

Submission ID #: 69306

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21138378>

Title: A Standardized Porcine Kidney Auto-Transplant Protocol for Studying Delayed Graft Function

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes, OR and lab**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 22

Number of Shots: 51

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Nader Abraham:** Our research focuses on developing methods to expand organ availability, reduce discard rates, treat ischemia-reperfusion injury, and rehabilitate grafts ex vivo.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Nader Abraham:** Delayed graft function affects ~40% of DCD kidney transplants; current preservation lacks effective interventions, which this protocol aims to address.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Nader Abraham:** This protocol objectively defines graft sufficiency and delayed function, while detailing a reproducible method to induce the condition in porcine models.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Nader Abraham:** Post-transplant graft function depends on controlled ischemic times, implant duration, and perioperative resuscitation, enabling consistent kidney preservation and reproducible model outcomes.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Nader Abraham:** Ex vivo organ assessment can evaluate graft viability and help predict post-transplant function following induced or prevented delayed graft function.

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

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Duke University

Protocol

2. Surgical Implantation of a Hickman Catheter and Foley Catheterization in a Swine Model

Demonstrator: Nader Abraham

- 2.1. To begin, place an anesthetized pig on its sides in a comfortable position [1]. After preparing the skin, use an 11-blade scalpel to make three superficial stab incisions [2] through the dermis to the subcutaneous fat layer between the shoulder blades, at the nape, and at the right anterior neck [3].
 - 2.1.1. WIDE: Talent placing an anesthetized pig on its side. **TXT: Anesthesia: Ketamine (22 mg/kg) and Midazolam (0.5 mg/kg) (i.m)**
 - 2.1.2. Talent making a skin incisions using a 11-blade scalpel.
 - 2.1.3. Shot of all 3 skin incisions.
- 2.2. Using a tissue tunneler, create a subcutaneous tunnel connecting the three incisions [1]. While the animal remains prone, thread the 9.6 French single lumen Hickman catheter from between the shoulder blades to the right anterior neck [2].
 - 2.2.1. Talent advancing the tissue tunneler beneath the skin between incision sites.
 - 2.2.2. Talent threading the Hickman catheter through the completed tunnel.
- 2.3. Turn the pig to the supine Trendelenburg position [1]. With an 18-gauge finder needle, aspirate the right external jugular vein using anatomic triangulation [2].
 - 2.3.1. Talent repositioning the animal supine in Trendelenburg position.
 - 2.3.2. Talent inserting the 18 G finder needle and aspirating the right EJ.
- 2.4. Thread a guidewire into the lumen using Seldinger technique [1]. Use the peel-away introducer to dilate the tract [2].
 - 2.4.1. advancing the guidewire into the vein.
 - 2.4.2. Talent dilating the venous tract with the introducer.
- 2.5. Then thread the Hickman catheter into the lumen of the external jugular vein [1] and secure the line on the back [2-TXT].
 - 2.5.1. Talent advancing the catheter into the lumen of the EJ.
 - 2.5.2. Shot of the line being secured on the back. **TXT: Confirm blood return and heparin lock the line**
- 2.6. To perform the manual approach of Foley placement, position the animal supine on the operating table [1]. Prepare the skin around the vaginal vault with betadine [2]. Then

flood the vaginal vault with 10 milliliters of betadine to reduce microbial load [3].

2.6.1. Talent positioning the animal supine on the operating table.

2.6.2. Talent applying betadine to the vaginal vault.

2.6.3. Shot of the vaginal vault being flooded with 10 mL betadine.

2.7. Manual identifying the urethral opening on the anterior vaginal wall [1]. Insert a 12 to 14-French lubricated Foley catheter stiffened with a wire into the urethral opening [2]. Advance the catheter 4 to 5 centimeters into the vaginal opening on the anterior vaginal wall [3].

