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Title: Liver Cold Storage and Transplantation in the Cold-Adaptive Daurian Ground Squirrels

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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? **Yes, all done**

   All shots except 2.1.1.

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

3. . **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: **30/07/2025**

When you are ready to submit your video files, please contact our China Location Producer, Yuan Yue.

Current Protocol Length
Number of Steps: 26
Number of Shots: 58 (57 Scope)

Introduction

1.1. **Zhishui Chen:** We study cold-adapted mammals like Daurian ground squirrels to create better organ preservation methods. Our goal is to understand their natural cold tolerance and improve human organ transplantation.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1* **SCOPE: 68444-** Zhishui Chen

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

~~1.2. **Ou Jingxing:** The most recent developments in the field of organ preservation and transplantation include machine perfusion and xenotransplantation. Yet how organ cold adaptation can be achieved remains understudied.~~

~~1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1* **SCOPE: 68444-** Jingxing Ou~~ **NOTE**: 1.2 is rejected due to heavy echo

What research questions will your laboratory focus on in the future?

1.3. **Bo Yang:** Our future work will explore how hibernators survive extreme cold and stress, and use that knowledge to improve organ preservation, extend storage time, and make transplants more successful.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.1* **SCOPE: 68444-** Bo Yang

Ethics Title Card

This research has been approved by the Peking University Institutional Committee for Animal Care and Use

Protocol

2. Donor Operation

Demonstrator: Zipei Wang

2.1. To begin, make the required incision extending from the xiphoid process to the pubic symphysis on the anesthetized animal **[1-TXT].**

2.1.1. SCOPE: 68444- 1 .mp4 01:50 – 02:00. **TXT: Anesthesia: Isoflurane; (Induction: 5%; Maintenance: 2%)**

2.2. Use cotton swabs to carefully free the infrahepatic inferior vena cava above the right renal vein from the surrounding tissues of the animal **[1]**. Then, ligate the right adrenal and lumbar veins draining into the inferior vena cava near the liver using 7-0 sutures **[2]**.

2.2.1. SCOPE: 68444- 1 .mp4 07:45 – 07:49

2.2.2. SCOPE: 68444- 1 .mp4 09:04 – 09:10

2.3. Isolate the proper hepatic artery, gastroduodenal artery, and common hepatic artery from the surrounding tissues using micro-forceps **[1-TXT]**.

2.3.1. SCOPE: 68444- 1 .mp4 15:36 – 15:40, 16:49 – 16:52， SCOPE: 68444- 2 .mp4 00:20 – 00:24

TXT: Administer 1.5 mL of heparinized saline (300 IU/mL)

Site: Penile vein (male donors) or the IHIVC (female donors)

3. Donor Liver Perfusion

3.1. Insert a 7-gauge intravenous needle into the aorta and secure it with a clamp **[1]**. Then, incise the diaphragm to expose the thoracic aorta **[2]** and place a vascular clamp on the thoracic aorta **[3]**.

3.1.1. SCOPE: 68444- 2 .mp4 05:45 – 06:02

3.1.2. SCOPE: 68444- 2 .mp4 06:16 – 06:20

3.1.3. SCOPE: 68444- 2 .mp4 06:25 – 06:35

3.2. ~~Perfuse the liver via the aortic cannula with 20 to 30 milliliters of cold University of Wisconsin solution at a flow rate of 180 to 210 milliliters per hour using an infusion pump **[1]**~~. After perfusing the liver, incise the intrathoracic vena cava and the infrahepatic inferior vena cava above the right renal vein to allow the perfusate to drain out **[2]**. During the perfusion, excise the gallbladder using surgical scissors **[3]**.

3.2.1. ~~SCOPE: setting the infusion pump and perfusing cold University of Wisconsin solution through the aortic cannula~~. **NOTE**: Not provided, VO altered

3.2.2. SCOPE: 68444- 2 .mp4 06:40 – 06:45, 07:30 – 07:40

3.2.3. SCOPE: 68444- 2 .mp4 07:40 – 07:52

3.3. Make a small incision on the anterior wall of the common bile duct **[1]** and insert the bile duct stent into the duct while securing it with a 7-0 silk suture **[2]**.

3.3.1. SCOPE: 68444- 2 .mp4 14:18 – 14:30

3.3.2. SCOPE: 68444- 2 .mp4 15:02 – 15:07 and 15:35 – 15:43.

3.4. Now, using micro-forceps, isolate the portal vein from the surrounding connective tissue **[1]**. Ligate and transect the pyloric vein with a 7-0 suture **[2]** and transect the portal vein trunk at the level of the splenic vein **[3]**.

