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Title: Kidney Procurement in a Preclinical Large Animal Model

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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? **No**

Current Protocol Length
Number of Steps: 18
Number of Shots: 40

Introduction

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

1.1. **Gerald Brandacher:** Our research aims to develop transformative new tissue and organ preservation techniques, such as using nature-inspired next-generation cryoprotective agents that allow for ice-free preservation of organs at subzero temperatures.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.14.2, 2.14.3.*

What technologies are currently used to advance research in your field?

1.2. **Byoung Chol Oh:** Innovative microsurgical small and large animal models are utilized as translational platforms to advance organ and tissue preservation, immune modulation, and immune monitoring.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1.*

What are the current experimental challenges?

1.3. **Siavash Khaki:** Technological advancements are currently transforming the field of transplantation. However, there are remaining challenges in establishing clinically relevant large animal settings to test these technologies in vivo.

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

How will your findings advance research in your field?

1.4. **Amanda Loftin:** By extending preservation capabilities from hours to several days, this research has the potential to alleviate one of the most daunting challenges in transplantation—the extremely limited time for organ storage—while also increasing the number of organs available for transplant.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 2.*

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

Ethics Title Card

This research has been approved by the Institutional Care and Use Committee of Johns Hopkins University~~, a United States Department of Agriculture (USDA) licensed, Office of Laboratory Animal Welfare (OLAW) assured, and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited institution~~

Protocol

2. Kidney and Blood Harvest Procedure

Demonstrator: Gerald Brandacher, Byoung Chol Oh, Eleni Drivas, and Siavash Khaki

NOTE: Authors did not provide files for 2.1-2.5 and requested the protocol start from 2.6

~~2.1. To begin, use clippers to remove the animal's hair from the surgical site and surrounding area **[1-TXT]**.~~

~~2.1.1. WIDE: Talent using clippers to shave the surgical site and surrounding area. **TXT: See text for sedation and anesthesia details**~~

~~2.2. Transfer the animal to the operating table **[1]**. Administer cefazolin intravenously at a dose of 20 to 22 milligrams per kilogram 10 minutes prior to the start of surgery **[2-TXT]**. Then, administer pantoprazole intravenously at a dose of 0.5 to 1 milligram per kilogram at the start of surgery **[3]**.~~

~~2.2.1. Talent placing the animal on the operating table.~~

~~2.2.2. Talent preparing and administering cefazolin intravenously. **TXT: Administer cefazolin every 90 min intraoperatively**~~

~~2.2.3. Talent preparing and administering pantoprazole intravenously.~~

~~2.3. Aseptically prepare the surgical site by alternating between chlorhexidine or betadine and 70 percent ethanol or saline, at least three times **[1]**.~~

~~2.3.1. Talent alternating between antiseptic solutions to prep the surgical site.~~

~~2.4. Continuously monitor fluid volume, heart rate, blood pressure, pulse oximetry, capnography, electrocardiography, and rectal temperature **[1-TXT]**. Use a heated underbody pad and warm-air blanket to prevent hypothermia **[2]**.~~

~~2.4.1. SCREEN: Display of vital signs on the monitoring equipment. **TXT: Record these values every 10 – 15 min**~~

~~2.4.2. Talent recording monitored values in a log sheet or software. *Videographer: If the values are recorded on a computer, please make sure the computer screen is clearly visible in the frame.*~~

~~2.4.3. Talent placing a heated underbody pad and warm-air blanket over the animal.~~

~~2.5. Prior to the initiation of surgery, confirm that the animal is within the appropriate plane of surgical anesthesia by assessing jaw tone, palpebral reflex, and the monitored parameters **[1].**~~

~~2.5.1. Talent checking jaw tone and palpebral reflex on the animal.~~

2.6. To begin, perform a median laparotomy on a surgically prepped anesthetized animal,incising about 25 to 30 centimeters to gain optimal access to both kidneys **[1-TXT]**. Insert a standard abdominal retractor **[2]**.

