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TITLE:

Left Lung Orthotopic Transplantation in a Juvenile Porcine Model for ESLP

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SUMMARY:

This protocol describes a juvenile porcine model of orthotopic left lung allotransplantation designed for use with ESLP research. Focus is made on anesthetic and surgical techniques, as well as critical steps and troubleshooting.

ABSTRACT:

Lung transplantation is the gold-standard treatment for end-stage lung disease, with over 4,600 lung transplantations performed worldwide annually. However, lung transplantation is limited by a shortage of available donor organs. As such, there is high waitlist mortality. *Ex situ* lung perfusion (ESLP) has increased donor lung utilization rates in some centers by 15%–20%. ESLP

has been applied as a method to assess and recondition marginal donor lungs and has demonstrated acceptable short- and long-term outcomes following transplantation of extended criteria donor (ECD) lungs. Large animal (*in vivo*) transplantation models are required to validate ongoing *in vitro* research findings. Anatomic and physiologic differences between humans and pigs pose significant technical and anesthetic challenges. An easily reproducible transplant model would permit the *in vivo* validation of current ESLP strategies and the preclinical evaluation of various interventions to reduce lung ischemia-reperfusion injury, a major cause of primary graft dysfunction—the foremost cause of morbidity and mortality post lung transplantation. Herein, this protocol describes a porcine model of orthotopic left lung allotransplantation. This includes anesthetic and surgical techniques, a customized surgical checklist, troubleshooting, modifications, and the benefits and limitations of the approach.

INTRODUCTION:

Lung transplantation is the preeminent long-term treatment for end-stage lung disease. Over 4,600 lung transplantations are performed worldwide annually[1]. However, lung transplantation currently has significant limitations. For one, the necessity for organs continues to eclipse available donors. Despite rates of lung transplantation increasing every year since 2012 due to the combined effects of more candidates being listed for transplant, an increase in the number of donors, and improved use of recovered organs, the transplant waitlist mortality has not decreased significantly[2]. Organ quality concerns represent another major limitation, with reported organ utilization rates as low as 20%–30%[3–5]. Finally, the trends in the post-operative outcomes of lung transplantation are less than satisfactory, with long-term graft and patient outcomes still lagging that of other solid organ transplantations[2].

An emerging technology, *ex situ* lung perfusion (ESLP), has the potential to mitigate these limitations. ESLP has been increasingly applied as a method to assess and recondition marginal donor lungs and has demonstrated acceptable short- and long-term outcomes following transplantation of extended criteria donor (ECD) lungs[6–10]. Consequently, ESLP has increased utilization rates in some centers by 15%–20%[6–11].

Proper ESLP research requires the *in vivo* validation of *in vitro* findings; however, there is limited literature on porcine lung transplantation models for ESLP[12–15]. Furthermore, available literature provides inadequate details regarding anesthetic management of Yorkshire pigs for lung transplantation, which can be highly unstable hemodynamically[12–15]. Establishing an easily reproducible model would permit the *in vivo* validation of current ESLP strategies and the preclinical evaluation of various interventions to reduce lung ischemia-reperfusion injury. The objective of the present study is to describe a porcine model of orthotopic left lung allotransplantation for use with ESLP. The protocol includes descriptions of the anesthetic and surgical techniques, a custom surgical checklist, and details regarding the troubleshooting experience and protocol modifications. The limitations and benefits of the left lung porcine transplantation model have also been discussed in this work. This manuscript does not outline the retrieval process of porcine lungs in 35–50 kg Yorkshire pigs, nor does it cover the establishment and termination of ESLP. This protocol exclusively addresses the recipient transplantation operation.

PROTOCOL:

All the procedures were performed in compliance with the guidelines of the Canadian Council on Animal Care and the guide for the care and use of laboratory animals. The protocols were approved by the institutional animal care committee of the University of Alberta. This protocol has been applied in female juvenile Yorkshire pigs between 35–50 kg. All individuals involved in ESLP procedures had received proper biosafety training.

1. Pre-surgical preparations/preparations and anesthesia

1.1. Administer intramuscular injections of ketamine (20 mg/kg) and atropine (0.05 mg/kg) as premedication for the recipient pig in the operating room.

1.2. Place the pig supine on a heated operating table to maintain normothermia and proceed with mask induction.

1.3. Titrate oxygen flow rate according to animal weight and the anesthetic system.

NOTE: Oxygen flow should be 20–40 mL/kg.

