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# Title: A High-Fidelity Porcine Model of Orthotopic Heart Transplantation Following Donation after Circulatory Death

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# Author Questionnaire

**1.** We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

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

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

**4. Proposed filming date:** To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **MM/DD/YYY**

When you are ready to submit your video LAB MEDIAs, please contact our Content Manager, Utkarsh Khare.

**Current Protocol Length**

Number of Steps: 16
Number of Shots: 32

# Introduction

1.1. **Jeffrey Keenan:** We present a high-fidelity porcine model of heart transplantation after circulatory death, enabling evaluation of DCD-related pathophysiology and supporting translational research to improve allograft recovery.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.2*

What are the most recent developments in your field of research?

1.2. **Abigail Benkert:** Since 2019, clinical adoption of DCD heart transplantation has grown in the U.S., with recent trials showing comparable non-inferior short-term survival to conventional brain death donation methods.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

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

1.3. **Abigail Benkert:** Outcomes after DCD heart transplantation have further been improved by advances in allograft reperfusion methods, including direct procurement and perfusion using normothermic ex-vivo perfusion devices and normothermic regional perfusion.

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

What research gap are you addressing with your protocol?

1.4. **Krish Dewan:** Primary graft dysfunction remains a primary driver of short-term mortality after DCD heart transplantation. While warm ischemic injury and metabolic derangements are proposed contributing factors, the underlying molecular mechanisms behind PGD remain unknown.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.10*

What advantage does your protocol offer compared to other techniques?

1.5. **Krish Dewan:** The porcine model replicates clinical DCD procedures with adjustments for species-specific anatomy, while ex-vivo perfusion supports development of targeted therapies to enhance allograft preservation and function.

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

**Ethics Title Card**

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

# Protocol

**NOTE: The protocol section was drafted from available footage**

**2. Sequential Workflow for Ex Vivo Heart Perfusion and Transplant Setup in Swine Models**

**Demonstrator:**

2.1. To begin, expose the donor heart, measure the length of the solid-state pressure-volume catheter from the cannulation site to the apex of the swine heart **[1]**. Introduce the catheter sequentially into the carotid artery and internal jugular vein **[2-TXT]**.

2.1.1. LAB MEDIA: 7Z7A0522: 00:00-00:06
*Video Editor: Please slow down the video to match the VO*

2.1.2. LAB MEDIA: 7Z7A0522: 00:42-00:48, 01:09-01:12 and 7Z7A0528: 00:00-00:04 **TXT: For left ventricular measurement, insert into arterial sheath and guide into left ventricle**

2.2. Prepare the donor heart for explant by cannulating the aortic root with a 7-French vent, securing it with a Rumel tourniquet, and placing a purse-string stitch in the right atrial appendage **[1]**.

2.2.1. LAB MEDIA: 7Z7A0535 00:00-00:17 **TXT: Flush and de-air the cardioplegia tubing before attaching to aortic root cannula**
*Video editor: Please slow down the video*

2.3. Cease mechanical ventilation to initiate the controlled circulatory death process. The heart may distend **[1-TXT]**. Wait for 10 minutes after confirmed death, defined by pulseless electrical activity and no signs of life, before proceeding **[2]**. Slow pulseless electrical activity persists in swine longer than in humans, resulting in increased allograft ischemic insult **[3].**

2.3.1. LAB MEDIA: 7Z7A0540 00:35-00:51 **TXT: Agonal phase starts when SBP < 50 mm Hg**

2.3.2. LAB MEDIA: 7Z7A0542 00:30-00:41, 00:44-00:47

2.3.3. LAB MEDIA: 20240606_103015 02:05-02:16

2.4. For ex-vivo perfusion device priming, use a number 11 blade to make a stab incision into the right atrial appendage and cannulate with the 34 French venous cannula **[1]**. Secure the cannula with a Rumel tourniquet and connect it to the ex vivo perfusion device's collection bag **[2]**. Collect approximately 1200 to 1500 milliliters of donor blood into the manufacturer-provided collection bag **[3]**.

2.4.1. LAB MEDIA: 7Z7A0543 00:23-00:32

2.4.2. LAB MEDIA: 7Z7A0543 2:16-2:26
2.4.3. LAB MEDIA: 7Z7A0543 2:44-2:53

2.5. Following collection of the donor blood, apply the aortic cross-clamp **[1]**.
2.5.1. LAB MEDIA: 7Z7A0544 00:12-00:17

2.6. Administer 1 liter of Del Nido cardioplegia into the aortic root, targeting a pressure of 60 to 100 millimeters of mercury **[1]**. Transect the inferior vena cava and left atrial appendage to vent both ventricles during cardioplegia delivery **[2]**.
2.6.1. LAB MEDIA: 7Z7A0545 00:02-00:10
2.6.2. LAB MEDIA: 7Z7A0544 00:42-00:55

2.7. Divide the inferior vena cava, superior vena cava, aorta just distal to the innominate artery, and pulmonary artery at the bifurcation. Then transect the donor left atrium, ensuring a sufficient cuff remains **[1]**.
2.7.1. LAB MEDIA: 7Z7A0547 00:18-00:24, 00:45-00:50, 01:14-01:23, 01:34-01:38, 03:31-04:43

2.8. Remove the heart and place it in a basin filled with cold sterile slush for back-table preparation **[1]**.
2.8.1. LAB MEDIA: 7Z7A0547 04:17-04:20
*Video Editor: Please slow down the video*

2.9. Place four equidistant pledgeted horizontal mattress sutures using 4-0 *(Four-oh)* prolene suture inside the distal end of the aorta **[1]**. Then insert the *ex vivo* perfusion aortic adapter and secure with 0-0 *(oh)* silk suture or umbilical tape **[2]**.
2.9.1. LAB MEDIA: 7Z7A0548 00:58-01:21
2.9.2. LAB MEDIA: 20240606_104529 14:20-14:42

2.10. Transport the allograft to the ex vivo perfusion device and connect the adapter **[1]**. Position the heart with the posterior side facing up and obtain perfusate samples, bloodwork, and biopsy samples during this stage **[2-TXT]**. In this model, the allograft remained on the ex vivo perfusion device at 34 degrees Celsius for 2 to 3 hours before implantation **[3]**.

