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Title: Development of a Neonatal Piglet Acute Lung Injury Model Recreating the Early Environment of Preterm Infant Lungs

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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? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

Videographer: Please record the computer screen for the shots labeled as SCREEN

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 22
Number of Shots: 39 (12 SC)

Introduction

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

1.1. **Marc-Olivier Deguise:** Dr. Thebaud's laboratory is pioneering the use of umbilical-cord-tissue-derived mesenchymal stromal cells for neonatal lung disease, also called bronchopulmonary dysplasia. Successful clinical translation is the focus of our current efforts.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the most recent developments in your field of research?

1.2. **Ewa Henckel:** We recently completed a phase-I clinical trial of intravenous UC-MSC administration in preterm infants at risk of developing BPD, providing important safety data for future efficacy-studies. However, important questions remain.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

What research gap are you addressing with your protocol?

1.3. **Ewa Henckel:** This clinically relevant large animal model of acute lung injury will allow optimization of the delivery and therapeutic capacity of UC-MSC and facilitate clinical translation of new therapies to patients.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Ewa Henckel:** Our neonatal acute lung injury model in newborn piglets mimics the early exposures of preterm human lungs with surfactant-depletion, hyperoxia, high-pressure-ventilation and inflammation being factors involved in the pathophysiology of BPD.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

How will your findings advance research in your field?

1.5. **Marc-Olivier Deguise:** This model offers insights of early BPD pathogenic processes. Proof-of-concept-studies for safety and efficacy in our piglets model will provide advances on therapeutic candidates for acute lung injury in preterm infants.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

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

Testimonial Questions (OPTIONAL):

Videographer: Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

1.6. **Ewa Henckel, MD, PhD**: The use of JoVE simplifies knowledge transfer of this complex model to not only new members joining our team, but other laboratories who wish to move a translational model of lung injury.

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.1*

Ethics Title Card

This research has been approved by the University of Ottawa Animal Care Committee and the Animal Care and Veterinary Service

Protocol

2. Induction of Acute Lung Injury (Multi-Hit Model)

Demonstrators: Marc-Olivier Deguise, Doreen Engelbert, Arul Vadivel, Shumei Zhong

2.1. To begin, turn on the suction apparatus and confirm that it is ready to use **[1]**. Install the lavage collection bucket in place **[2]**.

2.1.1. WIDE: Talent switching on the suction apparatus.

2.1.2. Talent placing the lavage collection bucket beside the surgical setup.

2.2. Weigh the absorbent pads before starting the lavage **[1]**. Then, position the pads under the head of the animal and beneath the surgical table to collect all leaking fluid during the lavage **[2]**. Set the ventilator to a positive end-expiratory pressure of 5 centimeters of water, peak inspiratory pressure of 25 centimeters of water **[3]**, respiratory rate of 25 per minute, and a fraction of inspired oxygen of 1 **[4-TXT]**.

2.2.1. Talent placing pads on a scale.

2.2.2. Talent spreading absorbent pads under the piglet's head and below the surgical table.

2.2.3. SCREEN: Show the ventilator settings being configured to PEEP 5, PIP 25. **Author's NOTE**: Please use the first take

SCREEN: Setting RR to 25/min, and FiO_2 1.0. **TXT: Anesthesia:**

Maintenance: Propofol (10 - 30 mg/kg/h) + Ketamine (5 - 10 mg/kg/h)

Rocuronium (5.5 mg/kg/hr)

Author's NOTE: Please use the first take

Videographer: Please record the computer screen for the shots labeled as SCREEN

2.3. Now, disconnect the ventilatory circuit from the endotracheal tube **[1]** and attach the lavage funnel apparatus **[2]**.

2.3.1. Talent detaching the ventilator tubing from the endotracheal tube. **Author's NOTE**: Step 2.3 to Step 2.8 were filmed from multiple different angles in order to best show the procedure

2.3.2. Talent connecting the funnel setup.

2.4. To instil saline into the lungs, gently pour 30 milliliters per kilogram of warm isotonic

saline into the funnel held approximately 30 centimeters above the anesthetized piglet **[1-TXT]**.

2.4.1. Talent slowly pouring in saline into the funnel while holding the funnel 30 centimeters above the piglet.

2.5. Bilaterally press on the lateral aspect of the ribcage area to provide a mechanical squeeze and massage the area **[1]**.

2.5.1. Talent pressing both sides of the piglet's ribcage to simulate a mechanical squeeze.

2.6. Then, lower the funnel below the piglet to begin fluid drainage **[1]** and slightly disconnect the funnel from the endotracheal tube to allow lavage fluid to flow into the collection bucket on the floor **[2]**.