1.4. Administer isoflurane at 4%–5% and reduce to 3% after 1–2 min.

1.5. Evaluate the depth of anesthesia, ensure the pig has no withdrawal reflex in response to a noxious stimulus. Repeat every 5 min.

1.6. Intubate the pig once the correct depth of anesthesia is confirmed. Use a custom 10 inch, flat blade laryngoscope and size 9 or 10 endotracheal tubes for pigs 40–50 kg.

1.7. Place a pulse oximeter probe on the tongue (preferred) or ear and target an oxygen saturation above 90%.

1.8. To maintain the anesthesia, adjust oxygen flow (20–40 mL/kg) and inhalant gas rate (1%–3%).

1.9. Keep the ventilator settings at a respiratory rate of 12–30 breaths/min, TV of 6–10 mL/kg, PEEP of 5 cm H_2O, and Peak Pressure of 20 cm H_2O.

NOTE: Although TVs are targetted as high as 10 mL/kg, 6–8 mL/kg are achieved. **Figure 1** provides a schematic overview of the negative pressure ventilation (NPV)-ESLP for the transplant protocol applied in the lab.

1.10. Shave, wash and aseptically prepare the incision site using iodine.

NOTE: Following sedation with Ketamine/Atropine, the analgesic regime involves administering 3mg/kg Ketamine IV q 1 h (range 1–3 mg/kg depending on patient parameters) and Hydromorphone 0.05 mg/kg IM q 2 h. Any longer duration between doses results in breakthrough pain response, such as elevated heart rate and abnormal breathing patterns / abdominal muscle movement.

2. Insertion of central venous and arterial lines

2.1. Insert a central line for fluid and heparin administration.

NOTE: Central line is also used to administer steroids, antibiotics, vasopressors, and inotropes. See **Figure 2A** for line positioning.

2.1.1 Use electrocautery to make a 5–8 cm midline incision centered over the trachea and extend cranially from the sternal notch.

2.1.2 Divide the skin and subcutaneous fat using cautery.

2.1.3 Divide the midline plane between the strap muscles, and then divide the connective tissue layers to identify the left or right carotid intravascular bundle lateral to the trachea.

2.1.4 Obtain proximal and distal control of the jugular vein using silk ties (size 2-0) as vessel loops.

2.1.5 Tie the cranial encircling tie and retract upwards on the proximal tie to control blood flow.

2.1.6 Make a small incision in the vein using Metzenbaum scissors (see **Table of Materials**) to accommodate a two-port, 7 Fr central line (~1/3 the vessel's circumference).

2.1.7 Simultaneously, release the tension on the proximal vessel loop, cannulate the vein, and then tie down to secure the cannula in the vein at a depth of 10 cm.

2.1.8 Flush the line with heparin, connect to an IV line of 0.9% normal saline, and administer the fluid if the pig is intravascularly depleted from dehydration.

NOTE: Heparin locks any unused ports.

2.1.9 Administer 500 mg of methylprednisone and 1 g of cefazolin IV.

2.2. Follow the same techniques to cannulate the common carotid artery using a 7 Fr arterial line for accurate blood pressure management.

3. Left lung procurement

3.1. Position the pig in a right lateral decubitus position.

3.2. Perform a left anterolateral thoracotomy (**Figure 2**).

3.2.1. Mark the thoracotomy incision (20 cm) using the following landmarks: use palpation to identify the tip of the left scapula; likewise, identify the xiphoid process inferior to the sternum with palpation. Connect the two as shown in **Figure 2B**.

3.2.2. Inject a total of 10 mL of 0.25% bupivacaine into the incisional line and two rib spaces above and below the incision.

3.2.3. Use electrocautery to dissect the skin, subcutaneous layers, and muscle layers. The latissimus dorsi must be divided. Identify the rib immediately below the incision and cauterize on top of the rib to expose the intercostal muscles while avoiding the intercostal neurovascular bundle.

3.2.4. Use a mosquito hemostat to puncture the intercostal muscles immediately above the rib, and then feel inside the chest for adhesions using a finger. Push the lung away using a Yankauer suction or finger (see **Table of Materials**) as you cauterize along the top edge of the rib to extend the thoracotomy.

3.2.4.1. Extend the thoracotomy anteriorly until 1 inch away from the sternum. Extend the thoracotomy posteriorly to the paraspinal muscles.

3.2.5. Insert a Cooley sternal retractor (see **Table of Materials**) to open the thoracotomy wide (10 cm) (**Figure 2C**). Retract the lung to expose the left hemi-azygous vein (**Figure 2D**).