2.7.1. Talent identifying the urethral opening.

2.7.2. Talent inserting the stiffened Foley catheter.

2.7.3. Talent pushing the catheter 4 to 5 cm into the vaginal opening on the anterior vaginal wall.

2.8. If manual insertion fails, insert a lubricated speculum to visualize the urethral opening [1]. After drying excess betadine insert the Foley catheter with or without the stiffening wire under direct visualization [2]. Inflate the balloon only after confirming urine return [3].

2.8.1. Talent inserting and opening the vaginal speculum.

2.8.2. Talent placing the Foley catheter under direct visualization.

2.8.3. Talent inflating the Foley balloon after urine return.

2.9. If nonoperative approaches fail, perform a laparotomy and insert an 18-gauge needle into the bladder [1]. Advance a 0.035-inch 1 meter wire through the bladder and urethra [2].

2.9.1. Talent inserting the needle into the bladder intraoperatively.

2.9.2. Talent advancing the guidewire through the bladder and urethra.

2.10. Thread the Foley catheter over the wire, inflate the balloon [1], then withdraw the wire vaginally [2].

2.10.1. Shot of the foley catheter being threaded over the wire and inflated.

2.10.2. Talent withdraws the wire vaginally.

2.11. Close the bladder puncture with a prolene 6-0 figure-of-eight suture [1]. Suture the body of the Foley and a 1 liter urine collection bag to the hide of the swine [2].

2.11.1. Shot of the bladder puncture being stitched close with figure of 8 suture.

2.11.2. Shot of the Foley and 1 L urine collection bag being stitched to the swine skin.

3. Right Nephrectomy and Graft Preparation After Circulatory Death Model

- 3.1. Prepare and drape the abdomen of the animal [1]. Perform a midline laparotomy from the xiphoid to the pubis [2].
 - 3.1.1. Talent preparing and draping the abdomen of the animal.
 - 3.1.2. Talent performing a midline abdominal incision from the xiphoid to the pubis.
- 3.2. Set up a Bookwalter retractor with four body wall blades to hold both sides of the abdominal wall and prevent bowel spillage [1].
 - 3.2.1. Talent placing and adjusting the Bookwalter retractor system.
- 3.3. Using warm saline moistened surgical towels, retract the bowel to the left side [1]. Replace the retractors above them to gently expose the right retroperitoneum containing the right kidney [2].
 - 3.3.1. Talent gently retracting bowel with moistened towels.
 - 3.3.2. Shot of retractors being replaced to gently expose the right retroperitoneum.
- 3.4. Now open the posterior peritoneum on the lateral side of the kidney [1]. Dissect the right ureter from the posterior peritoneum and off the right renal artery [2].
 - 3.4.1. Talent opening the posterior peritoneum on the lateral side of the kidney.
 - 3.4.2. Shot of the right ureter being dissected from the posterior peritoneum and off the right renal artery.
- 3.5. Divide the ureter between two 2-0 (Two-Oh) silk ties at the lower pole of the right kidney [1]. Leave a longer suture tail on the remaining ureter for later identification [2-TXT].
 - 3.5.1. Shot of the ureter being divided between two 2-0 silk ties at the lower pole of the right kidney.
 - 3.5.2. Shot of a longer suture tail on the remaining ureter. **TXT: Leave the remainder of the ureter covered under its peritoneal lining to reduce scarring**
- 3.6. Next, dissect the renal artery from the renal vein to its aortic origin to obtain maximal length [1]. Compress the inferior vena cava briefly [2] and roll it towards the left retroperitoneum to expose the origin of the right renal artery [3].
 - 3.6.1. Talent dissecting along the renal artery.
 - 3.6.2. Shot of the IVC being compressed.
 - 3.6.3. Shot of the IVC being rolled towards the left retroperitoneum to expose the origin of the right renal artery
- 3.7. Dissect the renal vein away from the adrenal gland cranially [1]. Then carefully lift the posterior peritoneal lining off the right renal vein until the larger adrenal draining veins

are visualized entering the inferior vena cava [2].