2.6.1. Talent lowering the funnel below the piglet.

2.6.2. Talent loosening the funnel connection and showing fluid draining into the bucket.

2.7. Next, insert the suction catheter into the endotracheal tube **[1]** and perform active suction for no more than 10 seconds while continuing ribcage massage to aid fluid removal **[2]**. Once confirmed, stop the lavage and proceed to the next step **[3]**

2.7.1. Talent inserting the suction catheter into the endotracheal tube.

2.7.2. Talent activating suction while massaging the ribcage.

2.10.2 Talent turning dissembling the lavage setup **NOTE**: 2.10.2 is moved here, VO is moved too

2.8. Now, reconnect the ventilatory circuit to the endotracheal tube **[1]** and let the piglet recover for at least 3 minutes between lavage rounds to reduce stress as well as risk of intolerance **[2]**.

2.8.1. Talent reattaching the ventilator tubing to the endotracheal tube.

2.8.2. Shot of the piglet calmly resting during the recovery period.

2.9. Start the next lavage round once the peripheral oxygen saturation returns to 100 percent **[1]**. During the lavage, the oxygen saturations levels can become as low as 5 **[2].** If saturation does not return to 100 percent, wait for stabilization and check the partial pressure of oxygen via blood gas analysis **[3-TXT]**.

2.9.1. SCREEN: Monitor showing SpO_2 levels returning to 100%.

Added shot: SCREEN: SpO2 levels going as low as 5

2.9.2. SCREEN: Accessing blood gas data to verify PaO_2. **TXT: Repeat the lavage step as required** **Author's NOTE:** Please blur the time appearing on the EPOCH analyser

2.10. Confirm that surfactant depletion injury is achieved when the partial pressure of oxygen remains below 100 millimeters of mercury for 15 minutes **[1]**. ~~Once confirmed, stop the lavage and proceed to the next step **[2]**~~.

2.10.1. SCREEN: cursor hovering over graph of PaO_2 staying below 100 mmHg over a 15-minute interval. **Author's NOTE:** Please blur the time appearing on the EPOCH analyser

2.10.2. Talent turning dissembling the lavage setup. **NOTE**: Move 2.10.2 after 2.7.2

3. Instillation of Intratracheal Endotoxin

Demonstrators: Marc Olivier Deguise, Doreen Engelberts

3.1. Prepare lipopolysaccharide or LPS from *Escherichia coli* at a dose of 1.5 milligrams per kilogram in normal saline **[1]** and aspirate a total of 2 milliliters into a 3-milliliter syringe **[2]**.

3.1.1. Talent inverting the tube with LPS solution to mix.

3.1.2. Talent drawing LPS into a syringe.

3.2. Fifteen minutes after the final lung lavage, prepare for the LPS instillation while the piglet is in the supine position **[1]**.

3.2.1. Talent adjusting the piglet's position and placing the LPS syringe beside the piglet.

3.3. To improve homogeneous distribution of LPS in the atelectatic lung, replace the standard endotracheal tube end with a Y-port adaptor to allow simultaneous ventilation and LPS administration **[1]**.

3.3.1. Talent disconnecting the ventilator tubing and attaching a Y-port adaptor to the endotracheal tube.

3.4. Apply a positive end-expiratory pressure of 10 centimeters of water for 1 minute **[1]**. Adjust the peak inspiratory pressure to maintain tidal volume at 7 milliliters per kilogram **[2]** and set the respiratory rate to 40 breaths per minute **[3]**.

3.4.1. SCREEN: PEEP being set to 10 centimeters of water for 1 minute.

3.4.2. SCREEN: Adjustments being made to PIP to maintain a tidal volume of 7 milliliters per kilogram.

3.4.3. SCREEN: Respiratory rate being set to 40 breaths/min.

3.5. Then, insert a catheter through the side port of the Y-adaptor into the endotracheal tube to a premeasured depth so the tip extends 1 to 2 millimeters beyond the tube **[1]**.

3.5.1. Talent feeding catheter through the side port.

3.6. Now, inject the LPS through the catheter **[1]** and flush the catheter with 1 milliliter of normal saline followed by a 9 milliliter air bolus to ensure complete delivery **[2]**.

3.6.1. Talent injecting LPS through the catheter.

3.6.2. Talent flushing the catheter with saline and then pushing in an air bolus using a syringe.

3.7. Then, remove the catheter and close the side port **[1]**.

3.7.1. Talent withdrawing the catheter and sealing the side port.

3.8. Disconnect the ventilator circuit from the endotracheal tube for 30 seconds **[1]**.

3.8.1. Talent disconnecting the ventilator circuit.

3.9. During the disconnection period, adjust the ventilator settings **[1]**.

3.9.1. TEXT ON PLAIN BACKGROUND:

FiO_2 (Fraction of Inspired Oxygen): 0.5

PEEP (Positive End-Expiratory Pressure): 6 cmH_2O

Set PIP (Peak Inspiratory Pressure) to maintain 7 mL/kg tidal volume

RR (Respiratory Rate): 40 - 60 breaths/min to achieve normal $PaCO_2$

3.10. Once the tidal volume is stabilized at 7 milliliters per kilogram, record the timepoint 0-hour physiological measurements and complete the case report form sheet **[1]**. Adjust the respiratory rate based on the partial pressure of carbon dioxide from the blood gas analysis **[2]**.