3.2.6. Circumferentially dissect the left hemiazygos vein using Metzenbaum scissors and a fine Lauer. Encircle the vessel with silk ties, and then ligate and transect it (**Figure 2E**). Keep a silk tie on the proximal stump for added control.

NOTE: Lauer is a right angle clamp or a celiac clamp used for tissue dissection.

3.2.7. Dissect out the left pulmonary artery (PA) and left pulmonary veins (PV). Encircle the veins in silk ties for control (**Figure 2F**).

NOTE: The superior PVs are very small and are suture ligated at their branch points or common trunk, depending on the individual anatomy. The left mainstem bronchus is deep to the PA and LA (left atrium), so occasionally, it cannot be dissected easily until the artery and veins have been clamped and transected (**Figure 2G**).

3.2.8. Administer 5000 units of heparin IV 5 min before clamping the PA.

NOTE: Heparin 5000 units IV is also administered 5 min before unclamping the PA. For every hour

after that, 1000 units of IV heparin is administered.

3.2.9. Clamp the PA (DeBakey cross-clamp), left inferior pulmonary vein (Satinsky clamp), and the left bronchus (Spoon Potts clamp) individually (see **Table of Materials**). Decrease tidal volumes to 5 mL/kg once the left bronchus is clamped.

3.2.10. Transect the PA, left inferior pulmonary vein, and the left bronchus. Leave at least 0.5 cm of tissue cuff to sew. Divide the left inferior pulmonary ligament and remove the left lung.

4. Termination of ESLP, division of left lung, and flushing with electrolyte solution

4.1. Clamp the ventilation tubing at maximal inspiration, terminate perfusion and ventilation, and disconnect the lungs from the ESLP device.

4.2. Weigh the lungs to determine the amount of edema formation.

NOTE: Edema is tissue swelling due to the accumulation of excess fluid.

4.3. Take a tissue biopsy of the accessory lobe, divide it into three equal pieces, and place one piece into each of the following: optimum cutting temperature (OCT) gel, formalin, and snap freeze in liquid nitrogen.

NOTE: This step is typically followed in the author's lab. The samples are then stored for future analysis: OCT and snap-frozen samples are kept in a -80 °C freezer, and formalin-stored samples are placed in a properly sealed container and stored in 4 °C refrigerators. Details of the specific ESLP protocol and tissue analysis are published elsewhere[16].

4.4. Divide the left donor lung from the right lung. Leave 1 cm of donor PA, 1 cm of donor bronchus, and adequate donor LA cuff (~0.5 cm circumferentially) to sew to the recipient LA (**Figure 2H**). Leave the left inferior PV and left superior PVs in continuity with the donor LA wall to facilitate later anastomoses.

4.5. Weigh the left lung.

4.6. Cannulate the donor left PA using a drop sucker connected to an IV line and flush 500 mL of extracellular, low potassium, dextran-based electrolyte preservation solution antegrade through the lung vasculature. Secure the cannula in the PA with a silk tie during the flush, and release when the flush is complete.

NOTE: The steps mentioned pertain to the specific ESLP device utilized for this work and may not be directly applicable to other devices.

5. Left lung transplantation

5.1. Insert the donor lung into the recipient's chest, beginning with the lower lobe. Do not force the lung into place.

NOTE: The lower ribcage may need to be lifted upwards to accommodate the donor lung by torquing on the sternal retractor. Ideally, the recipient is a few kilograms larger than the donor to facilitate a size match.

5.2. Perform the bronchial anastomosis first using 4-0 prolene on a TF needle (**Figure 2I**).

NOTE: A running, end-to-end anastomosis works well. Trim any excess length from the two anastomotic ends before sewing to avoid kinking caused by redundant tissue.

5.3. Perform the LA anastomosis second with 6-0 prolene on BV-1 needles using a running, end-to-end anastomosis. Again, trim excess tissue to avoid kinking.

NOTE: The LA is friable and benefits from the small BV-1 needle. Horizontal bites on the donor may be required to purchase adequate tissue and correct the size mismatched caused by sewing the donor IPV and SPV to the recipient IPV/LA opening.

5.4. Incorporate the donor SPVs into the inferior PV and LA anastomosis to allow left upper lung lobe venous drainage (**Figure 2J**).

NOTE: The branch superior pulmonary veins (SPVs) are less than 0.5 cm in diameter. The common SPV trunk is variable in length and is not routinely present, making direct anastomosis between the donor and recipient SPVs a poor option.

5.5. Complete the PA anastomosis with 6-0 prolene on BV-1 needles using a running, end-to-end anastomosis. Again, trim excess tissue to avoid kinking.

