**Submission ID #: 61591**
**Scriptwriter Name: Bridget Colvin**
**Project Page Link:** https://www.jove.com/account/file-uploader?src=18785303

# Title: Orthotopic Kidney Auto-Transplantation in a Porcine Model Using 24 Hours Organ Preservation and Continuous Telemetry

**Authors and Affiliations: Wen-Jia Liu*[1,2], Lisa Ernst*[2], Benedict Doorschodt[2], Jan Bednarsch[1], Felix Becker[3], Richi Nakatake[2], Yuki Masano[2], Ulf Peter Neumann[1,4], Sven Arke Lang[1], Peter Boor[5], Isabella Lurje[6], Georg Lurje[1,6], René Tolba[2], and Zoltan Czigany[1,2]**
*These authors have equally contributed to the work

[1]Department of Surgery and Transplantation, University Hospital RWTH Aachen, Faculty of Medicine
[2]Institute for Laboratory Animal Science and Experimental Surgery, University Hospital RWTH Aachen, Faculty of Medicine
[3]Department of General-, Visceral-, and Transplantation Surgery, University Hospital Muenster
[4]Department of Surgery, Maastricht University Medical Centre (MUMC)
[5]Institute of Pathology, University Hospital RWTH Aachen, Faculty of Medicine
[6]Department of Surgery, Campus Charité Mitte | Campus Virchow-Klinikum – Charité-Universitätsmedizin Berlin

**Corresponding Author:**
Zoltan Czigany
zczigany@ukaachen.de

**Co-Authors:**
wliu@ukaachen.de
lernst@ukaachen.de
bdoorschodt@ukaachen.de
jbednarsch@ukaachen.de
felix.becker@ukmuenster.de
rnakatake@ukaachen.de
ymasano@ukaachen.de
uneumann@ukaachen.de
svlang@ukaachen.de
pboor@ukaachen.de

 

isabella.barth@rwth-aachen.de
georg@lurje.net
rtolba@ukaachen.de

# Author Questionnaire

**1. Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

**2. Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

**3. Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

**Protocol Length**
Number of Shots: **53**

# Introduction

**1. Introductory Interview Statements**

**REQUIRED:**

1.1. **Zoltan Czigany**: This protocol describes all of the key steps to successfully establishing a porcine orthotopic kidney auto-transplantation model using an organ preservation time of 24 hours and peri-operative telemetry monitoring **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

**REQUIRED:**

1.2. **Wen-Jia Liu**: The major advantages of this large animal auto-transplantation model over ex vivo studies and small animal models are its surgical-anatomical and pathophysiological similarities to the clinical setting **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

**Introduction of Demonstrator on Camera**

1.3. **Zoltan Czigany**: Our team at University Hospital RWTH Aachen will be demonstrating the procedure **[1][2]**.

1.3.1. INTERVIEW: Author saying the above

1.3.2. LAB MEDIA: Demonstrator photo grid Author NOTE: Please edit the photgrid using the unedited pictures with names of each collaborating team member uploaded as "1_3_2_Step_DemonstratorPhotgrid_Rawpictures_Names

**Ethics Title Card**

1.4. Procedures involving animal subjects have been approved by the Governmental Animal Care and Use Committee, LANUV NRW, Recklinghausen, Germany.

# Protocol

## 2. Telemetry Implantation

2.1. After confirming the appropriate level sedation in an overnight-fasted, adult, female pig **[1-TXT]**, make a 3-5-centimeter incision in the groin **[2-TXT]** and dissect the femoral artery in a 360-degree fashion **[3]**.

2.1.1. WIDE: Talent checking sedation *Videographer: More Talent than pig in shot* **TEXT: Anesthesia: azaperone 4 mg/kg + atropine 0.1 mg/kg + ketamine 15 mg/kg i.m. -> 1.45-2% isoflurane + fentanyl 3-7.5 micrograms/kg/h**

2.1.2. Incision being made **TEXT: See text for full surgical preparation details**

2.1.3. Artery being dissected or red vessel loops being pulled through and fixed, depending on what looks better on film

2.2. Use a number 11 scalpel to make an arteriotomy **[1]** and insert the arterial sensor of the telemetry device **[2]**.

