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Title: Mouse Model of Surgical Uterine Injury and Subsequent Pregnancy Outcomes

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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**

   SCOPE: 2.1.3, 2.2-2.5

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

3. Filming location: Will the filming need to take place in multiple locations? **Yes. Different floors in the same building.**

   If **Yes**, how far apart are the locations? We may need to film interview questions in our main lab as opposed to the surgical suite. These spaces are both in the same building (Tupper Hall), separated by 2 floors.

Current Protocol Length
Number of Steps: 15
Number of Shots: 31

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Nathan L. Ng:** We are investigating how uterine scarring from C-sections or other procedures can result in several morbidities in subsequent pregnancies, including placenta previa, placenta accreta spectrum, and infertility.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.4*

What research gap are you addressing with your protocol?

1.2. **Elisa T. Zhang:** Understanding the mechanisms by which uterine injury leads to subsequent pregnancy defects has been challenging without animal models. Therefore, we have developed a new mouse model to address this research gap.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.5*

How will your findings advance research in your field?

1.3. **Elisa T. Zhang:** This animal model of uterine injury enables researchers to study uterine wound responses and how impaired healing causes developmental defects during pregnancy. This model also serves as a platform for testing interventional strategies.

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

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

1.4. **Elisa T. Zhang:** Our model allows researchers to study how the uterus heals, how defects arise in an injured uterus, what changes are present at the scar, and why some embryonic defects occur even in the uninjured uterine horn.

1.4.1. 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?

1.5. **Nathan L. Ng:** We will investigate how injuries lead to embryo implantation failure, how embryos resorb at the scar, and why only injuries during the later phases of the estrous cycle lead to defects like embryo misspacing.

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

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Elisa T. Zhang:** We have used JoVE publications in the past to learn new techniques and to reduce training time.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at University of California, Davis

Protocol

2. Surgical Preparation and Uterine Injury Induction in Anesthetized Mouse

Demonstrator: Elisa T. Zhang

VIDEOGRAPHER'S NOTE: Please use take 2 of the surgery

2.1. To begin, prepare the pre-operative area **[1].** Cover an anesthetized animal with a sterile drape, exposing only the snout **[2-TXT]**. ~~Cut a small square over the shaved region of the skin in preparation for the incision **[3].**~~

2.1.1. WIDE: Talent preparing the pre-operative area.

2.1.2. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4 00:24-00:31, 00:37-00:43

TXT: Anesthesia: Ketamine/xylazine injection (i.p)

~~2.1.3. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4~~

2.2. Use a pair of straight or curved iris scissors to cut a 2-to-3-millimeter incision in the dorsal skin while holding the skin with fine forceps **[1]**. Cut a 2-to-3-millimeter incision in the fascia overlaying the ovarian fat pad **[2]**.

2.2.1. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 01:38-01:58

2.2.2. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 02:11-02:26

2.3. Gently grip the ovarian fat pad with forceps and extract it from the fascia and skin incisions **[1]**. After exposing the upper third of the uterus, gently secure the uterotubal junction with forceps **[2]**. Then use a burred needle to create a small hole at the top of the uterus **[3]**.

2.3.1. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 06:29-06:39

2.3.2. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 06:55-07:04

2.3.3. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4.07:06-07:14

2.4. Then insert the needle and carefully scrape the anti-mesometrial surface of the uterine horn **[1]**. Continue scraping until both the endometrial and myometrial layers are fully perforated **[2]**.

2.4.1. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 07:15-07:30

2.4.2. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 07:38-07:54

2.5. Now gently return the injured uterine horn to the body cavity using forceps **[1]**. Suture the fascia closed with size 4-0 *(Four-oh)* suture and remove any excess thread **[2]**. Close the skin incision using a clip from a skin clip applicator **[3]**.

