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**Title: *In Utero* Fetal Lamb Model of Mitral Inflow Obstruction to Induce Hypoplastic Left Heart Syndrome-Like Physiology**

**Authors and Affiliations:**

**Chihiro Miyagi<sup>1</sup>, Kosuke Nakamae<sup>1</sup>, Tatsuya Watanabe<sup>1</sup>, Syed Faizullah Hussaini<sup>1</sup>, Satoshi Yuhara<sup>1</sup>, Junya Matsuda<sup>1</sup>, Daisuke Onohara<sup>1,2</sup>**

<sup>1</sup>Center for Regenerative Medicine, Research Institute at Nationwide Children's Hospital

<sup>2</sup>Department of Pediatrics, Ohio State University College of Medicine

**Corresponding Authors:**

Chihiro Miyagi

chihiromiyagi88@gmail.com

Daisuke Onohara

Daisuke.Onohara@nationwidechildrens.org

**Email Addresses for All Authors:**

[chihiromiyagi88@gmail.com](mailto:chihiromiyagi88@gmail.com)

[Daisuke.Onohara@nationwidechildrens.org](mailto:Daisuke.Onohara@nationwidechildrens.org)

[Kosuke.Nakamae@nationwidechildrens.org](mailto:Kosuke.Nakamae@nationwidechildrens.org)

[Tatsuya.Watanabe@nationwidechildrens.org](mailto:Tatsuya.Watanabe@nationwidechildrens.org)

[SyedFaizullah.Hussaini@nationwidechildrens.org](mailto:SyedFaizullah.Hussaini@nationwidechildrens.org)

[Satoshi.Yuhara@nationwidechildrens.org](mailto:Satoshi.Yuhara@nationwidechildrens.org)

[Junya.Matsuda@nationwidechildrens.org](mailto:Junya.Matsuda@nationwidechildrens.org)

## 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

☐ Incorrect

**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**

If **Yes**, we will need you to record using screen recording software.

We recommend using the screen capture program [OBS](#). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

As these files are necessary for finalizing your script, please upload all screen-captured video files to your project page as soon as possible.

**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: 04/18/2025, 05/20/2026, 06/02/2026, 06/09/2026

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

### Current Protocol Length

Number of Steps: 20

Number of Shots: 60

# Interviews

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## INTRODUCTION:

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

- 1.1. **Chihiro Miyagi:** Our research focuses on how fetal mitral inflow obstruction affects left ventricular growth and drives hypoplastic left heart syndrome-like physiology.

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

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Chihiro Miyagi:** This protocol can support studies of fetal cardiac development, hemodynamic remodeling, and prenatal therapeutic strategies.

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. **Chihiro Miyagi:** This protocol enables continuous measurement of fetal blood pressure, aortic flow, and ventricular responses during mitral inflow obstruction.

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. **Chihiro Miyagi:** The key challenge is safely positioning the balloon while avoiding atrial bleeding, tissue injury, and amniotic fluid loss.

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. **Chihiro Miyagi:** Researchers can add serial fetal imaging, postmortem morphometry, tissue sampling, and histological assessment after this procedure.

- 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) of the Abigail Wexner Research Institute at Nationwide Children's Hospital

# Protocol

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*Video editor: Please add title card before section 2*

## Title Card

### Animal Preparation:

- Q fever screening
- Overnight fasting
- Sedation: Diazepam 0.5 mg/kg + ketamine 4 mg/kg IV.
- Anesthesia Maintenance: Isoflurane 1%–5% in O<sub>2</sub> or propofol infusion 20–45 mg/kg/h
- Orogastric tube insertion for stomach/rumen decompression
- Venous catheter placement for fluids and drug delivery
- Arterial line placement for BP monitoring and blood sampling

**NOTE:** Shots are vertical, so can be titled for the video

## 2. Laparotomy and Hysterotomy in the Ewe

**Demonstrators:** Chihiro Miyagi, Kosuke Nakamae, and Daisuke Onohara

- 2.1. To begin, place the anesthetized sheep on a warming device in a supine position on a surgical table [1]. Sterilize the shaved abdominal area using chlorhexidine, povidone-iodine, or alcohol [2]. Administer cefazolin at 50 milligrams per kilogram body weight for antibiotic prophylaxis before incision [3].
  - 2.1.1. WIDE: Talent placing an anesthetized sheep on a warming device on a surgical table.
  - 2.1.2. Talent wiping the shaved abdominal area with chlorhexidine.
  - 2.1.3. Talent injecting cefazolin
- 2.2. After midline laparotomy to expose the uterus, palpate it to count the number of fetuses [1]. Gently exteriorize the uterus from the abdominal cavity [2] and select a hysterotomy site approximately ten to fifteen centimeters based on fetal size [3].
  - 2.2.1. Talent touching the uterus after midline incision to check the number of fetuses.
  - 2.2.2. Talent taking uterus out of abdominal cavity.
  - 2.2.3. CU: 10–15 cm hysterotomy site is being selected on the uterus.
- 2.3. Place a purse-string suture prior to hysterotomy to control amniotic fluid leakage [1]. Then use electrocautery to open the uterine wall layer by layer [2]. Place four Babcock

clamps along the uterine incision to secure all layers, including the amniotic membrane, to prevent amniotic fluid loss [3]. Use a 2-0 (*two-oh*) silk thread to place a continuous over-and-over suture along the incision edge to maintain the apposition of the amniotic membrane to the uterine wall. [4].