5.6. Remove the bronchial clamp and increase TVs to target 10 mL/kg.

5.7. Confirm heparinization, administer a potassium shift (40 mg of furosemide, 10 units of insulin, 100 mL of 25% dextrose solution), open the PA clamp partially, de-air, and tie the PA suture. Completely release the PA clamp after 10 min.

5.8. Meanwhile, de-air the LA, tie the sutures, and remove the LA clamp.

5.9. Take a reperfusion blood gas from the central line and a reperfusion tissue biopsy from the left middle lobe.

NOTE: To take a tissue biopsy, use a size 0-silk tie to encircle a 1 cm portion of the middle lobe apex, tie-down to ensnare the tissue, and then cut the isolated portion with Metzenbaum scissors. Divide the biopsy into three equal portions and manage as previously described.

5.10. Perform a left and right lung bronchoscopy to assess the bronchial anastomosis and to suction secretions. Insert a bronchoscope into the endotracheal tube using an adaptor connection.

5.10.1. Connect the scope to suction. Advance the bronchoscope into the left bronchus. Inspect the bronchial anastomosis (**Figure 2N**). Advance the scope down the bronchioles and suction any fluid. Repeat on the right side.

NOTE: Do not allow the oxygen saturation to fall below 90%. If saturations fall below this level, remove the scope and allow the pig a few minutes of uninterrupted ventilation to recover.

5.11. Insert a 20 Fr malleable chest tube (**Figure 2L**), close the thoracotomy in three layers (**Figure 2M**), and prone the pig as soon as the arterial blood gases (ABGs) are stable (**Figure 2O**).

5.12. Monitor the pig over 4 h in the prone position. Perform an ABG analysis every 30 min. Administer 1000 units of heparin every hour after reperfusion.

5.12.1. Take a 10 mL blood sample every hour for centrifugation and enzyme-linked immunosorbent assay (ELISA) analysis of inflammatory markers[16].

NOTE: Centrifugation parameters are detailed later.

6. Isolated Left Lung Assessment

6.1. Position the pig supine and perform a midline sternotomy for final isolated left lung assessment (**Figure 2P**).

6.2. Open the left pleura using Metzenbaum scissors and take a tissue biopsy from the left lower lobe as previously described (NOTE to step 5.9).

6.3. Open the accessory lobe pleura and dissect out the common vein using Metzenbaum scissors.

NOTE: This will be clamped later on.

6.4. Take a blood sample from the LA anastomosis using a 21 G needle. Direct the needle toward the left pulmonary veins and away from the common left atrium or accessory lobe trunk.

6.5. Open the right pleura to create space for the right hilar clamps (see **Table of Materials**). Dissect the right inferior pulmonary ligament up to the hilum. Ensure that a clamp can be placed around the hilum superiorly, inferiorly, and anteriorly.

NOTE: This ensures that the hilum is occluded, and all oxygenation is dependent on the left lung. The right lung will not ventilate at this time, which should be evident by a lack of

inflation/deflation with ventilator respirations. The right lower lobe can be lifted out of the chest to accomplish this.

6.6. Clamp the accessory lobe vein using a DeBakey aortic cross-clamp (see **Table of Materials**) to occlude any accessory lobe drainage into the standard LA (**Figure 2Q**).

6.7. Take the following serial blood samples from the left PV anastomosis with a 21 G needle directed toward the left lung: 0 min, 1 min, 2 min, 5 min, and 10 min after clamping.

NOTE: Five samples are taken to monitor for any trend in partial pressure of oxygen (PaO_2) (**Figure 2R**). The PaO_2 should remain relatively stable to represent proper left lung function. Five samples also provide insurance of a quality assessment if there is an issue with clotting of any samples or a problem arises with ABG analysis.

6.8. Transect the anastomoses, and remove the left lung. Transect the IVC to expedite exsanguination.

6.9. Weigh the donor lung to assess for edema formation and inspect it for overall appearance. Inspect the PA, bronchus, and LA cuff for signs of clot or other pathology within the donor lung and the recipient mediastinum.

6.10. Run the final gas analyses, centrifuge the perfusate samples, and store the tissue biopsies as previously described (NOTE to step 4.3).

NOTE: The centrifugation settings are: 112 x *g*, 9 acceleration, 9 deceleration, 4 °C, and 15 min duration.

