, 2-4L/min O <sub>2</sub> , 2-4L/min NO, IV: 0.5-1mg fentanyl, 10mg/kg/h ketamine, thiopentone if necessary	1L 4% dextrose, 0.18% saline, 0.5-1L 10% dextrose, PO day 1, 2: 1-2L 10% dextrose, 0.18% saline	0.5g/kg mannitol	IV&IM: temgesic as required	IV: 40mg gentamycin, 1gr ampicillin per-op <sup>6</sup> and PO day 1, ampicillin 1g 2 times daily PO day 2	La Manna (75)
IM: 2mg/kg xylazine, 8mg/kg tiletamine/zolazepam	Unmentioned	Endotracheal	1% isoflurane, 50% O <sub>2</sub> and room air	40mL/kg Plasmalyte A (total) per-op, 20mL/kg/day Plasmalyte A until 36 hours PO	100mL 20% mannitol	8 µg/kg/h fentanyl per-op, IV: 0.30mg/kg/12hr buprenorphine until 60 hours PO	2g cefazoline per-op <sup>7</sup>	Jochmans (43)
IM: 10-15mg/kg ketamine, 1-1.5mg/kg dormicum, 0.25-0.5mg/25kg atropine,	Mask: 2-4% isoflurane, O <sub>2</sub> 2-3L	Orotracheal, volume controlled 10-15ml/kg/min, O <sub>2</sub> :air 1:3 (40-45% O <sub>2</sub> , arterial pO <sub>2</sub> 220mmHg), 12-18 respiration/min, cut-off pressure 20mmHg	IV: 5-10µg/kg/hr sufentanyl, 10-15mg/kg/hr ketamine, 1-2mg/kg/hr dormicum, 0,1-0,15mg/kg/hr pavulon; bolus: 3-5µg/kg sufentanyl, 0,2-0,6mg/kg dormicum, 0,04-0,06mg/kg Pavulon, 2-4mg/kg ketamine,	5-10mL/kg/hr 0,9% NaCl adjusted to needs. PO day 1,2: 500mL 5% glucose and 500mL 0,9% NaCl increased according to input	None	IM: 2mL finadyne once PO, IV: 0.02-0.04mg/kg temgesic until 72 hours PO twice daily or prolonged when needed, aspégic 500mg daily, Lactulose 5mL twice daily	IV: 500mg augmentin twice daily	This article

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			80µg/kg medetomidine for recovery					
Unmentioned	15mg/kg ketamine, 0.02mg/kg atropine	Mechanical ventilation, volume controlled	2% halothane, fentanyl, pancuronium bromide	Unmentioned	Unmentioned	Unmentioned	Unmentioned	Lledo-Garcia (76)
SC: 0.02mg/kg buprenorphine	3mg/kg pentothal	Intubation	0.5-2% isoflurane in O <sub>2</sub>	Unmentioned	IV: 50mg furosemide, 500mg/kg mannitol	epidural: 0.1mg/kg morphine	IV: 500mg cefazolin per-op	Lin (55)
Unmentioned	IM: 2mg/kg ketamine, IV: 0.1mg/kg pancuronium bromide, 25mg/kg pentobarbital	Intubation	50% O <sub>2</sub> &NO, bolus pentobarbital and pancuronium bromide as need	10ml/kg/hr ringers lactate	Unmentioned	Unmentioned	50mg/kg cefazolin 1dd until PO 2	Inoue (77)
Unmentioned	25mg/kg thiopental	Positive-pressure mechanical ventilation	2% isoflurane, NO 2L/min, O <sub>2</sub> 2L/min,	30-35mL/kg/hr electrolytes per-op, 800mL/day electrolytes until PO day 3	IV: 1.25g mannitol, 10mg furosemide	Unmentioned	1g cefazolin per-op	Tahara (78)
Unmentioned	5mg/kg pentothal	Unmentioned	1% isoflurane in 40% O <sub>2</sub>	Unmentioned	Unmentioned	Unmentioned	Unmentioned	Salomon (79)
SC: 0.02mg/kg buprenorphine	IV: 2mg/kg ketamine, 0.2mg/kg diazepam	Unmentioned	Isoflurane in 100% O <sub>2</sub>	Unmentioned	Unmentioned	Epidural: 0.1mg/kg morphine	22mg/kg cefazolin per-op	Polyak (80)

