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# Title: A Model of Acute Lung Injury Following Visceral Ischemia-Reperfusion by Supra-Coeliac Aortic Cross Clamping in Rats

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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? **YES** , all done

**SCOPE: 2.6.1, 2.9.1-2.9.3**

**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: **08/31/2025**
**AUTHORS: Please film the introduction section**

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

**Current Protocol Length**

Number of Steps: 17
Number of Shots: 33

# Introduction

1.1. **Mickael Palmier:** We present a reproducible rat surgical model of acute lung injury after supra-celiac aortic cross-clamping, enabling detailed study of ischemia–reperfusion mechanisms in pulmonary injury **[1].**

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll: 2.11*

What advantage does your protocol offer compared to other techniques?

1.2. **Mickael Palmier:** Our protocol is feasible, reproducible, low-cost, and easy to learn, enabling rapid implementation, biomarker exploration, and testing of diverse pharmacological interventions **[1].**

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

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

1.3. **Mickael Palmier:** Our surgical model of acute lung injury will enable us to investigate various pharmacological strategies aimed at reducing the severity of pulmonary damage following aortic cross-clamping **[1].**

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll: 2.9*

**Ethics Title Card**

This research has been approved by the Regional University Ethics Committee at Rouen University Hospital

# Protocol

## 2. Microsurgical Induction of Aortic Ischemia-Reperfusion Injury and Analgesic Management in Rats

**Demonstrator:** Myriam Cherel

2.1. To begin, place an anesthetized rat in dorsal recumbency and immobilize the front and hind limbs with adhesive tape while maintaining physiological joint motion ranges **[1-TXT]**. Transfer the rat onto a warming pad set to 37 degrees Celsius and monitor rectal temperature **[2]**.

2.1.1. LAB MEDIA: Protocol.3.1.mov 00:00-00:30 **TXT: Analgesia: Buprenorphine (0.05 mg/kg, SQ) 1 h before the procedure; Apply Lidocaine hydrochloride gel 1% to the skin; Anesthesia:; 5% isoflurane for induction; 2-2.5% isoflurane for maintenance**

2.1.2. LAB MEDIA: Protocol.3.1.mov. 00:36-00:49

2.2. Apply ophthalmic eye ointment to the eyes to prevent dryness **[1]**. After shaving the abdominal fur, thoroughly disinfect the area using povidone-iodine solution and alcohol **[2]**.

2.2.1. LAB MEDIA: Protocol.3.3.1.mov. 00:13-00:23

2.2.2. LAB MEDIA: Protocol.3.3.2.mov 00:00-00:14

2.3. To perform the surgery, make a skin incision at the center of the abdomen to expose the linea alba **[1-TXT]**. Incise the abdominal muscle centered on the linea alba to prevent bleeding from surrounding muscle tissues **[2]**.

2.3.1. LAB MEDIA: Protocol_4.2.2.mov 00:00-00:20
**TXT: Perform surgery under a microsurgical loupe, if possible**

2.3.2. LAB MEDIA: Protocol_4.2.2.mov 00:56-01:20

2.4. Maintain the abdominal wall on both sides using self-retaining retractors **[1]**. Examine the cavity with cotton-tipped buds for any anomalies **[2]**, then gently mobilize the viscera, stomach, spleen, and liver to the right side of the abdomen to expose the supra-celiac aorta **[3]**.

2.4.1. LAB MEDIA: Protocol_4.2.2.mov 02:15-02:28

2.4.2. LAB MEDIA: Protocol_4.2.2.mov 02:36-02:50

2.4.3. LAB MEDIA: Protocol_4.3.2.mov 00:24-00:39, 00:53-01:04

2.5. Keep all viscera inside the abdominal cavity using a compress folded lengthwise and soaked in warm saline solution at 37 degrees Celsius **[1]**. Then cover all exposed viscera with a compress soaked in warm saline solution at 37 degrees Celsius to prevent hypothermia and dehydration **[2]**.

2.5.1. LAB MEDIA: Protocol_4.3.2.mov 01:11-01:26

2.5.2. LAB MEDIA: Protocol_4.3.2.mov 00:40-00:52

2.6. Identify and encircle the supra-celiac aorta with a 5-0 *(Five-Oh)* monofilament suture using dissecting and ligature forceps **[1-TXT]**.