3.7.1. Talent dissecting the renal vein from the adrenal gland.

3.7.2. Shot of the posterior peritoneal lining being lifted off the right renal vein until the larger adrenal draining veins are visualized

3.8. To simulate donor after circulatory death, administer 500 units per kilogram of heparin via the central line and allow circulation for 5 minutes [1]. Double tie the renal artery and vein at their origins using 2-0 silk ties or larger [2-TXT]. During ischemia use tenotomy scissors or electrocautery on the cut setting to skeletonize and separate the renal artery and vein from each other [3].

3.8.1. Talent administering heparin through the central line.

3.8.2. Talent tying off the renal vessels and starting a stopwatch. **TXT: Start 30 min warm ischemia**

3.8.3. Talent separating renal artery and vein using tenotomy scissors.

3.9. At the end of warm ischemia, divide the vessels and ureter [1]. Then cannulate the renal artery with a 3-millimeter straight cannula [2]. Flush the kidney with 250 milliliters of ice cold University of Wisconsin solution through the renal artery [3].

3.9.1. Talent dividing the vessels and the ureter.

3.9.2. Talent cannulating the renal artery with 3 mm straight cannula.

3.9.3. Talent perfusing the kidney with 250 mL cold preservation solution.

3.10. Preserve the graft using hypothermic unoxygenated machine perfusion for 24 hours [1]. Then irrigate the abdomen with 1 liter of balanced electrolyte solution [2].

3.10.1. Talent placing the kidney on machine perfusion.

3.10.2. Talent pouring 1 L balanced electrolyte solution into the abdomen.

3.11. Close the fascia and skin with continuous 1-0 Prolene looped sutures [1]. Then irrigate the wound [2] and close the skin with staples [3].

3.11.1. Talent stitching the fascia and skin with continuous 1-0 Prolene looped sutures.

3.11.2. Shot of solution being poured over the wound.

3.11.3. Talent stapling the skin shut.

Results

4. Results

4.1. Three out of five pigs implanted with the simulated DCD grafts had return of urine production by post-operative days 2 to 3 [2].

4.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight the black line labeled "Functional" on all 3 graphs (top)*

4.2. In 40% of the pigs, delayed graft function occurred, characterized by persistent anuria beyond postoperative day 3 [1], with progressive elevations in serum blood urea nitrogen [2], serum creatinine [3], and serum potassium above 8 milliequivalents per liter [4].

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the red line labeled "DGF" across postoperative days 3–7 on all three top graphs*

4.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the red line in the Serum BUN graph from day 3 onwards*

4.2.3. LAB MEDIA: Figure 3. *Video editor: Highlight the red line in the Serum Cr graph, from day 3 onwards*

4.2.4. LAB MEDIA: Figure 3. *Video editor: Highlight the red line in the Serum K graph from day 3 onwards*

4.3. Electrocardiogram recordings in pigs with delayed graft function and hyperkalemia showed peaked T-waves, consistent with potassium toxicity [1].

4.3.1. LAB MEDIA: Figure 3. (bottom)

Pronunciation Guide:

