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Title: A Syngeneic Murine Model of Endometriosis Using Naturally Cycling Mice

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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 similar? **Y**

   If you require a microscope but **do not** have a camera, JoVE will need to use our scope kit to film through a camera port or one of the oculars of your microscope. Please list the make and model of your microscope here: **Leica S4E; 10446339**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as reasonably possible.

   Videographer: Film the screen for all SCREEN shots as a backup.

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **41**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Elliott Richards**: Our murine model of endometriosis is a simplified and efficient system that integrates the best features of existing models and, perhaps most importantly, relies on microscopic quantification in lieu of subjective grading **[1]**.

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

REQUIRED:

1.2. **Elliott Richards**: Modeling endometriosis can be difficult, because there can be many confounding factors and sources of bias. In our approach, endometriosis is induced without ovariectomy or survival surgery and the endometrial lesions are objectively quantified **[1]**.

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

OPTIONAL:

1.3. **Jenna Rehmer**: These innovations provide a highly reliable and reproducible animal model of endometriosis that can be used to further endometriosis disease research **[1]**.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at The Learner Research Institute at The Cleveland Clinic.

Protocol

2. Donor and Recipient Mouse Preparation and Donor Mouse Endometrial Tissue Harvest

2.1. To facilitate ongoing estrous cycling, place urine-soaked bedding from a male mouse cage into the recipient female mouse cage periodically throughout development and again at 72 hours before endometrial tissue transplant **[1]**.

2.1.1. WIDE: Talent adding bedding to cage

2.2. When the donor female mice are between 22-24 days old, use a 1-milliliter syringe equipped with a 25-27-gauge needle to subcutaneously inject 2 international units of Pregnant Mare Serum Gonadotropin in 200 microliters of sterile saline into the lower mouse abdomen **[1-TXT]**.

2.2.1. Mouse being injected **TEXT: BALB/cJ mice were used for this demonstration**

2.3. Thirty-eight to forty-two hours after injection, use dissecting scissors to make a shallow, transverse incision through the skin and subcutaneous tissue of the euthanized mouse at the midline **[1]** and use blunt traction to open the skin on either side of the incision **[2]**.

2.3.1. Snip being made
2.3.2. Skin being opened

2.4. Before removing the uterus, trim the adjacent connective tissue **[1]** and transect each uterine horn just below its respective fallopian tubes **[2]**. Then transect the cervix to allow removal of the entire uterus en bloc **[3]**.

2.4.1. Shot of uterus, then connective tissue being trimmed
2.4.2. Horn being transected
2.4.3. Cervix being transected

2.5. Inspect the uterus carefully, removing any additional peripheral fat or connective tissue **[1]** and place the uterus in a droplet of cold PBS on a Petri dish for weighing **[2]**.

2.5.1. Uterus being inspected/tissue being removed
2.5.2. Talent placing uterus into dish, with PBS and balance visible in frame as possible

2.6. Transect each horn across the uterine fundus as close to the fundus as possible **[1]** and place the dish under a dissecting microscope **[2]**.

2.6.1. Horn being transected *Videographer: Important step*
2.6.2. Talent placing dish under microscope *Videographer: Important step*

2.7. Placing one blade of the dissecting scissors inside the lumen of the first horn, cut along the major axis of the tube, keeping in mind which side is the serosa and which side is the epithelium **[1]**. Then use forceps to grasp and peel away the endometrial layer **[2]** and mince the tissue into ten to twelve 1- x 1-millimeter fragments **[3]**.

2.7.1. SCOPE: Blade being inserted, then tube being incised *Videographer: Important step*
2.7.2. SCOPE: Endometrial layer being grasped and peeled *Videographer: Important step*
2.7.3. SCOPE: Tissue being fragment *Videographer: Important step*

2.8. Alternately, mince the tissue into 1- x 1-millimeter pieces without separating off the myometrium **[1]**.

2.8.1. SCOPE: Tissue being fragmented without myometrium removal *Videographer: Important step*

2.9. Then place the tissues into 500 microliters of saline in a new Petri dish **[1]** and harvest the endometrium from the second uterine horn as demonstrated **[2-TXT]**.

2.9.1. Talent placing tissues into dish, with saline container visible in frame
2.9.2. SCOPE: Horn being transected **TEXT: Record total tissue fragment number**

3. Peritoneal Tissue Fragment Injection

3.1. For peritoneal delivery of the tissue fragments into the recipient animal, use the blunt end of a 1-milliter syringe to collect the tissue pieces **[1]**. A total volume of 1 milliliter of tissue and saline should be collected **[2]**.

3.1.1. WIDE: Talent aspirating fragments *Videographer: Important/difficult step*
3.1.2. Shot of syringe filled with tissue and saline *Videographer: Important/difficult step*

3.2. Attach an 18-gauge needle to the syringe **[1]** and gently depress the plunger to load the fluid into the needle **[2]**. A mock injection into the Petri dish can be performed to ensure that all of the tissue will pass through the needle tip **[3]**.

3.2.1. Talent attaching needle to syringe
3.2.2. Plunger being depressed
3.2.3. Tissue being injected into dish

3.3. Either before or after the injection, use a bulb syringe to administer 10 microliters of saline to obtain a vaginal smear of the recipient animal for estrous cycle documentation **[1]**.

3.3.1. Talent adding sample to slide, with recipient mouse cage visible in frame

3.4. Holding the syringe at a 45-degree angle, intraperitoneally inject the fragments **[1-TXT]**. If fragments remain after the injection, draw an additional 200 microliters of saline into the syringe to allow the rest of the fragments to be injected **[2]**.

3.4.1. Syringe being held at 45° angle, then fragments being injected *Videographer: Important/difficult step* **TEXT: Caution: Do not inject subcutaneously**
3.4.2. Talent drawing saline into syringe *Videographer: Important/difficult step*

3.5. Once assured of no bleeding or complications, place the mouse back in the home cage with a normal diet **[1]**.

3.5.1. Talent placing mouse into cage

4. Endometriotic Lesion Harvest

4.1. Approximately 21 days after fragment injection, spray the recipient mouse abdomen with 70% ethanol **[1]** and tent the skin to allow the creation of a superficial, subcutaneous incision to bluntly open the abdomen **[2]**.

4.1.1. WIDE: Talent spraying mouse *Videographer: More Talent than mouse in shot*
4.1.2. Skin being tented/incised

4.2. Perform a complete survey of the abdominal wall and peritoneum **[1]** … pancreas and mesenteric fat **[2]** … and parauterine connective fat and tissue for gross lesions **[3-TXT]**.

4.2.1. Shot of abdominal wall and peritoneum (w/ lesions as possible)
4.2.2. Shot of pancreas and mesenteric fat (w/ lesions as possible)
4.2.3. Shot of parauterine connective fat and tissue (w/ lesions as possible) **TEXT: Ignore lesions observed in other regions**

4.3. Harvest each region of tissue entirely **[1]** and place the dissected tissue samples into an appropriately labeled cassette for formalin fixation and processing according to standard protocols **[2]**.

4.3.1. Tissue being dissected
4.3.2. Talent placing tissue into cassette

4.4. Then section the formalin blocks onto two slides per tissue area at two uniform depths **[1]**.

4.4.1. Shot of slide with mounted tissue sections

5. Endometriotic Lesion Scoring

5.1. To score the endometriotic lesions, scan the tissue sections at a 40x magnification **[1]** and archive the slides **[2]**.

5.1.1. WIDE: Talent loading slide(s) onto scanner
5.1.2. Talent archiving slide(s)

5.2. After scanning, use an appropriate digital slide reading software program **[1]** to define and mark the longest distance between the edges of each endometriotic lesion. A continuous lesion is defined by glands surrounded by stroma. The line does not necessarily traverse only the endometriotic tissue, but the two end points must be connected by continuous stroma and/or glands **[2]**.

5.2.1. Talent at computer, opening software, with monitor visible in frame
5.2.2. SCREEN: Distance being defined and marked *Videographer: Film the screen for all SCREEN shots as a backup.*

NOTE: Use the videographer's footage for all SCREEN shots, authors did not upload the screen capture videos.

5.3. Make a second line 90 degrees across the first line as demonstrated. If multiple non-contiguous lesions are encountered, give each their own X and Y measurements **[1]**.

5.3.1. SCREEN: Second line being made, X and Y measurements being assigned *Videographer: Film the screen for all SCREEN shots as a backup.*

5.4. Calculate the final score for each slide as the summation of the areas of each lesion and use the larger of the scores from the two sections on each slide as the final score for that tissue region **[1]**. Then total the scores from each region to give the final microscopic score for that animal **[2]**.

5.4.1. SCREEN: Final scores being calculated *Video Editor: please emphasize larger score*

5.4.2. SCREEN: Scores being totaled *Video Editor: please emphasize final score*
Videographer: Film the screen for all SCREEN shots as a backup.

Results

6. Results: Representative Endometriosis Lesion Histopathological Analyses

6.1. In this representative sample, histopathologic analysis after donor endometrium injection **[1]** revealed a classic architecture of endometriosis lesion **[2]**.

6.1.1. LAB MEDIA: Figure 3A
6.1.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize legion on left of image*

6.2. Fluorescent microscopy of the tissue sample confirmed that the lesion originated from the donor **[1]**.

6.2.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize red signal*

6.3. It is important to note that macroscopic examination for lesions alone is not sufficient for the quantification of the endometriosis disease burden **[1]**, as the gross "lesions" observed in these representative samples were ultimately determined not to be endometriosis upon histological examination **[2]**.

6.3.1. LAB MEDIA: Figure 5
6.3.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize "lesions" in images*

Conclusion

7. Conclusion Interview Statements

7.1. **Elliott Richards**: Collection of all of the tissue within the designated regions reduces bias by allowing for a systematic, objective approach to lesion quantification **[1]**.

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

7.2. **Jenna Rehmer**: Once this reliable and reproducible model has been achieved, researchers can use it investigate a multitude of endometriosis pathways and therapies **[1]**.

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