<sup>1</sup>AB-PO: antibiotics post-operative, <sup>2</sup>IM: intramuscular, <sup>3</sup>IV: intravenous, <sup>4</sup>PO: post-operative, <sup>5</sup>OR: operation, <sup>6</sup>SC: subcutaneous, <sup>7</sup>Per-op: perioperative

**Table 2.** Animal and preoperative specifications of large animal autotransplantation models mentioned in recent literature

Animal	Nr. <sup>1</sup>	Breed	Gender	Weight <sup>2</sup>	Accl. <sup>3</sup>	Fasting	Survival	Reference
Pig	10	White Landrace	Male	25 (16-46)	Unmentioned	24 hours	20 hours	Zacherl (24)
Pig	150	Large White	Unspecified	46-49	Unmentioned	Unmentioned	14 days	Baumert (70)
Pig	20	Large White	Female	35-70	14 days	Unmentioned	14 days	Nicholson (71)
Pig	16	White	Unspecified	25	7 days	Unmentioned	7 days	Badet (72)
Pig	34	German Landrace	Female	27	Unmentioned	Unmentioned	7 days	Treckman (73)
Pig	15	German Landrace	Female	20-30	Unmentioned	Unmentioned	7 days	Maathuis (74)
Pig	12	Large White	Female	Unspecified	7 days	Unmentioned	4 days	La Manna (75)
Pig	12	Unspecified	Female	24-51	2 days	12 hours	10 days	Jochmans (43)
Pig	-	Yorkshire X Landrace	Female	48-52	14 days	12 hours	8 days	This article
Minipig	14	Laboratory	Unspecified	35-40	Unmentioned	Unmentioned	1 hour	Lledo-Garcia (76)
Dog	12	Mongrel	Male	15-20	7 days	Overnight	14 days	Lin (55)
Dog	14	Mongrel	Unspecified	9-15	Unmentioned	Unmentioned	3 days	Inoue (77)
Dog	20	Hybrid (am. <sup>4</sup> Foxhound, Labrador, Beagle)	Female	11-13	Unmentioned	Overnight	14 days	Tahara (78)
Dog	18	Beagle	Unspecified	3-4 Years	24 hours	Unmentioned	3 days	Salomon (79)
Dog	12	Mongrel	Unspecified	15-22	7 days	Overnight	7 days	Polyak (80)

<sup>1</sup>number of animals used in the study, <sup>2</sup>Weight in kilograms unless mentioned as age, <sup>3</sup>Acclimatisation period, <sup>4</sup>American

minimizes hemodynamic influences in the animal. Post-operative IM administration of medetomidine and local bupivacaine allows time for skin closure and a smooth recovery.

