

Submission ID #: 69169

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Title: Doppler Ultrasonography for Live Imaging and Quantification of Ovarian Vascular Function in Mice

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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? **YES, all done**

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: 12/03/2025

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

Current Protocol Length

Number of Steps: 23

Number of Shots: 51

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Justine Galliou:** This research aims to characterize murine preovulatory ovarian blood flow using a non-invasive in vivo method.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the current experimental challenges?~~

- 1.2. **Justine Galliou:** The field lacks non-invasive methods to study ovarian vascular remodeling in real time without disrupting normal physiology in mice.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What significant findings have you established in your field?~~

- 1.3. **Justine Galliou:** Using Doppler ultrasonography, we determined that ovarian blood flow velocity increases rapidly after the LH surge in mice.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What advantage does your protocol offer compared to other techniques?~~

- 1.4. **Justine Galliou:** Doppler ultrasonography enables repeated, noninvasive assessment of ovarian hemodynamics during the preovulatory period, which other methods cannot achieve.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What new scientific questions have your results paved the way for?~~

- 1.5. **Justine Galliou:** Having characterized normal preovulatory hemodynamics, this protocol enables future examination of blood flow disruptions in murine models of ovarian disorders like PCOS.

- 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 Cornell University

Protocol

2. Preparation and Imaging Setup for High-Resolution Ultrasound in Mice

Demonstrator: Justine Galliou

- 2.1. To begin, switch on the main power located on the back of the imaging system [1]. After the system boots, toggle the computer standby switch on the left side of the cart to wake the monitor and computer [2].
 - 2.1.1. WIDE: Talent turning on the main power switch located at the back of the ultrasound imaging system.
 - 2.1.2. Talent toggling the computer standby switch on the left side of the imaging system cart.
- 2.2. Connect the MS-700 transducer to the active transducer port on the imaging unit located at the rightmost port [1]. Then align the locking pin on the transducer connector with the notch in the port, push the connector in fully, and turn the locking lever to the vertical position to secure it [2].
 - 2.2.1. Talent holding the MS-700 transducer and connecting it to the rightmost port on the imaging unit.
 - 2.2.2. Talent aligning the locking pin, pushing in the connector, and turning the locking lever into vertical position.
- 2.3. Launch the analysis software by clicking on the software icon [1].
 - 2.3.1. SCREEN: 69169-shot-2.3.1.MOV. 00:00-end
- 2.4. Click **New** to create a new study and choose the appropriate name from the drop-down menu [1]. In the **Study Information** window, enter the **study name**, **series name**, **application package** as **ovary**, and **measurement package** as **vascular** [2]. Once the program opens and the transducer is automatically detected, select the **ovary** application package [3].
 - 2.4.1. SCREEN: 69169-shot-2.4.1.MOV 00:03-end
 - 2.4.2. SCREEN: 69169-shot-2.4.2.MOV. 00:04-end
 - 2.4.3. SCREEN:69169-shot-2.4.3.MOV 00:00-end.
- 2.5. Next, apply a small amount of electrode cream to each of the electrodes to ensure proper contact for heart rate monitoring [1]. Once the animal is fully sedated, redirect gas flow by opening the stopcock to the nose cone on the imaging platform [2] and closing the stopcock to the induction chamber [3].