2.10.1. LAB MEDIA: 7Z7A0551 00:07-00:28
2.10.2. LAB MEDIA: 7Z7A0556 00:02-00:13 **TXT: Keep anterior surface in contact with device and defibrillation pads; Maintain temperature at 34 °C**
2.10.3. LAB MEDIA: 7Z7A0552 00:06-00:15

2.11. After preparing the heart for recipient cardiectomy, secure it with Rumel tourniquets and connect to the venous limb of the circuit **[1]**.

2.11.1. LAB MEDIA: 7Z7A0553 00:00-00:16

2.12. Apply the aortic cross-clamp proximal to the cannula and explant the recipient heart. Leave intact atrial cuffs for bi-atrial implantation **[1]**. Transect the aorta and pulmonary artery close to the root and place the explanted heart in cold PBS for laboratory processing **[2]**.
2.12.1. LAB MEDIA: 20240606_132254 02:30-02:48
2.12.2. LAB MEDIA: 7Z7A0555 00:00-00:21

2.13. Once the recipient's native heart has been explanted, begin bi-atrial implantation of the donor allograft and initiate the left atrial anastomosis using 4-0 *(four-oh)* prolene suture **[1]**, then complete the pulmonary artery **[2]**, aortic and right atrial anastomoses with continuous 4-0 prolene suture **[3]**.
2.13.1. LAB MEDIA: 7Z7A0571 01:26-02:00
2.13.2. LAB MEDIA: 7Z7A0574 01:37-01:45

2.13.3. LAB MEDIA: 7Z7A0576 01:16-01:25

2.14. After completing all anastomoses, release the aortic cross-clamp to reperfuse the allograft **[1]**. Ensure hemostasis of all anastomoses. After 60 minutes of reperfusion, attempt to wean the recipient from cardiopulmonary bypass **[2]**. The recipient animal was supported for 1 hour post separation from cardiopulmonary bypass **[3]**.
2.14.1. LAB MEDIA: 20240606_141754 13:43-13:49
*Video Editor: Please slow down the video*
2.14.2. LAB MEDIA: 7Z7A0584 00:02-00:19
2.14.3. LAB MEDIA: 7Z7A0587 00:09-00:14

# Results

## 3. Representative Results

3.1. In the DCD procurement phase, the elapsed time from cessation of life-sustaining measures in the donor pig to the declaration of death was approximately 14.25 minutes **[1-TXT]**. Operative cardiopulmonary bypass time in the recipient animals was around 3 hours with a cross-clamp time of on average 1.5 hours **[2]**.

3.1.1. LAB MEDIA: Table 1 **TXT: DCD: Donation after circulatory death**
*Video Editor: Please highlight row labeled " Time from cessation…."*

3.1.2. LAB MEDIA: Table 1 *Video Editor: Please highlight row labeled " CPB duration" and "Cross-clamp time"*

3.2. Post-transplant echocardiography using standard views revealed varying graft function, ranging from mild dysfunction with stable hemodynamics to severe dysfunction with instability **[1]**.

3.2.1. LAB MEDIA: Figure 3. *Video editor: Please sequentially emphasize the images from A to C.*

**Pronunciation Guide:**

**1. Porcine**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /ˈpɔːr.saɪn/
- **Phonetic Spelling**: POR-sinemerriam-webster.com+2merriam-webster.com+2merriam-webster.com+2

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**2. Orthotopic**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /ˌɔːr.θəˈtɒp.ɪk/
- **Phonetic Spelling**: or-thuh-TOP-ik

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**3. Cardiectomy**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /ˌkɑːr.diˈek.tə.mi/
- **Phonetic Spelling**: kar-dee-EK-tuh-meemerriam-webster.com

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**4. Anastomosis**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /əˌnæs.təˈmoʊ.sɪs/
- **Phonetic Spelling**: uh-NAS-tuh-MOH-sismerriam-webster.com

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**5. Cardioplegia**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /ˌkɑːr.di.oʊˈpliː.dʒə/
- **Phonetic Spelling**: kar-dee-oh-PLEE-juhmerriam-webster.com+1merriam-webster.com+1

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**6. Ischemic**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /ɪˈskiː.mɪk/
- **Phonetic Spelling**: ih-SKEE-mik

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**7. Perfusion**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /pərˈfjuː.ʒən/
- **Phonetic Spelling**: per-FYOO-zhun

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**8. Allograft**

- **Pronunciation link**: merriam-webster.com
- **IPA**: /ˈæl.əˌgræft/
- **Phonetic Spelling**: AL-uh-graftmerriam-webster.com+5merriam-webster.com+5merriam-webster.com+5

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**9. Biopsy**

- **Pronunciation link**: merriam-webster.com

- **IPA**: /ˈbaɪ.ɒp.si/
- **Phonetic Spelling**: BY-op-see