## 5. SURGICAL CONSIDERATIONS

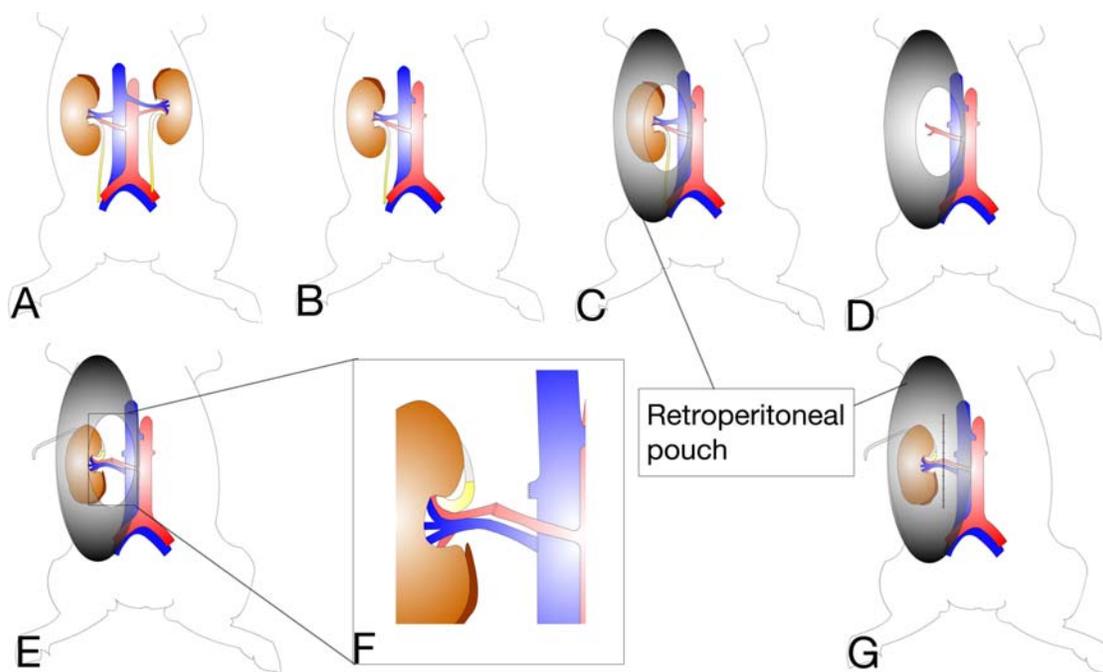
Autotransplantation entails unilateral graft retrieval, removal of the contralateral kidney, and subsequent graft implantation, potentially followed by survival. The experimental, surgical procedures may differ from those used in humans. Clinical NHBD kidney retrieval mostly comprises a transperitoneal, multi-organ donation procedure and for living related donation mainly a lumbar,

hand-assisted laparoscopic approach. Kidney graft implantation in humans occurs mainly in the iliac fossa via an inguinal approach and retroperitoneal route, using the external iliac artery and vein for the vascular anastomoses. In the porcine model, the transperitoneal approach is used while the vascular anastomoses are constructed using the caval vein and native renal artery, respectively. The porcine susceptibility for intestinal complications requires an adapted surgical protocol regarding handling of the intestines and vascular anastomoses (Figure 1).

### 5.1. Pre-nephrectomy

At first, sustainable vascular access can be obtained via tunneling and exteriorizing a jugular catheter to the

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**Figure 1.** Schematic illustration of the nephrectomy and transplantation procedure with A) the normal situation, B) after left nephrectomy where \*marks the careful opened retroperitoneum, C) after contralateral (right) nephrectomy with preservation of maximal arterial length, D) transplantation of the cranial to caudal flipped left kidney, E) close-up of the new vascular situation, and F) the final anatomical situation.

dorsum of the neck, allowing facile and relatively stress-free blood sampling during the survival period. Hereafter, exposure of the para-lumbar region is easily performed via a midline laparotomy, circumventing bilateral lumbar incisions (for contralateral kidney collection, see 'nephrectomy and graft implantation') and thus reducing post-operative discomfort.

Pre-existent intussusceptions are common in pigs and inspection prior to nephrectomy is important. Intestinal manipulation (e.g. retraction using surgical gauzes) is to be performed with great care and exteriorization avoided in view of the increased susceptibility for intestinal edema and ischemia. (21) Furthermore, intestinal post-operative fibrin deposits and adhesions can be avoided using a wetted fine cloth instead of gauzes.