2.2.1. Arteriotomy being made *Videographer: Important step*

2.2.2. Sensor being inserted *Videographer: Important step*

2.3. After making a subcutaneous pouch in the left flank, tunnel the telemetry transponder into the flank **[1]** and fix the transponder to the muscle fascia with a 3-0 polypropylene, single knot sutures **[2]**.

2.3.1. Shot of pouch, then transponder being inserted into pouch

2.3.2. Suture being placed

2.4. Next, make two 1-centimeter incisions on the left and right side of the thorax **[1]** and tunnel the red and white ECG (E-C-G) electrodes **[2-added]** to their designated location **[2]**.

2.4.1. Incision being made and/or showing the marked spots for the incision

2.4.2 Added shot: the electrodes are pulled through using the tunneling device

2.4.2. Electrode being inserted into the muscle **TEXT: ECG: electrocardiogram**

2.5. When the electrodes have been secured, commence registration of the telemetry data **[1]**.

2.5.1. Talent starting registration

2.6. Upon stable signal acquisition, close the groin incision with sutures and spray film dressing **[1]** and place a suitable-size porcine jacket onto the animal **[2-TXT]**.

2.6.1. Shot of closed wound closure, then spray dressing being applied

2.6.2. Talent placing jacket *Videographer: More Talent than pig in shot* **TEXT: Protect urine collection bag and catheters in designated jacket pockets**

**3. Nephrectomy and Kidney Graft Retrieval**

3.1. Fourteen days after the first surgery, make a 4-5-centimeter incision in the jugular groove on the right side of the anesthetized animal **[1]** and dissect the subcutaneous and muscle tissue to expose the jugular vein **[2]**.

3.1.1. WIDE: Talent making incision *Videographer: More Talent than pig in shot*

3.1.2. Jugular vein being exposed and/or blue vessel loops being pulled through and fixed, depending on what looks better on film

3.2. Use a peel-away sheet introducer or the Seldinger method to insert the jugular catheter **[1-TXT]**.

3.2.1. Vein getting punctured AND/OR Catheter being inserted AND/OR sheet introducer getting peeled away **TEXT: Tunnel catheter to back of animal as described** Author NOTE: tunneling in this case should not be shown

3.3. Secure the catheter with a 5-0 polypropylene suture **[1]** and close the incision in two layers **[2]**.

3.3.1. Suture being placed to secure catheter

3.3.2. Muscle or skin being sutured

3.4. Following surgical disinfection and sterile draping, perform a median laparotomy to open the abdomen **[1]** and use a standard abdominal retractor to expose the surgical field **[2]**.

3.4.1. Incision being made NOTE: show either skin AND/OR opening the abdomen with the cautery with one hand inside the abdomen

3.4.2. Retractor being placed

3.5. Open the peritoneal layer **[1]** and use a monopolar cautery, bipolar forceps, and fine scissors to dissect the left kidney and ureter from any adherent tissue **[2]**.

3.5.1. Peritoneum over the kidney being opened

3.5.2. Kidney being dissected

3.6. Then use a 3-0 polyglactin suture to ligate and divide the left ureter at least 10 centimeters distal to the kidney hilum **[1]**.

3.6.1. Ureter being ligated/divided

3.7. To retrieve the graft kidney, use vascular clamps to close the renal artery and vein close to the aorta and vena cava **[1]** and cut the vessels above the clamps **[2]**.

3.7.1. Vessels being clamped

3.7.2. Vessels being cut

3.8. Then give the kidney to the back-table team for additional preparation **[1]** and use a 5-0 polypropylene suture to close the stump of the renal artery and the renal vein **[2-TXT]**.