REPRESENTATIVE RESULTS:

All of the results are in the context of 4 h of reperfusion following 12 h of NPV-ESLP[16]. During lung explant, there are several clinical outcomes to anticipate (**Figure 3**). Typically, the pig will remain hemodynamically stable following a successful left lung explantation but may require a low dose infusion of phenylephrine (dose range: 2–10 mg/h) due to a vasodilatory response to surgery. Heart rate should target approximately 100–120 bpm, respiratory rate (RR) 8–30 for SpO_2 > 90%, mean arterial pressure (MAP) > 60 mmHg, normothermic (38 °C), and tidal volumes (TVs) are targeted at 5 mL/kg while on one-lung ventilation with peak pressures of 20–24 cm H_2O. During one-lung ventilation, the ventilation volumes were reduced by half to protect the left lung from overinflation. The respiratory rate was increased to target a physiologic end-tidal carbon dioxide level (**Figure 3**). Thus, **Figure 3** displays typical hemodynamic and ventilatory parameters during critical points of the transplant.

During lung implant, the following results are typical. The left lung will have absorbed fluid during the ESLP run and appears heavier and larger than the explanted lung. For this reason, the recipient should be slightly larger than the donor (2–4 kg), so the thorax can accommodate the somewhat edematous lung. The lung will require gentle pressure to insert into the chest through

the thoracotomy. It is easier to insert the lower lobe first, followed by the upper lobe. The bronchus is a direct end-to-end anastomosis and should be performed first. 4-0 prolene on a TF needle is recommended. The LA cuffs are highly friable but not too difficult to sew due to the redundancy and pliability of the tissue. 6-0 prolene on BV-1 needles work well for the LA anastomoses. The PA is the last anastomosis performed. This vessel can tear easily with little traction. If it tears, it is possible to open the pericardium and move the clamp proximally toward healthy tissue for sewing. Again, a 6-0 prolene on BV-1 needles works well for this anastomosis.

At the time of reperfusion, the following trends were noticed. Once the bronchus is unclamped and TVs are increased back to 10 mL/kg, the left lung will begin to inflate. Although the target was 10 mL/kg for tidal volumes, generally 6–8 mL/kg was attained, which is achieved gradually over the first 2–3 h of reperfusion, depending on the ESLP protocol used and the quality of the implanted lung. Rarely, there can be a small air leak, and this can be remedied with a simple stitch on the anterior wall. The posterior wall is more difficult to repair and will require packing. Great effort should be made to avoid air leaks from the bronchial anastomosis. Upon bronchoscopy, the right lung appears normal, and the left lung is typically edematous. The suture line is inspected, and approximately 50–100 mL of clear fluid is suctioned from the airways. The TV will drop significantly during suctioning from 300 s to 20 s, so this action should be performed quickly to allow the pig to recover. If arterial saturation drops below 90%, the bronchoscopy should be terminated, and the pig is allowed to recover over 1–2 min of ventilation. The first arterial blood gas (ABG) is typically normal because the right lung is functioning well as the left lung recovers.

The proactive administration of furosemide, dextrose, and insulin at the time of reperfusion serves to mitigate a dramatic rise in potassium through intracellular shifting. The potassium will predictably rise during 60–120 min of reperfusion (**Table 1**). **Table 1** demonstrates a sample of ABGs over transplantation with 4 h reperfusion following 12 h of normothermic negative pressure ventilation (NPV) ESLP. Approximately two to four shifts are required during 4 h reperfusion to keep potassium < 5 mmol/L. If the trend is upward and appears as a rapid change between two gases drawn at 30 min intervals, the target is K^+< 4.5 mmol/L. Shifts include 40 mg of furosemide, 100 mL of 25% dextrose (D25), and 10 units of regular insulin administered as IV push *via* the central line. Occasionally, the pig will require a low dose dobutamine infusion (1.5–5 mcg/kg/min) along with phenylephrine (2–10 mg/h) after 30–60 min of reperfusion to treat a developing vasoplegic response. It is preferable to use phenylephrine in this situation exclusively. Still, occasionally dobutamine is a useful supplemental inotrope to maintain a mean arterial pressure greater than 60 mmHg, mainly if the heart rate is bradycardic.

Upon thoracotomy closure and turning the pig prone, an improvement in ventilation and hemodynamics is demonstrated. The modification can be drastic and occur over 5–10 min, but occasionally the response takes 1 h. Tidal volumes increase as pressure/weight is taken off the right lung, and the left lung continues to ventilate with improved compliance and recruitment. A repeat bronchoscopy can be performed further to clear the airway after a change in position. Over the following 4 h, phenylephrine requirements decrease, TVs approach the target 10 mL/kg, and ABGs stabilize (**Table 1**). To reiterate, if TVs of 10 mL/kg are targetted, typically TVs in the range of 6–8 mL/kg are achieved (**Figure 3**).