2.3.1. CU: A purse-string suture is being placed

2.3.2. CU: The uterine walls are being opened layer by layer by using electrocautery.

2.3.3. Talent placing four Babcock clamps along the uterine incision.

2.3.4. CU: Continuous over-and-over suturing is being done along the incision.

2.4. Gently externalize the fetal head [1] and secure the uterine purse-string suture with a hemostat [2]. Stabilize the fetal head using gauze to maintain a supine position [3].

2.4.1. Talent externalizing the fetal head out of the uterine cavity.

2.4.2. CU: Hemostat being placed onto the purse-string suture.

2.4.3. Talent keeping gauze to the fetal head.

2.5. After inserting the intracardiac echocardiograph probe and providing intramuscular sedation for surgical immobilization, make a neck incision. [1] and expose the jugular vein and carotid artery [2]. Insert a fluid-filled catheter into the carotid artery for arterial pressure monitoring [3], and another one into the jugular vein for central venous pressure monitoring [4].

2.5.1. Talent making an incision on the neck after ICE probe insertion and IM injection.

2.5.2. CU: Talent exposing the jugular vein and carotid artery.

2.5.3. CU: A fluid-filled catheter is being inserted into the carotid artery.

2.5.4. CU: Another fluid-filled catheter is being inserted into the jugular vein.

2.6. Use 2-0 silk sutures to close the neck incision while externalizing the catheters [1]. Align the two pressure lines with an additional amniotic pressure line [2]. Use 3-0 (*three-oh*) silk sutures to secure them along the fetal neck [3].

2.6.1. Talent closing the neck incision with 2-0 silk sutures.

2.6.2. Talent aligning pressure lines are with the amniotic pressure line.

2.6.3. Talent securing the pressure line near the fetal neck with 3-0 silk sutures.

**NOTE: Insert the chapter title: Fetal Thoracotomy**

2.7. After positioning the fetus in the right lateral decubitus position, perform fetal thoracotomy at the fourth intercostal space [1]. Use a rib spreader to open the rib cage [2]. Then, open the pericardium [3] and suspend it with 4-0 (*four-oh*) silk stay sutures to expose the left atrium and pulmonary artery [4].

- 2.7.1. Talent making a thoracotomy at the fourth intercostal space.
- 2.7.2. CU: Rib cage is being opened with a spreader.
- 2.7.3. CU: Pericardium is being opened.
- 2.7.4. CU: Left atrium and pulmonary artery are being shown through lifting the pericardium with 4-0 silk sutures.

2.8. Dissect the pulmonary artery and ascending aorta [1] and encircle each with vessel loops [2]. Pass the six-millimeter flow probe cable through the second intercostal space, positioning it around the ascending aorta, ensuring adequate signal quality after connection to the flow measurement unit [3].

- 2.8.1. Talent dissecting the pulmonary artery and ascending aorta.
- 2.8.2. CU: Each vessel loop is placed around the pulmonary artery and ascending aorta.
- 2.8.3. CU: A 6 mm flow probe cable is being placed around the ascending aorta.

### 3. Balloon Implantation at the Left Atrium

**Demonstrator:** Chihiro Miyagi, Kosuke Nakamae

3.1. For fetuses older than gestational age 110, use a small-angled vessel clamp to occlude the left atrium slightly above the atrioventricular groove [1]. After placing a 6-0 (six-oh) polypropylene purse-string suture on the left atrial appendage, insert the custom-made balloon catheter [2] and secure it with a tourniquet [3].

- 3.1.1. Talent clamping LA with a small-angled vessel clamp.
- 3.1.2. Talent inserting the custom-made balloon catheter into the LA inside the purse-string suture placed on the LA wall.
- 3.1.3. CU: A tourniquet is being tightened to secure the purse-string suture.

3.2. For fetuses younger than gestational age 110, place two 6-0 polypropylene sutures on the balloon catheter, one at the tip and one at the base [1]. Anchor these sutures to the pericardium and the balloon cable at the corresponding positions [2].

- 3.2.1. CU: Balloon catheter is being sutured at the tip and the base.
- 3.2.2. CU: Sutures are being anchored to the pericardium and the balloon cable at corresponding positions

3.3. After securing the balloon, closing the chest and returning the fetus to the uterus, close the hysterotomy in three continuous layers [1]. Externalize the catheters and cables from the peritoneum and further out through the flank [2] and complete abdominal closure [3].

3.3.1. Talent closing the uterus in continuous layers

3.3.2. Talent is bringing the catheters and cables out from the peritoneum and further out through the flank.

3.3.3. Talent is closing the abdominal incision NOTE: Use file 3.3.3. Peritoneum and fascia closure

# Results

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## 4. Results

4.1. Representative data comparing the hemodynamic changes during balloon inflation between inside- and outside-left atrial configurations demonstrated a decrease in aortic pressure [1] and aortic flow with an increase in balloon size in both configurations [2].