2.6.1. Talent making a midline incision. **TXT: Analgesia: 2 mg/kg lidocaine**
AND
TEXT ON PLAIN BACKGROUND:
Animal preparation:
1. Cefazolin (i.v) : 20 - 22 mg/kg 10 mins before surgery, Pantoprazole (i.v): 0.5 - 1 mg/kg at start of surgery

2. Swab site with chlorhexidine/ betadine and 70 percent ethanol (3x)

3. Continuous monitoring of fluid volume, heart rate, blood pressure, pulse oximetry, capnography, electrocardiography, and rectal temperature

Video Editor: Please play both shots side by side in a split screen

2.6.2. Talent inserting an abdominal retractor.

2.7. Cover the colon and small bowel with towels soaked in warm saline **[1]**. Retract the bowels to the right for access to the left kidney or to the left for access to the right kidney **[2]**.

2.7.1. Talent placing warm saline-soaked towels over the colon and small bowel of the animal.

2.7.2. Talent retracting the bowels to expose the kidney.

2.8. Open the peritoneum overlying the kidney **[1]** and dissect around the kidney to free any adhesions **[2]**. Dissect the ureter **[3]** until 10 to 12 centimeters of length is obtained **[4]**.

2.8.1. Talent making an incision in the peritoneum.

2.8.2. Talent carefully dissecting around the kidney.

2.8.3. Talent dissecting the ureter.

2.8.4. Final dissected ureter.

2.9. Next, dissect the renal vein until its origin from the inferior vena cava is exposed **[1]**.

Similarly, dissect the artery until its origin from the aorta is exposed **[2]**.

2.9.1. Talent dissecting the renal vein until its origin from the inferior vena cava is exposed.

2.9.2. Talent dissecting the artery until its origin from the aorta.

2.10. After complete renal dissection, tie the ureter distally with a 2-0 *(two-zero)* silk ligature **[1]**. Cut proximally to the tie, leaving the proximal ureter end open for urine drainage **[2]**.

2.10.1. Talent placing a ligature around the ureter.

2.10.2. Talent cutting the ureter proximally to the tie.

2.11. Administer 100 International Units per kilogram of heparin intravenously and wait for 2 minutes to ensure adequate heparinization of the kidney **[1-TXT]**.

2.11.1. Talent injecting heparin intravenously. **TXT: Repeat this step prior to the resection of each kidney**

2.12. Clamp the renal artery close to the aorta using Satinsky vascular clamps **[1]**. Similarly, clamp the renal vein close to the inferior vena cava **[2]**. Remove the kidney graft by cutting the renal artery and vein close to the clamps **[3]**.

2.12.1. Talent placing vascular clamp on the renal artery close to the aorta.

2.12.2. Talent placing vascular clamp on the renal vein close to the inferior vena cava.

2.12.3. Talent removing the kidney graft by cutting the renal artery and vein close to the clamps.

2.13. ~~Immediately cannulate the renal artery with a 3-millimeter blunt tip perfusion cannula **[1]**.~~ Flush the kidney with ice-cold University of Wisconsin or UW *(U-W)* solution or Custodiol Histidine-tryptophan-ketoglutarate or HTK *(H-T-K)* preservation solution **[2]**.

2.13.1. ~~Talent inserting the blunt tip perfusion cannula into the renal artery.~~
NOTE: Shot not provided by authors

2.13.2. Talent flushing the kidney with preservation solution.

2.14. Remove the perfusion cannula **[1]** and place the kidney in a sterile organ bag filled with the same ice-cold preservation solution used for flushing **[2]**. Place the bag within a second sterile organ bag **[3-TXT]**.

2.14.1. Talent removing the perfusion cannula.

2.14.2. Talent placing the kidney in a sterile organ bag filled with preservation solution.

2.14.3. Talent placing the first organ bag with the kidney inside a second sterile organ bag. **TXT: Store the organ or initiate machine perfusion**

2.15. Ligate the renal artery stump with a 2-0 *(two-zero)* silk ligature **[1]** and close the renal vein stump using a two-layer running suture with 6-0 polypropylene **[2]**.

2.15.1. Talent ligating the renal artery stump.

2.15.2. Talent suturing the renal vein stump using a running suture technique.

~~**3. Blood Harvest Procedure**~~

3.1. To harvest blood for machine perfusion~~, retract the bowels to the right side after euthanizing the animal **[1-TXT]** and~~ identify the infrarenal abdominal aorta **[2]**. Free any large adhesions or tissue covering the vessel **[3]**.