At the time of the final isolated left lung assessment, a stable pattern of behavior has been observed. The pig is less tolerant hemodynamically in the supine position for sternotomy and may require additional vasopressor support. Inspection of the left lung reveals variable degrees of mild hyperemia from ischemic reperfusion injury (IRI). The right lung appears normal. Upon clamping the right hilum, the pig becomes sinus tachycardic (120–140 bpm), and 100% of the cardiac output is diverted to the left lung. Targeted tidal volumes are not decreased at this time as the entire process takes 10 min. The pig remains stable up to the 5 min mark, but the heart develops ventricular fibrillation between 5–10 min and manual cardiac massage is required to continue perfusing the left lung. The left lung is explanted, weighed, and the anastomoses are inspected for patency. The pig expires rapidly at the time of exsanguination, which coincides with the explantation of the previously transplanted lung.

A successful transplant has predictable findings after the experiment (**Table 1** and **Figure 4**). **Figure 4** displays typical P:F ratio changes and edema formation during the transplant protocol. Typically, the left lung will experience an approximate 35% (+/-15%) weight gain; however, residual blood in the circulation contributes to this weight. PF ratios drop by approximately 100 at reperfusion as the left lung is not immediately effective at oxygenation, but this discrepancy improves over 2–3 h. Upon isolated left lung assessment at 4 h, the PF ratio will remain stable or decline slightly. Generally, the isolated left lung gas at 10 min will be similar to the final gas analysis post 12 h ESLP (**Table 1**). However, this is entirely dependent on the ESLP protocol employed, and the extent of IRI incurred. An unsuccessful transplant can be caused by clotting of the LPA, which results in an infarcted lung that does not oxygenate. Likewise, the duration of the transplant surgery can affect the quality of the reperfused lung function. An implantation surgery should take between 30–60 min. Longer operations expose the donor lung to damaging warm ischemic time that exacerbates ischemic reperfusion injury and can confound the results of the experimental ESLP protocol. The specific ESLP protocol of a given experiment may produce a non-functioning lung that fails to oxygenate after transplantation despite patent anastomoses. Such isolated left lung gases will be very dark in color (deoxygenated) with a low partial pressure of oxygen (PaO_2).

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic of porcine left lung transplant protocol. Schematic representation of 12 h NPV-ESLP run followed by left lung transplantation in a Yorkshire pig.

Figure 2: Photos of porcine left lung transplant surgery protocol. (**A**) Internal jugular and common carotid line placement. (**B**) Thoracotomy incision. (**C**) Thoracotomy. (**D**) Left Hemi-azygous vein. (**E**) Ligated Left Hemi-azygous vein. (**F**) Isolation of pulmonary veins. (**G**) Clamped left atrial cuff, left bronchus, and left pulmonary artery. (**H**) Left donor lung with pulmonary vein, bronchial and PA cuffs. (**I**) Pulmonary artery anastomosis. (**J**) Left lung transplanted and unclamped. (**K**) Lung repositioned. (**L**) Chest tube positioned. (**M**) Thoracotomy closure. (**N**) Bronchial anastomosis. (**O**) Pig in prone position. (**P**) Sternotomy. (**Q**) Accessory lobe clamped

(Right lung clamped, but not shown). (**R**) Left pulmonary vein blood samples were drawn from pulmonary vein anastomosis (bleeding from prior puncture site).

Figure 3: Monitoring and ventilation parameters for porcine left lung transplant surgery. (**A**) Typical parameters for recipient pre-transplant. (**B**) Typical parameters at recipient left lung explant. (**C**) Typical parameters 4 h post left lung donor transplant.

Figure 4: P:F ratio and weight gain pre-and post-transplant. (**A**) PaO_2:FiO_2 ratios throughout the transplant. (**B**) Weight gain of left lung throughout transplant after 12 h of NPV-ESLP.

Table 1: Blood gas analysis performed following left lung transplant post 12 h of ESLP. Ca^+, calcium ion; Cl^-, chloride ion; Hb, hemoglobin; HCO_3^-, bicarbonate ion; K^+, potassium ion; Na^+, sodium ion; Osm, osmolarity; $paCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; sO_2, oxygen saturation; isolated left lung pre-clamp, right hilum open; Isolated left lung post-clamp, 1 min after right hilum clamped.