### 5.2. Nephrectomy and graft implantation

Graft implantation in the iliac fossa is not advised in the pig because it hampers postoperative mobility of the animal. Implantation in the contralateral retroperitoneal area is preferred, avoiding unnecessary re-exploration of the previous nephrectomy site in the retroperitoneum. The left kidney provides a long renal vein and the possibility of dissection of the renal artery at the aortal origin (Figure 1). Access to the retroperitoneal area and renal vascular hilus using a cautery knife prevents excessive lymph leakage and postoperative accumulation while pigs show higher lymphatic flow compared to humans. (41) Closing the retroperitoneum after nephrectomy with mattress sutures prevents lymphocele formation and intestinal adhesions,

and is therefore highly recommended. (42)

Prior to graft implantation, careful peritoneal and intestinal inspection is advised to identify possible complications as intussusceptions. The contralateral retroperitoneum is opened and lifted at the vascular hilus of the kidney, in order to be able to create a pouch for stabilization of the graft with the ureteral catheter and to prevent intestinal adhesions when the peritoneum is closed over the graft. Contralateral (right) nephrectomy is subsequently performed similarly as the left nephrectomy, providing room for placement of the kidney graft in the peritoneal pouch.

Dissection of the right renal artery distally at the cranio-caudal bifurcation facilitates anastomosis to the graft renal artery. (43) Flushing the arterial stump with heparin solution prior to completing the anastomosis is imperative in view of the pigs' markedly increased coagulative tendency in comparison to humans. (44) Cranio-caudal reversal of the graft conveniently exposes the renal vein simplifying the vascular anastomoses. An end-to-side anastomosis of the graft renal vein to the caval vein is constructed using a running Prolene® 7-0 suture, and an end-to-end anastomosis is performed for the renal artery using a running Prolene® 7-0 suture.

An uretero-cutaneostomy is performed using a large (18-20 Fr) urinary catheter for urine collection to prevent twisting or blockage by protein deposits. The catheter is tunneled through the abdominal wall via a premarked point

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**Figure 2.** The KARUNO pig jacket that enables 4 liter urine collection without the use of metabolic cages.

below the ribcage into the retroperitoneal pouch. It is important not to lift the ureter ventrally to prevent occlusion of the arterial and venous branches to the grafts dorsal pole. (45,46)

### 5.3. Post-transplantation

Flushing and inspection of the abdominal cavity with warm saline prevents the aforementioned fibrin deposits and possible intestinal complications during the survival period. Closure of the abdominal wall is best performed with a running suture (PDS II® 1.0) to withstand pressure on the wound when the pig is standing. (47) Freeing the skin from the underlying connective tissue to decrease wound tension (undermining) will increase post-operative pain significantly and should be discouraged. Post-operative urine collection during survival is possible (without a metabolic cage) using a “pig-jacket” (KARUNO, Figure 2), designed to hold a 4 L urinary bag. (48-50) The jacket allows overnight urine collection without the risk of overfilling and pressure rise in the renal pelvis and calices.

## 6. POSTOPERATIVE MANAGEMENT AND ANALGESIA

Recovery from general anesthesia usually is highly stressful at which time the natural instinct of the animal is to flee. It is therefore advisable to transfer the pig to its individual housing when it is almost able to stand and to assist the animal in assuming an upright position to alleviate stress and pulmonary complications (e.g. hyperventilation, stridor). When returned to the pen, water must be available for reasons addressed in section Feeding and Fasting. (26) Postoperatively, the pig frequently demands intake stimulation for which sweet flavored treats can be offered. When intake falls short despite these stimulants, IV fluids or glucose are administered as additives up to the third or fourth postoperative day via the jugular catheter. The increase in intake and indication for glucose IV can be based upon blood glucose levels. Taking the abovementioned considerations into account, the first

three to four postoperative days can be characterized as an intensive care period. Postoperative analgesia in this period is of utmost importance to provide basal welfare conditions for the animal, at the same time creating optimal conditions for the assessment of kidney function in the early postoperative phase.

Non-steroidal anti-inflammatory drugs (NSAIDs) like flunixin, meloxicam and ketoprofen have shown to be effective analgesics in the pig. (51,52) Whereas renal effects of meloxicam (selective COX 2-inhibition) and ketoprofen (nonselective COX 1- and 2-inhibition) in anesthetized normovolemic piglets have shown significant differences in urinary flow, selective COX 2-inhibition did not alter renal function when compared to a placebo group. (53) Nonselective COX-inhibition led to significant decreases in glomerular filtration rate (GFR) and renal blood flow (RBF). Clinical evidence suggests that the risk of acute kidney injury may be lower using selective COX-2 inhibitors. (54) If normotension cannot be adequately maintained, the risk of decreased GFR and RBF is increased.