2.6.1. SCOPE: Protocol_4.4.3.mov 00:00-00:20
**TXT: Verify the origins of the celiac trunk and the underlying mesenteric artery**

2.7. Identify the subrenal inferior vena cava through the transparent retroperitoneum **[1-TXT]**.

2.7.1. LAB MEDIA: Protocol_4.4.5.mov 00:00-00:07
**TXT: Release the anterior surface of the subrenal IVC to direct IV administration of pharmacological agents**

2.8. For the intravenous injection, use a 30-gauge needle and apply a small piece of hemostatic compress at the injection site to achieve hemostasis **[1-TXT]**.

2.8.1. LAB MEDIA: Protocol_4.10.1.mov 03:06-03:14.
**TXT: Administer injection before clamping for pharmacological treatment or after for post-conditioning**

2.9. Now clamp the celiac aorta with an atraumatic microvascular clamp for 40 minutes **[1]**. Verify the clamp's effectiveness by confirming the disappearance of aortic pulsations **[2]** and discoloration of the viscera and limbs multiple times during clamping **[3]**.

2.9.1. SCOPE: Protocol_4.6.1.5.mov 00:04-00:20

2.9.2. SCOPE: Protocol_4.6.1.5.mov 01:50-02:03

2.9.3. SCOPE: Protocol_4.6.1.5.mov 00:59-01:16.

2.10. Close the abdomen partially using three simple 5-0 braided absorbable sutures to reduce water and heat loss **[1]**. Then cover the incision with a sterile compress soaked in warm saline for the duration of ischemia **[2]**.

2.10.1. LAB MEDIA: Protocol_4.6.2.mov 00:27-00:57,02:10-02:20

2.10.2. LAB MEDIA: Protocol_4.6.2.mov 02:27-02:35

2.11. At the end of the ischemic period, reopen the abdomen and expose the aorta **[1]**. Remove the vascular clamp and verify resumption of aortic pulsations and recoloration of the viscera and limbs. This marks the beginning of the reperfusion period **[2]**.

2.11.1. LAB MEDIA: Protocol_4.7.1mov 00:15,00:38-00:50 .

2.11.2. LAB MEDIA: Protocol_4.7.1.mov 00:55-01:33.

2.12. Return the viscera to their original position **[1].** Then close the abdominal wall with a 5-0 braided absorbable suture on the linea alba **[2].** Use a 5-0 monofilament absorbable suture on the skin **[3]**.

2.12.1. LAB MEDIA: Protocol_4.8.1.1.mov 00:00-00:12

2.12.2. LAB MEDIA: Protocol_4.8.1.1.mov 00:18-00:36, 02:18-02:30

2.12.3. LAB MEDIA: Protocol_4.8.1.2.mov 00:40-00:50, 02:15-02:22

2.13. Place the rat in the left lateral decubitus position to prevent compression of the inferior vena cava during hypovolemia **[1]**. Then transfer it into an individual cage under a heat lamp until it awakens, which occurs approximately 10 minutes later **[2]**.

2.13.1. LAB MEDIA: Protocol_4.9.0.mov 00:00-00:06

2.13.2. LAB MEDIA: Protocol_4.9.1.mov 00:00-00:08

## 3. Blood and Tissue Collection, Molecular, Histological, and Gravimetric Analyses Following Aortic Ischemia-Reperfusion

3.1. For blood sampling, directly puncture the inferior vena cava below the renal veins using a 5-milliliter syringe equipped with a 23-gauge needle **[1]**.

3.1.1. LAB MEDIA: Protocol_5.1.1.mov 00:00-00:11

3.2. After the animal has been euthanized, harvest the left kidney **[1]**. Then harvest the left

hepatic lobe **[2]**, and the distal small intestine 1 centimeter before the cecum through the open abdomen **[3]**. Quickly perform a median sternotomy to expose and identify the heart and lungs **[4]**.

3.2.1. LAB MEDIA: Protocol_5.2.1..mov 00:12-00:40

3.2.2. LAB MEDIA: Protocol_5.2.1..mov 05:44-05:57

3.2.3. LAB MEDIA: Protocol_5.2.1..mov 01:16-01:30,

3.2.4. LAB MEDIA: Protocol_5.2.1..mov 02:40-02:50,03:34-03:45.

3.3. Free the left lung from its ligament and remove it entirely for histological analysis **[1]**. Then free the right multilobed lung from its ligaments and isolate the anterior lobe for nucleic acid and protein analysis **[2-TXT]**.