Supplementary File 1: Surgical safety checklist for left lung transplantation.

DISCUSSION:

Several critical surgical steps are involved in this protocol, and troubleshooting is needed to ensure successful transplantation and lung assessment. Juvenile porcine lungs are incredibly delicate compared to adult human lungs, so the operating surgeon must be cautious when handling porcine lungs. This is especially true after a 12 h run of ESLP as the organ will have taken on fluid volume and be susceptible to injury from excessive manipulation. Any undue pressure will cause atelectasis or trauma to the experimental lung that will affect assessment results. Likewise, the vascular structures are very delicate in the juvenile pig. It is critical to avoid torsion of the PA clamp as this can cause a tear or dissection of the tissue layers. A tear in the PA will necessitate opening the pericardium to access a more proximal portion of the left PA that can be anastomosed to the implanting lung. A DeBakey vascular clamp has a low profile that fits well in the surgical field, but this instrument can cause injury to the delicate PA if the surgeon is not careful. It is helpful to secure the clamp in position using a silk tie that is snapped to the drapes to prevent dislodgement or torsion. Bronchoscopy of the transplanted lung after unclamping of the bronchial anastomosis is also critical. There is often fluid within the donor lung airway after 12 h of ESLP and transplant. Suctioning this fluid is vital to ensure optimal recovery of left lung function and thereby assessment after 4 h of reperfusion. After bronchoscopy and the first ABG has returned with satisfactory potassium levels, it is critical to insert a chest tube, close the incision, and prone the pig. The pig's hemodynamics and ventilation are considerably more stable in the prone position, with the ribcage reapproximated. Elevated potassium > 5.5 mmol/L at this stage risks bradycardic arrest and will require emergent re-opening and manual cardiac massage to support perfusion, which is best avoided. Due to the significant risk of hyperkalemia and bradycardic arrest upon reperfusion, it is critical to perform serial ABGs beginning at reperfusion and recurring every 30 min until the 4 h exsanguination. ABGs give essential readings of oxygenation, partial pressure of carbon dioxide (PCO_2), potassium, and glucose. Monitoring these four components closely and treating them appropriately is vital to a successful experiment. A

continuous telemetry reading is also critical to monitor for peaked T waves associated with hyperkalemia and the anticipation of bradycardia. At the final stages of the experiment, it is crucial to clamp the right lung hilum and the accessory lobe before drawing final blood samples from the LA anastomosis. The right hilum supplies blood to the accessory lung lobe, and the accessory lobe drains adjacent to the left inferior pulmonary vein, often *via* a common trunk. The right hilum and accessory lobe need to be clamped separately to ensure no right lung function contributes to the sample LA gases through blood mixing. Drawing the left lung ABG sample from the PV anastomosis or just beyond it is suggested.

Several modifications have been made to this protocol along with significant troubleshooting of the described methods. Initially, it was attempted to perform the implantation *via* median sternotomy; however, the exposure was suboptimal due to the orientation of the pig PA, bronchus, and LA. The approach was successfully performed, but a thoracotomy was attempted on subsequent surgeries for improved exposure. This proved to be a superior surgical approach from visualization and technical perspective. Another essential modification was developing and implementing a surgical safety/protocol checklist (**Supplementary File 1**). There was a significant learning curve for all the team members involved, and these experiments are resource-intensive. A checklist was developed to guide the communication and document protocol development (**Supplementary File 1**). The checklist allowed to systemize and simplify the protocol for faster learning. The heparinization protocol was also modified. Two of the first ten transplants performed suffered from left lung ischemia due to clot formation in the left PA. Initially, 5000 units of heparin IV 5 min was administered before PA clamping and an additional 5000 units 5 min before PA unclamping. Dosing frequency was increased to include 5000 units every hour after PA unclamping, and there have not been any issues with bleeding or PA clotting since adopting this approach. A strategy that utilizes less heparin was developed to control expenses, with a dose of 5000 units IV heparin 5 min before PA clamping and 5 min before partial PA unclamping. This is followed by 1000 unit IV heparin boluses every hour for the remainder of the case. There was no access to ACT analysis, which would be the most accurate means of accessing adequacy of heparinization.