- 2.5.1. Talent applying electrode cream to each electrode on the imaging platform.
- 2.5.2. Talent opening the stopcock to the nose cone.
- 2.5.3. Talent closing the stopcock to the induction chamber.
- 2.6. Gently transfer the mouse onto the heated imaging platform [1]. Place the mouse in the prone position with the dorsal side facing upward [2-TXT].
 - 2.6.1. Talent placing the mouse on the heated imaging platform.
 - 2.6.2. Talent positioning the mouse prone with dorsal side up. **TXT: Secure the nose cone to maintain anesthesia**
AUTHOR'S NOTE: Shots 2.6.1-2.6.2 filmed as a single shot
- 2.7. Tape each foot in the electrode cream onto the corresponding electrode pad for physiological monitoring [1]. Apply ophthalmic ointment to the eyes to prevent corneal drying during anesthesia [2].
 - 2.7.1. Talent taping each paw to the corresponding electrode pad.
 - 2.7.2. Talent applying ophthalmic ointment to the eyes.
- 2.8. Confirm adequate anesthesia depth using the toe-pinch reflex method [1-TXT]. Monitor the rate of breathing, heartbeat, movements indicating discomfort, and maintain correct platform temperature every 5 minutes [2].
 - 2.8.1. Talent performing the toe-pinch test and observing no response. **TXT: Anesthesia: Isoflurane inhalation: 1.5 - 2 %**
 - 2.8.2. Talent monitoring vital signs and platform temperature.
AUTHOR'S NOTE: 2.8.2 was separated into 2 shots. One where the talent is monitoring vital signs and the other where talent is checking platform temperature
- 2.9. Next, shave the lower right quadrant of the back using an electric shaver from the right hindlimb to the midline, staying lateral to the spine [1]. Apply a thin layer of body hair remover cream [2]. After a minute, wipe off the cream thoroughly with damp gauze [3]. Then apply a generous and even layer of ultrasound gel to the shaved region [4].
 - 2.9.1. Talent shaving the mouse from the right hindlimb to midline of back.
 - 2.9.2. Talent applying body hair remover cream to the shaved region.
 - 2.9.3. Talent wiping off the cream with damp gauze.
 - 2.9.4. Talent applying ultrasound gel to the shaved area.
- 2.10. Position the transducer probe in transverse mode relative to the mouse on the mechanical stand with the orientation notch facing to the left of the operator [1]. Secure the probe in place using the clamp [2].
 - 2.10.1. Talent orienting the transducer in transverse mode with notch to the left and

Talent clamping the transducer in place.

AUTHOR'S NOTE: Shots 2.10.1-2.10.2 were filmed together

3. High-Resolution Doppler Ultrasound Imaging of Ovarian Vasculature in Mice

Demonstrator: Justine Galliou

3.1. Press the **B-Mode** key on the control panel to activate the standard grayscale imaging window [1]. Lower the transducer gently onto the shaved area coated with ultrasound gel [2].

3.1.1. Talent pressing the **B-Mode** button on the control panel.

3.1.2. Talent lowering the transducer onto the gelled skin.

AUTHOR'S NOTE: Move 3.1.2 after 3.2.2

3.2. Ensure the mouse and platform are flat and that the transducer is perpendicular to the surface [1]. Then pull the skin to the left, and position the transducer near the upper shaved region, closer to the midline of the back [2]. Align the left edge of the probe with the spine, and the right edge over the right flank [3].

3.2.1. Shot showing the probe held perpendicular to the mouse and platform.

3.2.2. Talent pulling skin left and positioning the transducer near the upper shaved region.

3.2.3. Shot of the probe being aligned over the right flank.

3.3. To identify the ovary, first identify the kidney, a large light grey ovoid structure with cortex and medulla [1]. Push the imaging platform slightly toward the mouse's head to reveal the ovary and periovarian adipose tissue [2].

3.3.1. SCREEN: 69169_screenshot_1_3.3.1.mp4. 00:00-end

Added shot 3.3.2: Talent moving the platform forward

3.3.2. SCREEN: 69169_screenshot_2_3.3.3.mp4 and [69169 screenshot 3 3.3.3.mp4](#)

3.4. ~~Wait for the frame count to increase up to 100 frames [1].~~ Press the **Cine Store** button to capture the B-mode video [2]. Now, press the key for **Power Doppler Mode** on the control panel to visualize blood vessels with high sensitivity to low-velocity flow [3].

~~3.4.1. SCREEN: Frame counter increasing on the imaging monitor.~~

AUTHOR'S NOTE: Delete shot-

3.4.2. Talent pressing **Cine Store** button on the console.

3.4.3. Talent pressing the Power Doppler key.

3.5. Adjust the **Doppler Gain** to 32 to 55 decibels, **Sensitivity** to 5, **Dynamic Range** to 15 DR, and **Velocity** to 1 kilohertz [1].