3.4.1. SCOPE: 68444- 2 .mp4 16:12 – 16:35

3.4.2. SCOPE: 68444- 2 .mp4 19:48 – 20:00

3.5. Ligate and transect the gastroduodenal artery distally **[1]** and create a small incision on the anterior wall of the common hepatic artery **[2]**. Insert the hepatic artery stent into the common hepatic artery and secure it with a 7-0 silk suture **[3]**.

3.5.1. SCOPE: 68444- 3 .mp4 02:15 – 02:23

3.5.2. 68444 3 .mp4 02:45 – 02:50，03:22-03:28

3.6. Isolate the left diaphragmatic vein from the suprahepatic inferior vena cava and ligate it with a 7-0 *(7-Oh)* suture **[1]**

3.6.1. SCOPE: 68444- 2 .mp4 10:58 – 11:05, 11:30 – 11:40,

3.7. Dissect the suprahepatic inferior vena cava as close to the diaphragm as possible using fine surgical instruments **[1]**. Lagating the hepato-splenic ligament using a 7-0 suture **[2-TXT]**.

3.7.1. SCOPE: 68444- 2 .mp4 12:40 – 12:44

3.7.2. SCOPE: 68444- 3 .mp4 05:18 – 05:20 and 05:40-05:45 **TXT: Place the liver in cold University of Wisconsin solution**

~~3.8. Place the liver into a 7-centimeter steel bowl containing cold University of Wisconsin solution to store at 4 degrees Celsius **[1].**~~

3.8.1. ~~SCOPE: placing the liver into a cold solution-filled steel bowl~~. **NOTE**: Not provided, VO moved as on screen text

4. Ex Vivo Graft Preparation

4.1. Secure the handle of the portal vein cuff with a vascular clamp and stabilize it using mosquito forceps **[1]**. Carefully thread the portal vein through the cuff, ensuring it is not twisted **[2]** and fold the portal vein over the cuff and secure it in place with a 7-0 suture **[3]**.

4.1.1. SCOPE: 68444- 03 .mp4 17:43 – 17:50

4.1.2. SCOPE: 68444- 03 .mp4 17:50 – 17:55

4.1.3. SCOPE: 68444- 03 .mp4 18:15 – 18:22, 20:10 – 20:18

4.2. Ligate the cystic duct securely **[1-TXT]**.

4.2.1. SCOPE: 68444- 04 .mp4 04:00 – 04:05, 04:10 – 04:13, 04:28 – 04:33 **TXT: Flush the liver graft with 8 - 10 mL saline through the portal vein cuff**

~~4.3. Flush the liver graft with 8 to 10 milliliters of cold saline through the portal vein cuff to remove residual University of Wisconsin solution after cold storage **[1-TXT].**~~

4.3.1. ~~SCOPE: flushing cold saline through the portal vein cuff into the liver graft. **TXT: Perform this step just before anhepatic phase (5.3).**~~ （DELATE） **NOTE**: Not provided, VO moved as on screen text

4.4. Place a single 7-0 silk suture around the infrahepatic inferior vena cava between the attached cuff and the right inferior lobe of the liver **[1]**.

4.4.1. SCOPE: 68444- 07 .mp4 08:57 – 09:02

4.5. Insert 8-0 *(8-oh)* stay sutures at both lateral corners of the suprahepatic inferior vena cava from the exterior to the interior **[1]**.

4.5.1. SCOPE: 68444- 07 .mp4 13:01 – 13:06, 13:37 – 13:43

5. Recipient Operation for Graft Installation

5.1. Carefully pass a blunt forceps behind the supra-hepatic inferior vena cava and place a 7-0 *(7-oh)* silk suture around it **[1]**. Ligate the common bile duct proximally with a 7-0 silk suture and transect it above the ligature **[2]**. ~~Use a cotton-tip applicator to gently separate the bile duct from the portal vein **[3]**.~~

5.1.1. SCOPE: 68444- 09 .mp4 07:25 – 07:45

5.1.2. SCOPE: 68444- 08 .mp4 14:11 – 14:35

~~5.1.3. SCOPE: using a cotton tip to separate the bile duct from the portal vein.~~ ~~（DELATE）~~ **NOTE**: Not provided, VO moved to next shot in concise format

5.2. After separating the bile duct, transect the gastroduodenal artery and proper hepatic artery between the ligations **[1]**. At the end of the common hepatic artery, form a Y-shaped bifurcation and leave a 2-centimeter length of suture on the proper hepatic artery ligature for later use **[2]**.