2.5.1. SCOPE: SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 08:08-08:28

2.5.2. SCOPE**:** SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 08:58-09:40
2.5.3. SCOPE**:** SCOPE---Take-2-(preferred-take)_1_lowres.mp4. 11:11-11:21

Results

3. Representative Results

3.1. A uterine wound at 3 days post-injury showed an open lesion with the uterine lumen visibly exposed **[1]**. At 1-month post-injury, the uterine wound had closed with minimal visible scarring at the prior injury site **[2]**.
- 3.1.1. LAB MEDIA: Figure 10A. *Video editor: Please highlight the area marked by the orange and green arrows*
- 3.1.2. LAB MEDIA: Figure 10B. *Video editor: Emphasize the location marked by the orange arrow*

3.2. Injured uterine horns showed resorbing embryos and intrauterine bleeding, visible as darkened areas and hemorrhage **[1]**. A normal implantation outcome featured a single placenta and embryo clearly separated within the uterine horn **[2]**.
- 3.2.1. LAB MEDIA: Figure 10C. *Video editor: Highlight the regions indicated by arrows showing the resorbing embryos and areas of maternal hemorrhage.*
- 3.2.2. LAB MEDIA: Figure 10D. *Video editor: Focus on the orange and green arrow*

3.3. Uterine injury resulted in close pairs of embryos with fused placentas, illustrating embryo misspacing **[1]**. A misspaced embryo pair displayed one underdeveloped embryo and a normal amniotic sac, reflecting adverse developmental outcomes **[2]**.
- 3.3.1. LAB MEDIA: Figure 10E. *Video editor: Highlight the white and orange arrows*
- 3.3.2. LAB MEDIA: Figure 10F. *Video editor: Focus on the orange and white arrows*

Pronunciation Guide:

1. **Uterine**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/uterine
   IPA: /ˈjuːtərɪn/ or /ˈjuːtəˌraɪn/
   Phonetic Spelling: YOO-tur-in or YOO-tuh-rine
2. **Placenta previa**
   Pronunciation link (Placenta):
   https://www.merriam-webster.com/dictionary/placenta
   Pronunciation link (Previa):
   https://www.howtopronounce.com/previa
   IPA: /pləˈsɛntə ˈpriːviə/
   Phonetic Spelling: pluh-SEN-tuh PREE-vee-uh

3. **Placenta accreta**
   Pronunciation link (Accreta):
   https://www.howtopronounce.com/accreta
   IPA: /pləˈsɛntə əˈkriːtə/
   Phonetic Spelling: pluh-SEN-tuh uh-KREE-tuh
4. **Myometrial**
   Pronunciation link:
   https://www.merriam-webster.com/medical/myometrium
   IPA: /ˌmaɪoʊˈmiːtriəl/
   Phonetic Spelling: my-oh-MEE-tree-uhl
5. **Endometrial**
   Pronunciation link:
   https://www.merriam-webster.com/medical/endometrium
   IPA: /ˌɛndoʊˈmiːtriəl/
   Phonetic Spelling: en-doh-MEE-tree-uhl
6. **Estrous**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/estrous
   IPA: /ˈɛstrəs/
   Phonetic Spelling: ES-truhs
7. **Anti-mesometrial**
   Pronunciation link: No confirmed link found
   IPA: /ˈæn.taɪ ˌmiːz.oʊˈmiː.tri.əl/
   Phonetic Spelling: AN-ty mee-zoh-MEE-tree-uhl
8. **Ketamine**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/ketamine
   IPA: /ˈkɛtəˌmiːn/
   Phonetic Spelling: KET-uh-meen
9. **Xylazine**
   Pronunciation link:
   https://www.merriam-webster.com/medical/xylazine
   IPA: /ˈzaɪləˌzin/
   Phonetic Spelling: ZY-luh-zeen
10. **Intraperitoneal (i.p.)**
    Pronunciation link:
    https://www.merriam-webster.com/medical/intraperitoneal
    IPA: /ˌɪntrəˌpɛrɪtəˈniːəl/
    Phonetic Spelling: in-truh-PEH-rih-tuh-NEE-uhl
11. **Uterotubal**
    Pronunciation link: No confirmed link found
    IPA: /ˌjuːtəroʊˈtjuːbəl/
    Phonetic Spelling: YOO-tuh-roh-TOO-buhl

12. **Fascia**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/fascia
    IPA: /ˈfæʃiə/
    Phonetic Spelling: FASH-ee-uh
13. **Hemorrhage**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/hemorrhage
    IPA: /ˈhɛmərɪdʒ/
    Phonetic Spelling: HEM-uh-rij
14. **Amniotic**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/amniotic
    IPA: /ˌæmniˈɑːtɪk/
    Phonetic Spelling: am-nee-AH-tik
15. **Resorption**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/resorption
    IPA: /rɪˈzɔrpʃən/
    Phonetic Spelling: rih-ZORP-shun