- 3.5.1. Talent Adjusting Doppler Gain, Sensitivity, Dynamic Range, and Velocity on control panel.
- 3.6. Move the platform gently back and forth to get the best view of the ovarian vasculature [1] and identify the ovarian artery entering the ovarian hilum [2], medullary vessels in the central medulla [3], and cortical vessels around developing follicles [4].
Video Editor: For shots 3.6.2-3.6.4, please label the video as given in the [labeled-1h-power-doppler.pdf](#)
- 3.6.1. Talent moving the platform gently back and forth.
- 3.6.2. SCREEN: 69169_screenshot_4_3.6.2-3.6.4.mp4 00:00-end
- 3.6.3. SCREEN: 69169_screenshot_4_3.6.2-3.6.4.mp4 00:00-end
- 3.6.4. SCREEN: 69169_screenshot_4_3.6.2-3.6.4.mp4 00:00-end
- 3.7. Press **Cine Store** to save the displayed video of the ovary and vasculature in Power Doppler mode [1].
- 3.7.1. Shot of Cine Store being pressed.
- 3.8. To distinguish arterial from venous flow, press **Color Doppler Mode** [1]. Red shows flow toward the transducer and blue shows flow away [2-TXT].
- 3.8.1. Talent pressing Color Doppler Mode.
- 3.8.2. SCREEN: 69169_screenshot_5_3.8.2.mp4. 00:00-end
TXT: Vessel with the higher average velocity is the ovarian artery
- 3.9. ~~While in Color Doppler mode,~~ Press the **Pulsed Wave (PW)** (*Pulsed-Wave-P-W*) Doppler button to bring up the sample gate lines . Press it again to activate the Doppler spectral waveform [1].
- 3.9.1. SCREEN : 69169_screenshot_6_3.9.1.mp4 00:00-end
- 3.10. After positioning the sample gate in the center of the vessel lumen, adjust the insonation angle to be less than or equal to 60 degrees and parallel to flow using the angle knob [1].
- ~~3.10.1. SCREEN: 69169_Screenshot_7_3.10.1_and-3.11.1.mp4 Sample gate being moved to center of vessel.~~
- NOTE: Deleted shot since it is repeated in 3.11.1**
- 3.10.2. Talent turns the knob to adjust the insonation angle.
- 3.11. For the ovarian artery, place the gate just before it enters the ovary [1]. For the medullary vessel, place it at the central bifurcation [2]. For cortical vessels, place it at the base of a growing follicle [3].
- 3.11.1. SCREEN: 69169_Screenshot_7_3.10.1_and-3.11.1.mp4 00:00-end

3.11.2. SCREEN: 69169_screenshot_8_3.11.2.mp4. 00:00-end

3.11.3. SCREEN: 69169_screenshot_9_3.11.3.mp4. 00:00-end

3.12. Press **Cine Store** to save both the ultrasound image and spectral waveform [1].

3.12.1. Talent pressing the Cine Store function.

3.13. When imaging is complete, wipe off ultrasound gel from the back [1] and gently remove the mouse from the imaging platform [2]. and place the mouse on a heating pad until fully awake [3].