4.1.1. LAB MEDIA: Figure 5A and 5C *Video Editor: Please emphasize the line corresponding to “AoP” in both graphs.*

4.1.2. LAB MEDIA: Figure 5B and 5D *Video Editor: Please emphasize the line corresponding to “mean Ao Flow” in both graphs.*

4.2. A decrease in the left ventricular end-diastolic volume [1], left ventricular stroke volume [2], and left ventricular ejection fraction was observed during balloon inflation in both inside- and outside-left atrial configurations [3].

4.2.1. LAB MEDIA: Figure 6A and 6D *Video Editor: Please emphasize the red line corresponding to “LVEDV” in both graphs.*

4.2.2. LAB MEDIA: Figure 6A and 6D *Video Editor: Please emphasize the blue line corresponding to “LVSV” in both graphs*

4.2.3. LAB MEDIA: Figure 6C and 6F *Video Editor: Please emphasize the blue line corresponding to “LVEF” in both graphs.*

### 1. Electroporation

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

IPA: /ɪˌlek.troʊ.pəˈreɪ.jən/

Phonetic Spelling: ih-lek-troh-puh-ray-shuhn

### 2. EBY100

Pronunciation link: No confirmed link found

IPA: No verified IPA available

Phonetic Spelling: ee-bee-why-one-zero-zero

### 3. Glycerol

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

IPA: /ˈɡlɪs.əˌrɔːl/

Phonetic Spelling: glis-uh-rawl

4. Erlenmeyer  
Pronunciation link: <https://www.merriam-webster.com/dictionary/Erlenmeyer%20flask>  
IPA: /'ɜː.lənˌmaɪ.ə/  
Phonetic Spelling: er-len-my-er
5. Micropipette  
Pronunciation link: <https://www.merriam-webster.com/dictionary/micropipette>  
IPA: /ˌmaɪ.kroʊ.pəɪˈpet/  
Phonetic Spelling: my-kroh-pie-pet
6. Spectrophotometer  
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>  
IPA: /ˌspek.troʊ.fəˈtɑː.mə.tə/  
Phonetic Spelling: spek-troh-fuh-tom-uh-ter
7. Optical density  
Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>  
IPA: /'ɑːp.tɪ.kəl ˈden.sə.ti/  
Phonetic Spelling: op-tih-kuhl den-suh-tee
8. Microcuvette  
Pronunciation link: No confirmed link found  
IPA: No verified IPA available  
Phonetic Spelling: my-kroh-kyoo-vet
9. Conditioning buffer  
Pronunciation link: <https://www.merriam-webster.com/dictionary/conditioning>  
IPA: /kənˈdɪʃ.ə.nɪŋ ˈbʌf.ə/  
Phonetic Spelling: kuhn-dish-uh-ning buf-er
10. Sorbitol  
Pronunciation link: <https://www.merriam-webster.com/dictionary/sorbitol>  
IPA: /'sɔːr.bəˌtɔːl/  
Phonetic Spelling: sor-bih-tol
11. Electrocompetent  
Pronunciation link: No confirmed link found  
IPA: No verified IPA available  
Phonetic Spelling: ih-lek-troh-kom-puh-tuhnt
12. Resuspend  
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>  
IPA: /ˌriː.səˈspend/  
Phonetic Spelling: ree-suh-spend
13. Centrifugation  
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>  
IPA: /ˌsen.trəˈfjuːˈgeɪ.jən/  
Phonetic Spelling: sen-truh-fyoo-gay-shuhn
14. Plasmid  
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>  
IPA: /ˈplæz.mɪd/  
Phonetic Spelling: plaz-mid
15. Linearized  
Pronunciation link: <https://www.merriam-webster.com/dictionary/linearize>

IPA: /'lɪn.i.ə.raɪzd/

Phonetic Spelling: lin·ee·uh·ryzd

16. Cuvette

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

IPA: /kju:'vet/

Phonetic Spelling: kyoo·vet

17. TRP1

Pronunciation link: No confirmed link found

IPA: No verified IPA available

Phonetic Spelling: tee·ar·pee·one

18. Fluorescence-activated cell sorting

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

IPA: /flʊ:'res.əns 'æk.tə'veɪ.tɪd sel 'sɔ:r.tɪŋ/

Phonetic Spelling: floor·ess·uhns ak·tuh·vay·tid sel sor·ting

19. Hemagglutinin

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

IPA: /,hi:.mə'glu:.tʃən.ɪn/

Phonetic Spelling: hee·muh·gloo·tuh·nin

20. Fluorescein isothiocyanate

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

IPA: /,flʊr.ə'si:.ɪn ,aɪ.səʊ,θaɪ.əʊ'saɪ.ə.neɪt/

Phonetic Spelling: floor·uh·see·in eye·soh·thy·oh·sy·uh·nayt

21. Phycoerythrin

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

IPA: /,faɪ.kəʊ.ə'rɪθ.rɪn/

Phonetic Spelling: fy·koh·uh·rith·rin