3.8.1. Talent handing kidney to the back-table team *Videographer: Important step*

3.8.2. Stump being sutured *Videographer: Important step* **TEXT: See text for full post-operative care details**

**4. Back-Table and Organ Preservation**

4.1. Immediately after retrieval, use a standard 14-gauge peripheral catheter to cannulate the renal artery **[1]** and flush the kidney with at least 500 milliliters of ice-cold organ preservation solution **[2]**.

4.1.1. WIDE: Talent cannulating artery and placing tourniquet to fix the catheter

4.1.2. Talent rinsing kidney, with solution container visible in frame

4.2. After the flush, wrap the graft in sterile organ bags **[1]** for storage in organ preservation solution with a target cold ischemic time of 24 hours at 4 degrees Celsius using a computer-controlled cooling circuit **[2]**.

4.2.1. Talent wrapping graft

4.2.2. Talent placing wrapped graft into computer-controlled cooling device

**5. Contralateral Nephrectomy and Orthotopic Kidney Auto-Transplantation**

5.1. For orthotopic auto-transplantation of the harvested kidney, the next day, use percutaneous puncture and the Seldinger technique to place an arterial catheter into the right femoral artery **[1-TXT]** and reopen the median laparotomy **[2]**.

**5.1.1.** WIDE: Talent placing catheter *Videographer: More Talent than pig in shot* **TEXT: Anesthesia: Adapted to the restricted renal metabolism as described in the text protocol**

5.1.2. Retractor being placed into incision

5.2. Two-five minutes before vascular clamping, intravenously inject 100 international units/kilogram of natrium-heparin **[1]** and subsequently remove the contra-lateral right kidney as demonstrated **[2]**.

5.2.1. Natrium-heparin being injected

5.2.2. Shot of clamped vessels, then kidney being removed

5.3. Place the preserved graft kidney into the surgical field **[1-TXT]** and initiate the administration of 0.1-1 microgram/kilogram/minute of norepinephrine as a continuous infusion, using the mean arterial blood pressure and heart rate to monitor the efficiency **[2-TXT]**.

5.3.1. Kidney being placed *Videographer: Difficult step* **TEXT: Flush the graft with 500 mL saline before implantation**

5.3.2. Norepinephrine being administered *Videographer: Difficult step* **TEXT: Keep MAP >80-90 mm Hg**

5.4. For end-to-end anastomosis of the renal vein, use 5-0 polypropylene to place two corner stitches **[1]** and suture the back wall in a continuous fashion **[2]**.

5.4.1. Corner stitch being placed OR Shot of corner stitches *Videographer: Important step*

5.4.2. Back wall being sutured *Videographer: Important step*

5.5. After completing the back wall, use one end of the cranial corner stitch to suture the front wall in a cranio-caudal direction **[1]** and flush the vein with a 100 international units/milliliter of heparinized saline solution **[2]**.

5.5.1. Front wall being sutured *Videographer: Important step* **TEXT: Anatomical variation seen: Figure 3 Panel H**

5.6. For end-to-end anastomosis of the renal artery, use a 6-0 polypropylene cranial corner stitch **[1]** before using the parachute technique to suture the back wall in a continuous fashion as demonstrated **[2]**.

5.6.1. Cranial corner stitch being placed OR showing few back wall stitches *Videographer: Important step*

5.6.2. Vessels ends and back wall being parachuted together *Videographer: Important step*

5.7. Suture the front wall with the other end of the double-armed, 6-0 polypropylene suture **[1]** and flush the artery with 100 international units/milliliter of heparinized saline solution **[2]**.

5.7.1. Front wall being sutured

5.7.2. Artery being flushed

5.8. After anastomosis completion, tie the two threads at the caudal corner **[1-TXT]** and sequentially open the venous vascular and arterial clamps to allow reperfusion of the kidney **[2]**.