3.3.1. LAB MEDIA: Protocol_5.2.1..mov 03:48-04:06, 04:15-04:23.

3.3.2. LAB MEDIA: Protocol_5.2.1..mov 05:10-05:33. **TXT: Store the remaining portion in a cup with warm physiological serum for gravimetry**

3.4. Extract the heart as a whole block, including sections of the aorta, pulmonary artery, and both vena cavae **[1-TXT]**.

3.4.1. LAB MEDIA: Protocol_5.2.1..mov 04:54-05:08.
**TXT: Sample a piece of the left ventricle for nucleic acid and protein analysis**

# Results

## 4. Results

4.1. Blood gas analysis following supracoeliac aortic cross clamping showed reduced pH **[1]**, decreased partial oxygen pressure **[2]**, increased partial carbon dioxide pressure **[3]**, and lower bicarbonate levels compared to normal ranges **[4]**.

4.1.1. LAB MEDIA: Table 2. *Video editor: Highlight the row labeled "pH" in the column "After Supracoeliac Aortic cross clamping"*

4.1.2. LAB MEDIA: Table 2. *Video editor: Highlight the row labeled "$PaO_2$ (mmHg)" in the column "After Supracoeliac Aortic cross clamping"*

4.1.3. LAB MEDIA: Table 2. *Video editor: Highlight the row labeled "$PaCO_2$ (mmHg)" in the column "After Supracoeliac Aortic cross clamping"*

4.1.4. LAB MEDIA: Table 2. *Video editor: Highlight the row labeled "$HCO_3^-$ (mmol/L)" in the column "After Supracoeliac Aortic cross clamping"*

4.2. The histopathologic lung injury score increased markedly in the supracoeliac aortic cross clamping group **[1]**. Polymorphonuclear neutrophils per square millimeter were significantly elevated after supracoeliac aortic cross clamping **[2]**.

4.2.1. LAB MEDIA: Table 3 *Video editor: Highlight the "Histopathologic lung injury score" row and column "Supracoeliac Aortic cross clamping"*

4.2.2. LAB MEDIA: Table 3 *Video editor: Highlight the row labeled "PNN"* for *column " Supracoeliac Aortic cross clamping"*

4.3. Intravascular leukocyte counts were significantly higher in the aortic cross clamping group compared to the sham group **[1]**.

4.3.1. LAB MEDIA: Table 3 *Video editor: Highlight the row labeled "Intravascular cells" for column "Supracoeliac Aortic cross clamping"*

4.4. Pulmonary inflammation markers were also significantly elevated after supracoeliac aortic cross clamping **[1]**. Additionally, pulmonary edema, as measured by gravimetric analysis, was found to be increased after aortic clamping **[2].**

4.4.1. LAB MEDIA: Table 4. Video editor: Highlight the rows labeled "TNF-alpha" and "IL-1 β" *for column "Supracoeliac Aortic cross clamping"*

4.4.2. LAB MEDIA: Figure 3 *Video Editor: Please highlight the red squares*

**Pronunciation Guide:**

**Recumbency**
Pronunciation link:
https://www.merriam-webster.com/dictionary/recumbency
IPA: /rɪˈkʌmbənsi/
Phonetic Spelling: ri-kum-buhn-see

**Physiological**
Pronunciation link:
https://www.merriam-webster.com/dictionary/physiological
IPA: /ˌfɪziəˈlɑːdʒɪkəl/
Phonetic Spelling: fiz-ee-uh-lah-juh-kl

**Ophthalmic**
Pronunciation link:
https://www.merriam-webster.com/dictionary/ophthalmic
IPA: /ɑfˈθælmɪk/
Phonetic Spelling: ahf-thal-mik

**Povidone-iodine**
Pronunciation link:
https://www.merriam-webster.com/dictionary/povidone-iodine
IPA: /ˈpoʊ.vɪˌdoʊn ˈaɪ.əˌdaɪn/
Phonetic Spelling: poh-vi-dohn eye-uh-dine

**Linea alba**
Pronunciation link:
https://www.howtopronounce.com/linea-alba
IPA: /ˈlɪniə ˈælbə/
Phonetic Spelling: lin-ee-uh al-buh