Auto-Transplant

Pronunciation link: <https://www.merriam-webster.com/dictionary/autotransplant>

IPA: /ˌɔːtoʊˈtrænsˌplænt/

Phonetic Spelling: aw-toh-trans-plant

Porcine

Pronunciation link: <https://www.merriam-webster.com/dictionary/porcine>

IPA: /ˈpɔːrˌsaɪn/

Phonetic Spelling: por-syne

Nephrectomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/nephrectomy>

IPA: /nəˈfrɛktəmi/

Phonetic Spelling: nuh-frek-tuh-mee

Ischemia-Reperfusion

Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemia>

IPA: /ɪˈskiːmiə/

Phonetic Spelling: ih-skee-mee-uh

Reperfusion

Pronunciation link: <https://www.merriam-webster.com/dictionary/reperfusion>

IPA: /ˌriːpərˈfjuːʒən/

Phonetic Spelling: ree-per-fyoo-zuhn

Ex Vivo

Pronunciation link: <https://www.merriam-webster.com/dictionary/ex%20vivo>

IPA: /ˌɛks ˈviːvoʊ/

Phonetic Spelling: eks vee-voh

Ischemic

Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemic>

IPA: /ɪˈskiːmɪk/

Phonetic Spelling: ih-skee-mik

Perioperative

Pronunciation link: <https://www.merriam-webster.com/dictionary/perioperative>

IPA: /ˌper.iˈɑːp(ə)rətɪv/

Phonetic Spelling: peh-ree-op-uh-ruh-tiv

Catheterization

Pronunciation link: <https://www.merriam-webster.com/dictionary/catheterization>

IPA: /ˌkæθətəˈreɪʒən/

Phonetic Spelling: kath-uh-tuh-ruh-zay-shuhn

Subcutaneous

Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>

IPA: /ˌsʌbkjuːˈteɪniəs/

Phonetic Spelling: sub-kyoo-tay-nee-uhs

Jugular

Pronunciation link: <https://www.merriam-webster.com/dictionary/jugular>

IPA: /ˈdʒʌgjələr/

Phonetic Spelling: jug-yuh-ler

Trendelenburg

Pronunciation link: <https://www.merriam-webster.com/dictionary/Trendelenburg>

IPA: /trɛnˈdɛlənbʊrg/

Phonetic Spelling: tren-del-uhn-burg

Seldinger

Pronunciation link: <https://www.merriam-webster.com/dictionary/Seldinger>

IPA: /ˈsɛldɪŋər/

Phonetic Spelling: sel-ding-er

Laparotomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/laparotomy>

IPA: /ˌlæpəˈrɑːtəmi/

Phonetic Spelling: lap-uh-raa-tuh-mee

Prolene

Pronunciation link: <https://www.merriam-webster.com/dictionary/Prolene>

IPA: /ˈproʊliːn/

Phonetic Spelling: proh-leen

Retroperitoneum

Pronunciation link: <https://www.merriam-webster.com/dictionary/retroperitoneum>

IPA: /ˌrɛtroʊˌpɛrɪtəˈniːəm/

Phonetic Spelling: reh-troh-peh-ruh-tuh-nee-uhm

Aortic

Pronunciation link: <https://www.merriam-webster.com/dictionary/aortic>

IPA: /eɪˈɔːrtɪk/

Phonetic Spelling: ay-or-tik

Adrenal

Pronunciation link: <https://www.merriam-webster.com/dictionary/adrenal>

IPA: /əˈdriːnəl/

Phonetic Spelling: uh-dree-nuhl

Cannulate

Pronunciation link: <https://www.merriam-webster.com/dictionary/cannulate>

IPA: /ˈkæn.jəˌleɪt/

Phonetic Spelling: kan-yuh-layt

Hypothermic

Pronunciation link: <https://www.merriam-webster.com/dictionary/hypothermic>

IPA: /ˌhaɪpəˈθɜːrmɪk/

Phonetic Spelling: hy-puh-thur-mik

Anuria

Pronunciation link: <https://www.merriam-webster.com/dictionary/anuria>

IPA: /ænˈjʊriə/

Phonetic Spelling: an·yoor·ee·uh

Electrocardiogram

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrocardiogram>

IPA: /ɪˌlektroʊˈkɑːrdiəˌgræm/

Phonetic Spelling: ih·lek·troh·kar·dee·uh·gram

Hyperkalemia

Pronunciation link: <https://www.merriam-webster.com/dictionary/hyperkalemia>

IPA: /ˌhaɪpərˈkæˈliːmiə/

Phonetic Spelling: hy·per·kuh·lee·mee·uh