NOTE: VO has been edited to reflect the order of the moved shots

3.13.1. Talent lifting the mouse off the platform.

3.13.2. Talent wiping the back clean.

AUTHOR'S NOTE: Move 3.13.2 before 3.13.1

3.13.3. Talent placing mouse on heating pad.

Results

4. Results

- 4.1. Power Doppler imaging revealed a visible increase in perfusion within the medullary vessels at 1 hour post-human chorionic gonadotropin injection [1], followed by a return to baseline at 8 hours [2] and further decrease by 12 hours post-injection [3].
 - 4.1.1. LAB MEDIA: Figure 2A and B. *Video editor: Highlight the image row for 1 hour post-hCG*
 - 4.1.2. LAB MEDIA: Figure 2A and B. *Video editor: Highlight the image row for 8 hours post-hCG*
 - 4.1.3. LAB MEDIA: Figure 2A and B. *Video editor: Highlight the image row for 12 hours post-hCG*
- 4.2. Color Doppler imaging enabled clear differentiation between the ovarian artery and surrounding vein and improved visualization of flow direction within ovarian vessels [1].
 - 4.2.1. LAB MEDIA: Figure 2C.
- 4.3. Representative PW Doppler waveforms displayed distinct cycles with measurable peak systolic and end diastolic velocities in the ovarian artery of a hormonally responsive mouse [1], whereas a non-responsive mouse exhibited low-signal traces with no detectable waveform [2].
 - 4.3.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the three repeated waveform peaks and troughs labeled PSV and EDV in the upper trace.*
 - 4.3.2. LAB MEDIA: Figure 3D.
- 4.4. At 1 hour post-human chorionic gonadotropin injection, blood flow velocity increased in the ovarian artery, medullary vessels, and cortical vessels in mice that responded to stimulation [1], but no such increase was observed in the non-responsive mouse [2].
 - 4.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the peak at 1 hour post-hCG across all three vessel types (left, middle, right panels) in the blue, pink, and purple lines*
 - 4.4.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the orange dashed line (Mouse 4) across all three panels*
- 4.5. In non-responsive mice, the velocity in cortical vessels dropped below the detection threshold at selected time points, indicated by assigned placeholder values [1].
 - 4.5.1. LAB MEDIA: Figure 4A (rightmost panel). *Video editor: Highlight the orange dashed line showing points at 8 hours and 11 hours post-hCG*

Pronunciation Guide:

❓ Doppler

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

IPA: /'dɑːplə/

Phonetic Spelling: daa·pluhr

❓ Ultrasonography

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

IPA: /,ʌltrə'sɑː'nɑːgrəfi/

Phonetic Spelling: uhl·truh·saa·naa·gruh·fee

❓ Quantification

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

IPA: /,kwɑːntəfə'keɪʃən/

Phonetic Spelling: kwon·tuh·fuh·kay·shuhn

❓ Ovarian

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

IPA: /oʊ'veəriən/

Phonetic Spelling: oh·vair·ee·uhn

❓ Vascular

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

IPA: /'væskjələr/

Phonetic Spelling: vas·kyuh·luhr

❓ Murine

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

IPA: /'mjʊr,əɪn/

Phonetic Spelling: myoor·ine

❓ Preovulatory

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

IPA: /,priː'ɑːvjələr,tɔːri/

Phonetic Spelling: pree·ov·yuh·luh·tohr·ee

❓ Hemodynamics

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

IPA: /,hiːmoʊdaɪ'næmɪks/

Phonetic Spelling: hee·moh·dye·na·miks

❓ **Noninvasive**

Pronunciation link:
[webster.com/dictionary/noninvasive](https://www.merriam-webster.com/dictionary/noninvasive)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/noninvasive)

IPA: /ˌnɑːnɪnˈveɪsɪv/

Phonetic Spelling: non·in·vay·siv

❓ **Polycystic Ovary Syndrome (PCOS)**

Pronunciation link:
[webster.com/dictionary/polycystic%20ovary%20syndrome](https://www.merriam-webster.com/dictionary/polycystic%20ovary%20syndrome)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/polycystic%20ovary%20syndrome)

IPA: /ˌpɑːliˈsɪstɪk ˈoʊvəri ˈsɪndroʊm/

Phonetic Spelling: pah·lee·sis·tik oh·vuh·ree sin·drohm

❓ **Isoflurane**

Pronunciation link:
[webster.com/dictionary/isoflurane](https://www.merriam-webster.com/dictionary/isoflurane)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/isoflurane)

IPA: /ˌaɪsoʊˈflʊreɪn/

Phonetic Spelling: eye·soh·floor·ane

❓ **Transducer**

Pronunciation link:
[webster.com/dictionary/transducer](https://www.merriam-webster.com/dictionary/transducer)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/transducer)

IPA: /trænzˈduːsə/

Phonetic Spelling: tranz·doo·suhr

❓ **Periovarian**

Pronunciation link:
[webster.com/dictionary/periovarian](https://www.merriam-webster.com/dictionary/periovarian)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/periovarian)

IPA: /ˌpɛriˈoʊvəriən/

Phonetic Spelling: pehr·ee·oh·vair·ee·uhn