5.2.1. SCOPE: 68444- 08 .mp4 17:00 – 17:12, 19:16 – 19:26

5.2.2. SCOPE: 68444- 09 .mp4 00:00 – 00:10,

5.3. Free the right and left branches of the portal vein **[1]**. Then, place 5-centimeter 7-0 stay sutures on each branch to aid in later handling **[2]**.

5.3.1. SCOPE: 68444- 09 .mp4 04:35 – 04:46

5.3.2. SCOPE: 68444- 09 .mp4 05:10 – 05:18, 05:48 – 05:55

5.4. Now, clamp the infrahepatic inferior vena cava above the right renal vein **[1]**, followed by clamping the portal vein above the pyloric vein **[2]**. Inject 1.5 milliliters of saline into the right portal vein within 10 seconds using a 2-milliliter syringe to flush the blood from

the liver **[3]**.

5.4.1. SCOPE: 68444- 09 .mp4 09:22 – 09:28

5.4.2. SCOPE: 68444- 09 .mp4 10:10 – 10:14

5.4.3. SCOPE: 68444- 09 .mp4 11:49 – 12:05

5.5. To retract the diaphragm, gently pull the 7-0 silk suture placed around the suprahepatic inferior vena cava **[1]**. Then, apply a bulldog clamp to the suprahepatic inferior vena cava, including a portion of the diaphragm in the clamp **[2]**.

5.5.1. SCOPE: 68444- 09 .mp4 13:22 – 13:25

5.5.2. SCOPE: 68444- 09 .mp4 13:25 – 13:38

5.6. Next, transect the suprahepatic inferior vena cava just above the liver **[1]**. Secure the 7-0 stay sutures on both portal vein branches and transect the portal vein above these ligatures **[2]**. Then, transect the infrahepatic inferior vena cava close to the liver parenchyma and promptly remove the liver by cutting any remaining ligamentous attachments **[3]**.

5.6.1. SCOPE: 68444- 09 .mp4 13:47 – 14:05

5.6.2. SCOPE: 68444- 09 .mp4 12:25 – 12:30, 12:45 –12:50, 14:49 –14:51

5.6.3. SCOPE: 68444- 09 .mp4 14:58 – 15:05

5.7. Suture the posterior wall with 8 to 10 continuous stitches beginning at the left corner **[1]**. Continue with the anterior wall closure using 10 to 12 stitches moving from right to left with the same suture **[2-TXT]**.

5.7.1. SCOPE: 68444- 09 .mp4 20:10 – 20:18, SCOPE: 68444- 10 .mp4 00:02 – 00:29,

5.7.2. SCOPE: 68444- 10 .mp4 01:05 – 01:13, 04:10 – 04:35 **TXT: Flush the suprahepatic inferior vena cava cavity to remove air**

5.8. For portal vein reconstruction, use the previously placed 5-centimeter stay sutures to gently elevate both portal vein bifurcations by applying upward traction with vessel clamps **[1-TXT]**.

5.8.1. SCOPE: 68444- 10 .mp4 05:50 – 06:10 **TXT: Make an incision at the midpoint of the portal vein branches**

5.9. Flush the portal vein lumen with heparinized saline at a concentration of 300

international units per milliliter **[1]**. Insert the cuff into the portal vein and secure it using a 7-0 silk suture **[2]**. Sequentially release the clamps on the portal vein and the suprahepatic inferior vena cava to reestablish graft perfusion and conclude the anhepatic phase **[3]**.

5.9.1. SCOPE: 68444- 10 .mp4 06:25 – 06:30

5.9.2. SCOPE: 68444- 10 .mp4 08:10 – 08:30

5.9.3. SCOPE: 68444- 10 .mp4 08:50 – 08:54, 09:05 – 09:10

5.10. Then, make a small incision at the bifurcation of the Y-configuration at the terminus of the recipient's common hepatic artery **[1]**. Flush the common hepatic artery lumen with heparinized saline at 300 international units per milliliter **[2]**. Insert the arterial stent into the common hepatic artery and secure it with a 7-0 silk suture **[3]**.

5.10.1. SCOPE: 68444- 11 .mp4 04:07 – 04:15

5.10.2. SCOPE: 68444- 11 .mp4 04:18 – 04:22, 04:43 – 04:48

5.10.3. SCOPE: 68444- 11 .mp4 05:50 – 05:55, 06 :05 – 06:07, 06 :45 – 06:48

5.11. Tie the stay sutures on both the donor and recipient sides to stabilize the stent and prevent its displacement **[1]**. Release the microvascular clip to restore arterial blood flow **[2]**.