Postoperative analgesia using systemic opioids, like buprenorphine, has shown to be effective in pigs, although high dosages are necessary for adequate analgesic effects. Importantly, gastrointestinal side-effects like constipation or ileus could interfere during this period, especially as pigs appear to be very susceptible to gastrointestinal complications after abdominal surgery. Fortunately, laxatives as lactulose or movicol have a sweet taste and will therefore be readily consumed by pigs. Postoperative analgesia using epidural morphine has been described in pigs after abdominal surgery and potentially unlocks a new era of postoperative pain management in laboratory animals. (55,56) A particularly interesting development would be the implementation of ‘depodur’ as a long-acting epidural formulation, avoiding the use of systemic opioids and their impact on intestinal motility. (57) Tramadol has been reported to improve the quality of induction and improve duration of anti-nociception in ketamine-xylazine

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anesthesia in young pigs. (58) However, as a major downside, this analgesic treatment cannot be complemented with opioids when desired. Due to this disadvantage and the possible renal side-effects resulting from NSAIDs, we recommend using opioids in the post-operative phase. Finally, pigs should be mobilized and receive proper fluid management in combination with the aforementioned to prevent gastro-intestinal complications as obstipation.

### 7. RECOMMENDATIONS FOR MONITORING AND MAINTAINING PORCINE HEALTH

Maintenance of a high quality of porcine health is indispensable throughout transplantation procedures to minimize variations in the transplantation results. The most important elements to secure the health condition of the pig are delineated in this paragraph.

Pigs are unaccustomed to restraining associated with the physical examinations as should be performed prior to each experiment. Therefore, visual inspection is relatively important to avoid physiological changes caused by the excitement of handling. If physical contact is necessary, the observations should be judged in relation to the amount of stress the pig is experiencing. As mentioned in paragraph 3, chronic stress syndrome develops rapidly, and therefore, regular and at least preoperative inspections are performed taking account of attentiveness, posture, skin color, nutritional status, body condition, wounds, and other striking abnormalities such as diarrhea, coughing, sneezing, visible nasal discharge, or swollen joints. Impaired mobilization causes a problematic welfare assessment in the survival period. Vocalizations, interestingly, are an important indicator of health and should therefore be compared pre- and post-operatively. (59,60) Furthermore, pigs should react promptly to noises and movements. Abnormalities and changes of these physical parameters should lead to exclusion from the experiment.

The respiratory rate of pigs of 50kg ranges from 25 to 40 breaths per minute whereas heart rates vary from 75 to 110 (peak 140) beats per minute in a stable surrounding. (61) Body temperature varies between 38.5 to 39.5 °C while temperatures above 41°C should be lowered using wetted, or alcohol soaked towels because of the pigs' inability to sweat. (61) Hypothermic pigs will bend their legs under their body and show pale skin with rough, raised hairs. Healthy pigs, in contrast, will lie comfortably in a sternal or lateral position with their legs stretched.

Proper acclimatization and minimizing the number of caretakers will facilitate post-operative handling as it needs to be emphasized that pigs are generally very susceptible to changes in their environment. Solitary pigs tend to refrain from eating and demand extra stimulation, underscoring the need to synchronize behavior with pen mates. Mobilization in the pen or, advisably, a larger area, will stimulate bowel movement and enhance wellbeing as experienced during our animal experiments. Sounds (e.g. radio, caretakers) help filter noises from outside and reduce stress, whereas thorough perioperative cleaning of pens

increases stress due to loss of a familiar surrounding. Postoperative food and water intake should be strictly controlled as an aberrant pattern is considered an indicator of discomfort and stress.