3.10.1. SCREEN: Display of ventilator panel showing stabilized tidal volume at 7 milliliters per kilogram.

3.10.2. Talent recording readings in the form and looking at blood gas data.

3.11. Next, during the 6-hour observation period, switch to volume-controlled ventilation with the same settings as demonstrated earlier to maintain normal partial pressure of carbon dioxide **[1]**.

3.11.1. SCREEN: Changing the ventilator mode to volume control.

3.12. Use hourly blood gas measurements to guide respiratory rate adjustments for the remainder of the experiment **[1]** and continuously adjust peak inspiratory pressure to maintain tidal volume at 7 milliliters per kilogram **[2]**.

3.12.1. SCREEN: pointing to the blood gas readings.

3.12.2. SCREEN: PIP being modified on ventilator to keep tidal volume consistent.

Results

4. Results

4.1. Multi-hit animals exhibited a significantly increased oxygenation index between 8 and 12 over the 6-hour period, indicating moderate to severe lung injury **[1]**, while their partial pressure of oxygen to fraction of inspired oxygen ratio dropped markedly **[2]**. The respiratory system compliance was reduced by over 50% **[3]** compared to control animals **[4].**

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4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the red line in panel A* .

4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the red line in panel B* .

4.1.3. LAB MEDIA: Figure 2. *Video editor: Highlight the red line in panel C*.

4.1.4. LAB MEDIA: Figure 2. *Video editor: Highlight the BLUE line in panel C*.

4.2. Multi-hit lungs showed clear macroscopic signs of patchy lung injury concentrated in the posterior central region **[1]** compared to controls **[2].**

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4.2.1. LAB MEDIA: Figure 3 panel B. *Video editor: Highlight the dark RED area in panel B of the multi-hit lung (extreme left image).*

4.2.2. LAB MEDIA: Figure 3 panel A. *Video editor: Highlight the lung image on the extreme left in panel A (control)*.

4.3. Histological analysis revealed marked neutrophilic infiltration and thickening of alveolar septa in multi-hit animals, indicating severe structural damage, including proteinaceous debris deposition in alveolar spaces **[1].**

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4.3.1. LAB MEDIA: Figure 3. *Video editor: Zoom into panel D AND F*

4.4. Neutrophils made up more than 75% of the bronchoalveolar lavage fluid cell population in multi-hit animals 6 hours after injury **[1]**

4.4.1. LAB MEDIA: Figure 4. *Video editor: Emphasize the red bar in panel A*.

4.5. Levels of interleukin-6 were highly elevated in bronchoalveolar lavage fluid and in lung tissue of multi-hit animals **[1]** compared to controls, reflecting an intense inflammatory response **[2].**

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4.5.1. LAB MEDIA: Figure 4. *Video editor: Highlight the RED bars in panels B and C*.

4.5.2. LAB MEDIA: Figure 4. *Video editor: Highlight the BLUE bars B and C*

1. lavage
Pronunciation link (Merriam-Webster):
https://www.merriam-webster.com/dictionary/lavage How To Say Guide+6How To Pronounce+6How To Pronounce+6Oxford English Dictionary+15Merriam-Webster+15Oxford English Dictionary+15
IPA: /ləˈvɑʒ/ or /ˈlævɪdʒ/
Phonetic spelling: luh-VAHZH or LAV-ij

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2. endotracheal
No confirmed Merriam-Webster or Oxford entry found—common term though.
IPA: /ˌɛnˌdoʊˈtreɪkiəl/
Phonetic spelling: en-doh-TRAY-kee-uhl

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3. rocuronium
No confirmed link.
IPA: /ˌroʊkjʊˈroʊniəm/
Phonetic spelling: roh-KYOO-ROH-nee-uhm

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4. lipopolysaccharide
Pronunciation link (Merriam-Webster):
https://www.merriam-webster.com/dictionary/lipopolysaccharide Definitions+5Collins Dictionary+5Wikipedia+5YouTube+11Merriam-Webster+11OpenMD+11
IPA: /ˌlɪpoʊˌpɑlɪˈsækəˌraɪd/
Phonetic spelling: lipo-pol-ee-SAK-uh-ried

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5. intratracheal
No link found.
IPA: /ˌɪn.trəˈtreɪkiəl/
Phonetic spelling: in-truh-TRAY-kee-uhl

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6. atelectatic
No link found.
IPA: /ˌeɪˌtɛlˈtæktɪk/
Phonetic spelling: ay-tel-TAK-tik

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7. bronchoalveolar
No link found.
IPA: /ˌbrɒŋkoʊˈælviələr/
Phonetic spelling: BRONG-koh-AL-vee-ohler

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8. interleukin-6
No link found (but interleukin itself is common).
IPA: /ˌɪn.tərˈluːkɪn sɪks/
Phonetic spelling: in-ter-LOO-kin six