**Viscera**
Pronunciation link:
https://www.merriam-webster.com/dictionary/viscera
IPA: /ˈvɪsərə/
Phonetic Spelling: vis-er-uh

**Supra-celiac** (as in supra-celiac aorta)
Pronunciation link:
https://www.howtopronounce.com/supraceliac
IPA: /ˌsuːprəˈsiːliæk/
Phonetic Spelling: soo-pruh-see-lee-ak

**Mesenteric**
Pronunciation link:
https://www.merriam-webster.com/dictionary/mesenteric
IPA: /ˌmɛzənˈtɛrɪk/ or /ˌmɛsənˈtɛrɪk/
Phonetic Spelling: mez-en-ter-ik or mess-en-ter-ik

**Retroperitoneum**
Pronunciation link:
https://www.howtopronounce.com/retroperitoneum
IPA: /ˌrɛtroʊˌpɛrətnˈiəm/
Phonetic Spelling: reh-troh-peh-rih-tee-nee-uhm

**Intravenous**
Pronunciation link:
https://www.merriam-webster.com/dictionary/intravenous
IPA: /ˌɪntrəˈviːnəs/
Phonetic Spelling: in-truh-vee-nuhs

**Hemostasis**
Pronunciation link:
https://www.merriam-webster.com/dictionary/hemostasis
IPA: /ˌhiːmoʊˈsteɪsɪs/
Phonetic Spelling: hee-moh-stay-sis

**Atraumatic**
Pronunciation link:
https://www.merriam-webster.com/dictionary/atraumatic
IPA: /ˌeɪtrɔːˈmætɪk/
Phonetic Spelling: ay-traw-mat-ik

**Ischemia**
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-zhun

**Decubitus** (as in lateral decubitus position)
Pronunciation link:
https://www.merriam-webster.com/dictionary/decubitus
IPA: /dɪˈkjuːbɪtəs/
Phonetic Spelling: dih-kyoo-bih-tuhs

**Cecum**
Pronunciation link:
https://www.merriam-webster.com/dictionary/cecum
IPA: /ˈsiːkəm/
Phonetic Spelling: see-kuhm

**Sternotomy**
Pronunciation link:
https://www.howtopronounce.com/sternotomy
IPA: /stərˈnɑːtəmi/
Phonetic Spelling: ster-nah-tuh-mee

**Histological**
Pronunciation link:
https://www.merriam-webster.com/dictionary/histological
IPA: /ˌhɪstəˈlɑːdʒɪkəl/
Phonetic Spelling: his-tuh-lah-jih-kl

**Gravimetric**
Pronunciation link:
https://www.merriam-webster.com/dictionary/gravimetric
IPA: /ˌgrævəˈmɛtrɪk/
Phonetic Spelling: grav-uh-meh-trik

**Supracoeliac** (alternate spelling in Results)
Pronunciation link:
https://www.howtopronounce.com/supracoeliac
IPA: /ˌsuːprəˈsiːliæk/
Phonetic Spelling: soo-pruh-see-lee-ak

**Histopathologic**
Pronunciation link:
https://www.merriam-webster.com/dictionary/histopathologic
IPA: /ˌhɪstoʊˌpæθəˈlɑːdʒɪk/
Phonetic Spelling: his-toh-path-uh-lah-jik

**Polymorphonuclear**
Pronunciation link:
https://www.merriam-webster.com/dictionary/polymorphonuclear
IPA: /ˌpɑːlɪˌmɔːrfəˈnuːkliər/
Phonetic Spelling: pah-lee-mor-foh-noo-klee-er

**Neutrophils**
Pronunciation link:
https://www.merriam-webster.com/dictionary/neutrophil
IPA: /ˈnuːtrəˌfɪl/
Phonetic Spelling: noo-truh-fil

**Pulmonary**
Pronunciation link:
https://www.merriam-webster.com/dictionary/pulmonary
IPA: /ˈpʊlməˌnɛri/ or /ˈpʌlməˌnɛri/
Phonetic Spelling: pull-muh-neh-ree or puhl-muh-neh-ree

**Edema**
Pronunciation link:
https://www.merriam-webster.com/dictionary/edema
IPA: /ɪˈdiːmə/
Phonetic Spelling: ih-dee-muh