3.1.1. ~~Talent retracting the bowels to access the infrarenal abdominal aorta. **TXT: Euthanasia: Exsanguination; See text for details**~~
NOTE: Shot not provided by authors

3.1.2. The infrarenal abdominal aorta.

3.1.3. Talent dissecting adhesions and clearing tissue covering the vessel.

3.2. Insert the blood collection bag needle directly into the aorta **[1]**. Hang the bag below the animal to facilitate filling **[2]**. ~~Once the bag is full, remove the needle from the aorta **[3]** and apply pressure to the puncture site **[4]**.~~

3.2.1. Talent inserting the blood collection needle into the aorta.

3.2.2. Blood bag hanging below the animal, filling with blood.

3.2.3. ~~Talent removing the needle from the aorta after blood collection.~~
NOTE: Shot not provided by authors

3.2.4. ~~Talent applying pressure to the puncture site.~~
NOTE: Shot not provided by authors

~~3.3. For a second blood bag, insert a new needle 1 to 2 centimeters proximally to the previous puncture site **[1-TXT]**.~~

~~3.3.1. Talent inserting a second needle proximally to the previous puncture site. **TXT: Repeat with additional blood bags advancing each needle 1 – 2 cm proximally**~~
NOTE: Shot not provided by authors

Results

4. Representative Results

4.1. Kidneys were successfully retrieved for transplantation, machine perfusion, and primary cell culture experiments **[1]**. Notably, successful kidney retrieval was achieved in all experimental groups, implying no complications with this surgical procedure model **[2]**.

4.1.1. LAB MEDIA: Figure 1. *Video Editor: Highlight the blue bar when the VO says, "transplantation", green bar when the VO says "machine perfusion", and grey bar when the VO says, "primary cell culture experiments".*

4.1.2. LAB MEDIA: Figure 1. *Video Editor: Highlight 100% on the y-axis.*

4.2. The gross appearance of kidney grafts varied at different stages, as described in this figure **[1]**. The native kidney appeared deep red and vascularized **[2]**. The kidney graft, after flushing with ice-cold preservation solution, showed a pale and swollen appearance **[3]**.

4.2.1. LAB MEDIA: Figure 2.

4.2.2. LAB MEDIA: Figure 2. *Video Editor: Highlight A.*

4.2.3. LAB MEDIA: Figure 2. *Video Editor: Highlight B.*

4.3. After machine reperfusion, the kidney graft regained a pinkish hue with visible vascular flow **[1]**. After transplantation and reperfusion, it appeared darker with visible anastomosed vessels **[2].**

4.3.1. LAB MEDIA: Figure 2. *Video Editor: Highlight C.*

4.3.2. LAB MEDIA: Figure 2. *Video Editor: Highlight D.*

4.4. ~~A summary of anatomic variations found in pig kidneys is shown in this table **[1].**~~ 64% of kidneys exhibit standard anatomy of one renal artery and one renal vein **[2].** The most common variations included one artery with two veins **[3],** one artery with three veins **[4],** two arteries with one vein **[5],** and two arteries with two veins **[6].**

~~4.4.1. LAB MEDIA: Table 2.~~

NOTE: Shot removed to prevent redundancy

4.4.2. LAB MEDIA: Table 2. *Video Editor: Highlight "1 Artery, 1 Vein" row.*

4.4.3. LAB MEDIA: Table 2. *Video Editor: Highlight "1 Artery, 2 Veins" row when the VO says, "one artery with two veins".*

4.4.4. LAB MEDIA: Table 2. *Video Editor: Highlight "1 Artery, 3 Veins" row.*

4.4.5. LAB MEDIA: Table 2. *Video Editor: Highlight "2 Artery, 1 Veins" row.*

4.4.6. LAB MEDIA: Table 2. *Video Editor: Highlight "2 Artery, 3 Veins" row.*

4.5. Bilaterally typical anatomy was observed in 46% of pigs **[1]**, while 22% had one atypical kidney on the left **[2],** 14% on the right **[3]**, and 19% had both kidneys atypical **[4].**