5.11.1. SCOPE: 68444- 11 .mp4 06:50 – 07:18

5.11.2. SCOPE: 68444- 11 .mp4 07:58 – 08:05

5.12. Then, make a small incision on the anterior wall of the recipient's common bile duct **[1]**. Insert the bile duct stent into the lumen and secure it using a 7-0 silk suture **[2]**. Finally, tie the stay sutures on both donor and recipient bile ducts to hold the stent securely in place and prevent displacement **[3]**.

5.12.1. SCOPE: 68444- 11 .mp4 12:50 – 13:00

5.12.2. SCOPE: 68444- 11 .mp4 13:40 – 13:50, 14:03-14:06

5.12.3. SCOPE: 68444- 11 .mp4 14:20 – 14:40

Results

6. Results

6.1. Recipients of liver grafts preserved in cold UW solution for 24 hours exhibited a 100% survival rate at 7 days post-transplantation, regardless of whether they were in a hibernation state prior to transplantation or not **[1].**

6.1.1. LAB MEDIA: Figure 9. *Video editor: Highlight the blue and red lines*.

6.2. In contrast, all SD rats receiving liver grafts from other SD rats, also preserved for 24 hours in cold UW solution, succumbed within 12 hours post-transplantation **[1]**.

6.2.1. LAB MEDIA: Figure 9. *Video editor: Highlight the GREEN line*.

- **Infrahepatic**

Pronunciation link:
https://www.merriam-webster.com/medical/infrahepatic
IPA: /ˌɪn-frə-hɪˈpætɪk/
Phonetic Spelling: in-fruh-hi-PAT-ik

- **Inferior vena cava**

Pronunciation link:
https://www.merriam-webster.com/dictionary/vena%20cava
IPA: /ɪnˈfɪriəˠ ˌveɪnə ˈkeɪvə/
Phonetic Spelling: in-FEER-ee-er VAY-nuh KAY-vuh

- **Renal**

Pronunciation link:
https://www.merriam-webster.com/dictionary/renal
IPA: /ˈriːnəl/
Phonetic Spelling: REE-nuhl

- **Adrenal**

Pronunciation link:
https://www.merriam-webster.com/dictionary/adrenal
IPA: /əˈdriːnəl/
Phonetic Spelling: uh-DREE-nuhl

- **Lumbar**

Pronunciation link:
https://www.merriam-webster.com/dictionary/lumbar
IPA: /ˈlʌmˌbɑːr/
Phonetic Spelling: LUM-bar

- **Ligate**

Pronunciation link:
https://www.merriam-webster.com/dictionary/ligate
IPA: /ˈlaɪˌgeɪt/
Phonetic Spelling: LYE-gayt

- **Heparinized**

Pronunciation link:
https://www.merriam-webster.com/medical/heparinize
IPA: /ˈhɛpərəˌnaɪzd/
Phonetic Spelling: HEP-uh-ruh-nyzd

- **Saline**

Pronunciation link:
https://www.merriam-webster.com/dictionary/saline
IPA: /ˈseɪˌliːn/
Phonetic Spelling: SAY-leen

- **Penile**

Pronunciation link:
https://www.merriam-webster.com/dictionary/penile
IPA: /ˈpiːnaɪl/
Phonetic Spelling: PEE-nyle

- **Proper hepatic artery**

Pronunciation link:
https://www.merriam-webster.com/medical/hepatic%20artery
IPA: /ˈprɑːpɚ həˈpætɪk ˈɑːrtəri/
Phonetic Spelling: PROH-per huh-PAT-ik AR-ter-ee

- **Gastroduodenal (artery)**

Pronunciation link:
https://www.merriam-webster.com/medical/gastroduodenal

IPA: /ˌgæstroʊˌduːəˈdɛnəl/
Phonetic Spelling: GAS-troh-DOO-uh-DEN-uhl

- **Common hepatic artery**

Pronunciation link:
https://www.merriam-webster.com/medical/hepatic%20artery
IPA: /ˈkɑːmən həˈpætɪk ˈɑːrtəri/
Phonetic Spelling: KOM-ən huh-PAT-ik AR-ter-ee

- **Micro-forceps**

Pronunciation link:
https://www.merriam-webster.com/dictionary/forceps
IPA: /ˈmaɪkroʊ ˈfɔːrˌsɛps/
Phonetic Spelling: MY-kroh FOR-seps

- **Aorta**

Pronunciation link:
https://www.merriam-webster.com/dictionary/aorta
IPA: /eɪˈɔːrtə/
Phonetic Spelling: ay-OR-tuh

- **Diaphragm**

Pronunciation link:
https://www.merriam-webster.com/dictionary/diaphragm
IPA: /ˈdaɪəˌfræm/
Phonetic Spelling: DYE-uh-fram

- **Thoracic**

Pronunciation link:
https://www.merriam-webster.com/dictionary/thoracic
IPA: /θəˈræsɪk/
Phonetic Spelling: thuh-RASS-ik

- **Cannula** (as in aortic cannula)

Pronunciation link:
https://www.merriam-webster.com/dictionary/cannula
IPA: /ˈkænjələ/
Phonetic Spelling: KAN-yuh-luh

- **Perfuse / Perfusion**

Pronunciation link:
https://www.merriam-webster.com/dictionary/perfuse
https://www.merriam-webster.com/dictionary/perfusion
IPA: /pəˈfjuːz/ ; /pəˈfjuːʒən/
Phonetic Spelling: per-FYOOZ ; per-FYOO-zhən

- **Perfusate**

Pronunciation link:
https://www.merriam-webster.com/medical/perfusate
IPA: /ˈpɚˌfjuːzeɪt/
Phonetic Spelling: PER-fyoo-zayt

- **Intrathoracic**

Pronunciation link:
https://www.merriam-webster.com/medical/intrathoracic
IPA: /ˌɪntrə-θəˈræsɪk/
Phonetic Spelling: in-truh-thuh-RASS-ik

- **Gallbladder**

Pronunciation link:
https://www.merriam-webster.com/dictionary/gallbladder
IPA: /ˈgɔːlˌblædɚ/
Phonetic Spelling: GAHL-blad-er

- **Common bile duct**

Pronunciation link:
https://www.merriam-webster.com/medical/common%20bile%20duct
IPA: /ˈkɑːmən baɪl dʌkt/
Phonetic Spelling: KOM-ən BYLE dukt

- **Stent**

Pronunciation link:
https://www.merriam-webster.com/dictionary/stent
IPA: /stɛnt/
Phonetic Spelling: stent

- **Portal vein**

Pronunciation link:
https://www.merriam-webster.com/medical/portal%20vein
IPA: /ˈpɔːrtəl veɪn/
Phonetic Spelling: POR-tl vayn

- **Pyloric (vein)**

Pronunciation link:
https://www.merriam-webster.com/dictionary/pyloric
IPA: /paɪˈlɔːrɪk/
Phonetic Spelling: pie-LOR-ik

- **Splenic (vein)**

Pronunciation link:
https://www.merriam-webster.com/dictionary/splenic

IPA: /ˈspliːnɪk/
Phonetic Spelling: SPLEE-nik

- **Diaphragmatic**

Pronunciation link:
https://www.merriam-webster.com/dictionary/diaphragmatic
IPA: /ˌdaɪəfrægˈmætɪk/
Phonetic Spelling: dye-uh-frag-MAT-ik

- **Suprahepatic**

Pronunciation link:
https://www.merriam-webster.com/medical/suprahepatic
IPA: /ˌsuːprə-hɪˈpætɪk/
Phonetic Spelling: SOO-pruh-hi-PAT-ik

- **Hepato-splenic (hepatosplenic)**

Pronunciation link:
https://www.merriam-webster.com/medical/hepatosplenic
IPA: /ˌhɛˌpætoʊˈspliːnɪk/
Phonetic Spelling: HEP-uh-toh-SPLEE-nik

- **Anhepatic**

Pronunciation link:
https://www.merriam-webster.com/medical/anhepatic
IPA: /ˌæn-hɪˈpætɪk/
Phonetic Spelling: an-hi-PAT-ik

- **Cystic duct**

Pronunciation link:
https://www.merriam-webster.com/medical/cystic%20duct
IPA: /ˈsɪstɪk dʌkt/
Phonetic Spelling: SIS-tik dukt

- **Transect / Transection**

Pronunciation link:
https://www.merriam-webster.com/dictionary/transect
https://www.merriam-webster.com/dictionary/transection
IPA: /trænˈsɛkt/ ; /trænˈsɛkʃən/
Phonetic Spelling: tran-SEKT ; tran-SEK-shən

- **Parenchyma**

Pronunciation link:
https://www.merriam-webster.com/dictionary/parenchyma
IPA: /pəˈrɛŋkɪmə/
Phonetic Spelling: puh-RENG-kih-muh

- **Bifurcation**

Pronunciation link:
https://www.merriam-webster.com/dictionary/bifurcation
IPA: /ˌbaɪfəˈkeɪʃən/
Phonetic Spelling: BYE-fer-KAY-shən

- **Lumen**

Pronunciation link:
https://www.merriam-webster.com/dictionary/lumen
IPA: /ˈluːmən/
Phonetic Spelling: LOO-mən