5.8.1. Thread(s) being tied ~~**TEXT: Target warm ischemia <40 min**~~

5.8.2. Venous clamp then arterial clamp being opened

Added shot: extra shot of the well perfused kidney with pulsating artery etc AND/OR showing that the initially pale kidney gets rapidly red again NOTE: Show extra shot if it fits well

5.9. Following reperfusion, topically administer 5 milliliters of papaverine to the outside wall of the renal artery **[1]** and intravenously administer of 250 milliliters of 20% glucose solution and a single 80-milligram dose of furosemide **[2]**.

5.9.1. Papaverine being applied

5.9.2. Furosemide being delivered

5.10. Next, pass a 12-French pediatric urine catheter through the abdominal wall and skin **[1]** and use ligatures to secure the catheter in the ureter **[2]**.

5.10.1. Catheter being pulled through abdominal wall

5.10.2. Catheter being secured in the ureter

5.11. Close the peritoneal layer over the kidney to prevent dislocation of the graft and kinking of the vascular anastomoses **[1]** and close the abdomen in 4 layers as demonstrated **[2]**.

5.11.1. Peritoneum over the kidney being closed

5.11.2. Abdominal tissue being sutured

5.12. Finally, use color Doppler ultrasound to ensure adequate arterial and venous perfusion of the kidney graft **[1]** before returning the fully recumbent animal to the housing facility **[2]**.

5.12.1. LAB MEDIA: LabMedia_Doppler: Doppler ultrasound of kidney graft

5.12.2. LAB MEDIA: "Step 5_12_2_Labmedia_normal_Camera" and "Step 5_12_2_Labmedia_Thermocamera": Video of pig within housing Author NOTE: Please use the combination of the video files "Step 5_12_2_Labmedia_normal_Camera" and "Step 5_12_2_Labmedia_Thermocamera". I have prepared a suggestion using amateur video editing tools how these could be edited and merged (but we of course welcome any other suggestion). This please find as "Merge_Thermal_plus_Normal_final" video file as an example. Please mind that the raw thermal images are rotated 90 degrees compared to the normal camera footage and the time frame of registration was the same but the raw videos thermal and normal are yet to be rotated, edited and synced based on the movement of the animals.

# Protocol Script Questions

**A.** Which steps from the protocol are the most important for viewers to see?

2.2., 3.8., 5.4-5.6.

**B.** What is the single most difficult aspect of this procedure and what do you do to ensure success?

5.3., 5.12. The most difficult aspect of success is to ensure a good graft reperfusion and good initial kidney function after anastomosis.

# Results

**6. Results: Representative Findings After Orthotopic Kidney Auto-Transplantation**

6.1. In this representative set of 5 porcine orthotopic kidney auto-transplantation experiments **[1]**, the transponder implantation and auto-transplantation procedures were successful in each animal, with sufficient telemetry signals registered over the observation period **[2]**.

6.1.1. LAB MEDIA: Table 1

6.1.2. LAB MEDIA: Table 1 *Video Editor: please emphasize Figure 5 F*

6.2. Following abdominal closure, color Doppler ultrasound revealed a satisfactory arterial and venous perfusion of the kidney in all cases **[1]**.

6.2.1. LAB MEDIA: Figures 4B-4E *Video Editor: please emphasize Figure 4D and E*

6.3. Serum potassium and creatinine values **[1]** peaked on post-operative days 3-4 and showed gradual recovery afterwards **[2]**.

6.3.1. LAB MEDIA: Figures 5A and 5B

6.3.2. LAB MEDIA: Figures 5A and 5B *Video Editor: please emphasize data lines at POD3 and POD4 in both graphs*

6.4. The blood pH remained within normal range **[1]** and the urine output recovered to normal values over the first four postoperative days **[2]**.

6.4.1. LAB MEDIA: Figures 5C and 5E *Video Editor: please emphasize Figure 5C*

6.4.2. LAB MEDIA: Figures 5C and 5E *Video Editor: please emphasize Figure 5E data line*

# Conclusion

**7. Conclusion Interview Statements**

7.1. **Zoltan Czigany**: This protocol can be used to answer various questions about the effects of organ preservation techniques or ex vivo and in vivo therapies in a clinically significant large animal model **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera