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Title: Transuterine Fetal Tracheal Occlusion Model in 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 something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes

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

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 videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, 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? **No**

Current Protocol Length

Number of Steps: 22
Number of Shots: 46

Introduction

1. Introductory Interview Statements

NOTE: Authors has submitted self-filmed interview statements.

REQUIRED:

1.1. **Emrah Aydin:** The most apparent advantages of this technique are reversibility, reduction in cost of the animals and maintenance, and a shorter pregnancy period. Moreover, animal mortality is reduced because there is no need for hysterotomy and there are wider opportunities for genetic work up.

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

1.2. **Emrah Aydin:** This technique can be used for investigating the pathophysiology of the congenital diaphragmatic hernia. It can also be applied to understanding other diseases in the lung such CHAOS and pulmonary hypoplasia

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

Ethics Title Card

1.3. The procedure was approved by the Cincinnati Children's Research Foundation Institutional Animal Care and Use Committee (IACUC).

Protocol

2. Preparation

2.1. Begin by allowing the mice to mate in the same cage from 6:00pm to 9:00 am the next day **[1-TXT]**.

2.1.1. Talent keeping the mice in the cage for mating. **TEXT: WT C57BL/6 mice**

2.2. To determine E0, observe the vaginal plug **[1-TXT]**, which has a homogeneous outer zone attached to the vaginal wall and an inner zone that is fibrous and includes some spermatozoa that form entangled masses mixed with the fibers of the plug material **[2]**.

2.2.1. Talent observing the vaginal plug. **TEXT: E0-Embryonic day 0**

2.2.2. Vaginal plug.

2.3. Record the weight of the mice at the time of mating and on E10 to ensure ongoing pregnancy and perform the surgery on E16.5 **[1]**. Sterilize the instruments that are going to be used during the surgery **[2]**.

2.3.1. Talent recording the weight of the mice.

2.3.2. Talent sterilizing the instruments. *Videographer take a single shot of all the sterilized instruments.*

2.4. Pre-heat the surgery platform to 24 degrees Celsius **[1]** and prepare warm saline prior to surgery **[2]**. Create a warm environment for recovery and leave wet food inside the cage for the early feeding **[3]**.

2.4.1. Talent pre-heating the surgery platform

2.4.2. Talent pre-heating the surgery platform.

2.4.3. Talent leaving wet food inside the cage.

3. Laparotomy

3.1. Clean the abdominal surface with alcohol and povidone-iodine and maintain sterile conditions throughout the operation **[1]**.

3.1.1. Talent cleaning the abdominal surface.

3.2. Perform a vertical incision for the laparotomy of pregnant dams **[1]** and cut all layers separately **[2]**. Identify the uterine horns on each side and determine the candidate fetuses for the surgery **[3]**.

3.2.1. Talent performing a vertical incision on pregnant dams.

3.2.2. Talent cutting all layers.

3.2.3. Talent identifying the horns and determining the fetuses for surgery.

3.3. Operate on two fetuses in each uterine horn if there is an even number of fetuses on each side, and on 1 fetus in each uterine horn if there is an odd number **[1]**.

3.3.1. Fetuses in a uterine horn.

4. Tracheal occlusion

4.1. Using 2.5x magnification glasses for visualization, position the uterine horn in a transverse fashion **[1]**. *Videographer: This step is important!*

4.1.1. Talent positioning the uterine horn in transverse fashion.

4.2. Position the pups, facing upward, between two fingers using the eyes and the tail as a guide **[1]**. Apply gentle pressure to the pup's head to allow extension of the head and visualization of the neck **[2]**. *Videographer: This step is difficult and important!*

4.2.1. Talent taking the pups between two fingers.

4.2.2. Talent applying gentle pressure to the pup's head to visualize the neck.

4.3. Perform tracheal occlusion, or TO, using an atraumatic needle and a 6 by 0 polypropylene suture. Keep the placenta on the side and far from the entrance and exit points of the needle **[1]**. *Videographer: This step is difficult and important!*

4.3.1. Talent positioning the needle.

4.4. Insert the needle transversely through the side of the uterus away from the placenta through the one-third anterior part of the neck, then move the needle gently to the midline of the neck. Direct it to the anterior part, then exit the neck between the trachea and opposite the carotid sheath and uterus **[1]**. *Videographer: This step is difficult and important!*

4.4.1. ECU: Talent inserting the needle through the side of the uterus and moving it to the midline of the neck, then exiting the neck.

4.5. Knot the suture, taking care to maintain the integrity of the membranes and uterine wall **[1-TXT]**. *Videographer: This step is important!*

4.5.1. Talent knotting the suture. **TEXT: Keep the umbilical cord safe**

4.6. Replace the uterine horn in the abdomen **[1]** and inject 2 milliliters of warm sterile saline into the peritoneal cavity before closure **[2]**.

4.6.1. Talent replacing the uterine horn.

4.6.2. Talent injecting warm saline into the peritoneal cavity.

4.7. Place a running 5 by 0 polyglactin suture to close the abdominal wall **[1]** and close the skin with a non-running silk suture **[2]**.

4.7.1. Talent closing the abdominal wall with polyglactin suture.

4.7.2. Talent closing the skin with silk suture.

4.8. Apply 0.1 microgram per kilogram of buprenorphine intraperitoneally for analgesia **[1]** and allow the dam to recover in a warm incubator **[2]**.

4.8.1. Talent applying buprenorphine to the animal.

4.8.2. Talent placing the animal in warm incubator for it to recover.

4.9. Observe that the operated animal can feed themselves and keep it alone in its individual cage **[1]**.

4.9.1. Talent observing the feeding ability of animal.

5. Harvest

5.1. Harvest all the fetuses at E18.5 by cesarean section **[1]** and check their viability by watching their movements **[2]**. Weigh all the fetuses **[3]**.

5.1.1. Harvested fetuses.

5.1.2. Talent observing the movements of the fetuses.

5.1.3. Talent weighing the fetuses.

5.2. Perform a vertical incision on the thorax for a thoracotomy **[1]** to dissect the lungs **[2]** and weigh them to calculate total lung to body weight ratio **[3-TXT]**.

5.2.1. Talent performing vertical incision.

5.2.2. Talent removing the lungs.

5.2.3. Talent weighing the lungs. **TEXT: LBWR = (left lung weight + right lung weight)/body weight x 100**

6. Histology and tissue processing for protein and DNA analyses

6.1. Snap-freeze the tissue in liquid-nitrogen, optimal cutting temperature compound, and dry ice **[1]**. Cut the samples in sections of 10 micrometers using a cryostat **[2]** and mount them on poly-lysine-coated slides **[3]**.

6.1.1. Talent snap-freezing the tissue.

6.1.2. Talent cutting sections of the tissue using cryostat.

6.1.3. Talent mounting the sections on slides.

6.2. Bake the slides overnight at 60 degrees Celsius **[1]** and stain the baked slides with hematoxylin and eosin **[2]**. Image the slides at 10 to 20x magnification using a widefield microscope **[3]**.

6.2.1. Talent keeping the slides for baking overnight.

6.2.2. Talent staining the slides with hematoxylin and eosin.

6.2.3. Talent mounting the slides on the microscope.

6.3. For protein and DNA analyses, snap-freeze the tissue **[1]** and homogenize it in 300 microliters of radioimmunoprecipitation assay buffer **[2]**.

6.3.1. Talent snap-freezing the tissue.

6.3.2. Talent homogenizing the tissue in radioimmunoprecipitation assay buffer.

6.4. Centrifuge at 18,000 times *g* and 4 degrees Celsius for 5 minutes **[1]**. Then, extract and quantify protein, DNA, and RNA **[2]**.

6.4.1. Talent centrifuging the tissue.

6.4.2. Talent taking the tissue out of the centrifuge.

Results

7. Histological analyses of the fetal lung tissue dissected post TO

7.1. The mean body weight, lung weight, and LBWR were higher in the TO group than in the control group **[1]**.

7.1.1. LAB MEDIA: Table 1. *Video editor focus on Column B and Column C.*

7.2. Lung DNA amounts and the DNA to protein ratio were higher in the TO group **[1]**. No difference was observed in lung RNA **[2]** and protein amounts were lower in the TO group than in the control group **[3]**.

7.2.1. LAB MEDIA: Figure 2 B and C

7.2.2. LAB MEDIA: Figure 2D

7.2.3. LAB MEDIA: Figure 2E

7.3. Histological analyses of the E18.5 control lungs showed the late canalicular or early saccular stage of lung development with developing airspaces and thickened interstitium between epithelial surfaces **[1]**. The lungs in the TO group had dilated central and distal airspaces with subjectively higher numbers of nuclei **[2]**.

7.3.1. LAB MEDIA: Figure 2F and 2G.

7.3.2. LAB MEDIA: Figure 2F and 2G. *Video editor focus on the black square region.*

Conclusion

8. Conclusion Interview Statements

8.1. **Emrah Aydin:** When attempting this protocol, fix the uterus in a way that the eyes of the fetus are visible and the neck is extended so that the needle does not disrupt the adjacent structures.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2 and 4.4*

8.2. **Emrah Aydin:** The removal of the transuterine suture with the live birth of the fetus in the nitrofen and knockout models of CDH will be the future applications of this technique.

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