In summary, close clinical observations, minimal changes in caretakers, sounds, and smells and stimulation of feeding and mobilization in the postoperative phase will minimize variations in outcome. This careful control of porcine health will greatly enhance the quality of the experiment and subsequent outcome.

### 8. DISCUSSION

Although the kidney autotransplantation model is often deemed established, the way it is applied greatly influences the outcome of a transplantation study. A standardized use of type of animal, weight, sex, preoperative- and anesthetic protocols would not only be beneficial for the animals, but also improves comparability of studies among various research groups (Table 2).

Similarity between human and animal anatomy and physiology is the basis for devising a proper large animal model, along with other aspects such as the availability of pathogen-free (SPF) animals, low-costs, and the complexity of care and housing. Historically, large animal models comprise non-human-primates (NHP), dogs, mostly beagle or mongrel, and pigs. (4,5) NHP seem closest to man, however, internal renal anatomy differs between species in form and number of renal pyramids. (62,63) Furthermore, NHP are expensive regardless of SPF-status, and only a minimum of pathogens are excluded in SPF-animals. (64) In addition, NHP require specialized care and housing and, most importantly, institutes and individuals are often faced with societal condemnation and protest emanating from the use of NHP for research purposes.

Dogs feature a dissimilar anatomy in the arterial supply to the renal dorsal pole (comprising multiple supplying arteries) as compared to humans and display resilience to IR injury potentially resulting in misrepresentative extrapolations to the clinical setting. (65,66) Furthermore, to obtain comparable size and morphology of the renal pelvis and calices, dogs taller than 70cm must be used. (67) SPF-status animals are plenty, but costs remain sixfold that of pigs (personal communication, Harlan, The Netherlands; van BeekTopigs BV, Lelystad, The Netherlands). Knowledge of housing and care is widespread, but as with NHP, societal pressure contributes to the decline in the use of dogs. (5)

In pigs, basic renal function is comparable to man, but distal bifurcation of the renal artery is in cranio-caudal direction instead of antero-dorsally in man, resulting in a different orientation of the kidneys' avascular plane. (12,45,46,68,69) Despite these differences, the susceptibility of pigs to IR-induced organ damage is equal to that of man and societal resistance to the use of pigs for research is limited due to its large-scale agricultural use. This keeps costs low and availability high, creating an

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advantage of the porcine model above the NHP or canine model.

The impact of age, sex, surgical and anesthesiological techniques of which potential pharmaceutical interactions are most prominent, are challenging when using pigs. Underreporting of the details of the models used in literature is a major issue in interpretation of transplantation results (in 10 of 15 models; Table 2). Despite their side-effects, isoflurane was applied in 7 and halothane in 3 of 13 models in which inhalant anesthesia was applied for maintenance of anesthesia. The use of diuretics after transplantation of the graft clouds renal function, but has been used in 6 models. Post-operative systemic morphine or its analogs requires the use of laxatives to prevent obstipation, but this was not mentioned in all models reported. This suggests that not only underreporting is an issue but also incorrect implementation of the model.

In conclusion, the female pig of 50kg, or 5 months of age, is highly suitable for the kidney autotransplantation model. Using properly designed perioperative protocols, confounding effects on transplant outcome can be minimized. We have provided guidelines for perioperative care, anesthesia and surgical techniques proposing a more uniform use of the porcine kidney autotransplantation model. A standardized method will facilitate future planning of large animal studies in renal transplantation research and provides a basis to compare results of different research groups.

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**Abbreviations:** NHBD: non-heartbeating donor, DGF: delayed graft function, PNF: primary non-function, IR: ischemia-reperfusion, IM: intramuscular, IV: intravenous, NSAID: non-steroidal anti-inflammatory drugs, GFR: glomerular filtration ratio, RBF: renal blood flow, SPF: specific pathogen free, NHP: non-human primate, NO: nitric oxide

**Key Words:** *Sus scrofa*, Transplantation, Housing, Surgery, Anesthesia, Preoperative Care, Postoperative Care, Review

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