4.5.1. LAB MEDIA: Table 2. *Video Editor: Highlight the table at the top (Bilateral Kidney Anatomy table).*

4.5.2. LAB MEDIA: Table 2. *Video Editor: Highlight Right Typical, Left Atypical.*

4.5.3. LAB MEDIA: Table 2. *Video Editor: Highlight Left Typical, Right Atypical row.*

4.5.4. LAB MEDIA: Table 2. *Video Editor: Highlight "Both Atypical" row.*

Pronunciation Guide:

 Laparotomy
Pronunciation link: https://dictionary.cambridge.org/pronunciation/english/laparotomy Cambridge Dictionary+1
IPA: /ˌlæpəˈrɑt̬əmi/
Phonetic: *lap-uh-ROH-tuh-mee*
 Median (as in "median laparotomy")
No confirmed link found
IPA: /ˈmiːdiən/
Phonetic: *MEE-dee-uhn*
 Retract / Retraction
No confirmed link found
IPA: /rɪˈtrækt/, /rɪˈtrækʃən/
Phonetic: *ri-TRAKT*, *ri-TRAK-shuhn*
 Peritoneum
No confirmed link found
IPA: /ˌpɛrɪˈtoʊniəm/
Phonetic: *per-ih-TOH-nee-uhm*
 Ureter
No confirmed link found
IPA: /jʊˈriːtər/
Phonetic: *yoo-REE-ter*
 Inferior vena cava
- *Inferior* — no confirmed link
  IPA: /ɪnˈfɪriər/
  Phonetic: *in-FEER-ee-er*
- *Vena cava* — "vena" /ˈviːnə/, "cava" /ˈkɑvə/
  Phonetic: *VEE-nuh KAH-vuh*

 Heparinization
No confirmed link found
IPA: /hɛpəˌrɪnˈeɪʃən/
Phonetic: *hep-uh-rih-NAY-shuhn*
 Satinsky (as in Satinsky vascular clamps)
No confirmed link found
IPA (approx.): /səˈtɪnski/
Phonetic: *suh-TIN-skee*
 Cannulate / Cannulation
No confirmed link found
IPA: /ˈkæn.jəˌleɪt/ (verb), /ˌkæn.jəˈleɪʃən/ (noun)
Phonetic: *KAN-yuh-layt*, *kan-yuh-LAY-shuhn*
 Perfusion
No confirmed link found

IPA: /pərˈfjuːʒən/
Phonetic: *per-FYOO-zhuhn*

Custodiol / Histidine-tryptophan-ketoglutarate (HTK)

- Custodiol — no confirmed link
  IPA (approx): /kʌsˈtoʊdiˌoʊl/
  Phonetic: *kus-TOH-dee-ohl*
- Histidine — no confirmed link
  IPA: /ˈhɪstɪˌdiːn/
  Phonetic: *HIS-tih-deen*
- Tryptophan — no confirmed link
  IPA: /ˈtrɪptəˌfæn/
  Phonetic: *TRIP-toh-fan*
- Ketoglutarate — no confirmed link
  IPA (approx): /ˌkiːtoʊˈgluːtərəˌteɪt/
  Phonetic: *kee-toh-GLOO-tuh-rayt*

Ligature / Ligating

No confirmed link found
IPA: /ˈlɪgətʃər/ (ligature), /ˈlɪgeɪtɪŋ/ (ligating)
Phonetic: *LIG-uh-chur*, *LIG-ay-ting*

Polypropylene

No confirmed link found
IPA: /ˌpɑliˈprɑpəliːn/
Phonetic: *pol-ee-PROP-uh-leen*

Exsanguination

No confirmed link found
IPA: /ˌɛksˌsæŋgwɪˈneɪʃən/
Phonetic: *eks-SANG-gwi-NAY-shuhn*

Capnography

Pronunciation link: https://forvo.com/word/capnography/ Forvo.com
Merriam-Webster gives a related form *capnograph* and *capnographic*, from which *capnography* is derived: /kap-ˈnä-grə-fē/ Merriam-Webster
IPA: /kæpˈnɑgrəfi/
Phonetic: *kap-NAH-gruh-fee*