The unclamping of the PA was also modified from a sudden unclamping to an approach that gradually reintroduces full flow to the transplanted lung over 10 min. The left inferior PV and LA cuff remain clamped upon PA unclamping to allow for antegrade de-airing. Full PA flow produced significant pressure on the delicate LA suture lines and considerable pressure within the lung vasculature, which appeared damaging. Prolonged PA unclamping allows for the antegrade de-airing of the LA with a gradual increase in flow as opposed to sudden unclamping and a sudden increase in flow. Prolonged unclamping protects the suture lines and lung endothelium from sudden increase in pressure. Even with ESLP, an ischemic insult to the transplanted lung and cell death contributes to a significant release of potassium into the pig's circulation following ischemic-reperfusion. For managing hyperkalemia proactively, the protocol was modified to pre-emptively shift potassium at the time of reperfusion by administering furosemide 40 mg IV, 100 mL of 25% dextrose (D25), and 10 units of regular insulin. This maintains target potassium on the ABGs within the first hour of reperfusion, and the pig can be safely proned earlier in the experiment, which helps with graft function. From a hemodynamic perspective, the protocol is

modified to use phenylephrine as the predominant vasopressor support. Vasopressin was found to be less effective and was thus abandoned. A low dose drip of dobutamine was occasionally run to increase cardiac output, along with a phenylephrine infusion to maintain blood pressure. Still, dobutamine is used sparingly due to its arrhythmogenic properties. Finally, the assessment of the isolated left lung was modified. After clamping the right lung hilum, the LA gases were initially drawn from the body of the LA after lifting the heart cephalad; however, gas mixing from the accessory lobe drainage into the LA produced falsely high PaO_2 readings. Now, samples are drawn distal to the LA anastomosis line after clamping the right lung and the accessory lobe individually. These samples are taken at 0, 1, 2, 5, and 10 min after clamping the right hilum and are a more accurate representation of the isolated left lung function. Manual cardiac massage may be required between the 5–10 min mark. The most recent protocol improvement pertains to the superior pulmonary vein (SPV) anastomoses. Initially, the recipient SPVs were oversewed due to their small caliber and propensity to clot. Still, the donor's upper lobe occasionally suffered congestion as collateral drainage was variable and inadequate between pigs. To remedy this, the donor SPV and IPV were incorporated into the recipient's IPV/LA anastomosis, eliminating any issue with venous drainage and lung congestion. This protocol will continue to benefit from further modification as experience grows.

There are several limitations with this method of left lung transplantation. The model has only been assessed with a 4 h period, which only considers the transplanted lung function in the acute post-operative period following 12 h of ESLP. This protocol was designed with the animal's recovery in mind; however, it has yet to be tested in that capacity. The technical operation requires considerable surgical skill and necessitates a trained surgeon or highly independent surgical trainee to perform. There are many opportunities for fatal errors to occur that would compromise the entire experiment, and proper surgical technique is needed to avoid or correct such hazards. The only true assessment of the transplanted lung occurs at the very end of reperfusion. The native right lung is capable of meeting the oxygen requirements of the pig and producing satisfactory ABGs. When the right lung is completely clamped at the hilum, it is prevented from receiving fresh oxygen, fresh deoxygenated blood supply, and oxygenated blood drainage. This is a pivotal moment to determine the transplanted left lung function as 100% of cardiac output is redirected toward the transplanted lung, which becomes solely responsible for systemic oxygenation.

There are multiple benefits of this method concerning existing/alternative methods. After reviewing the literature[12–15], this method is the most detailed and reproducible after an initial learning curve of 1 or 2 pigs in the hands of a junior cardiac surgical trainee or fully qualified surgeon. The operation is straightforward; however, the hemodynamics of the pig (including its susceptibility for lethal arrhythmias) creates a learning opportunity for those accustomed to operating on adult humans, which are more robust from a cardiopulmonary perspective. The methods for isolated left lung functional assessment, although brief, are easy to perform and highly reproducible. In particular, this methodology provides more details about anesthetic management than is currently available in the literature.

This method is essential and has significant applications for ESLP and lung transplantation

research. ESLP is the most crucial development in lung transplantation since the introduction of antirejection medication, with some centers already benefitting from the increased organ utilization rates afforded by this technology[6–12]. Further advancement in this field of research is needed to decrease waitlist mortality and expand the accessibility of ESLP platforms. *In vitro* analysis with ESLP benefits from the *in vivo* assessment and confirmation of a large animal model. Large animal models that confirm *in vitro* findings are often necessary for clinical research trial approval for developing labs. This method provides a reliable and relatively straightforward transplant method for labs performing ESLP research.

ACKNOWLEDGMENTS:

This research is funded on behalf of the University Hospital Foundation.

DISCLOSURES:

DHF holds patents on *Ex situ* organ perfusion technology and methods. DHF and JN are founders and major shareholders of Tevosol, Inc.

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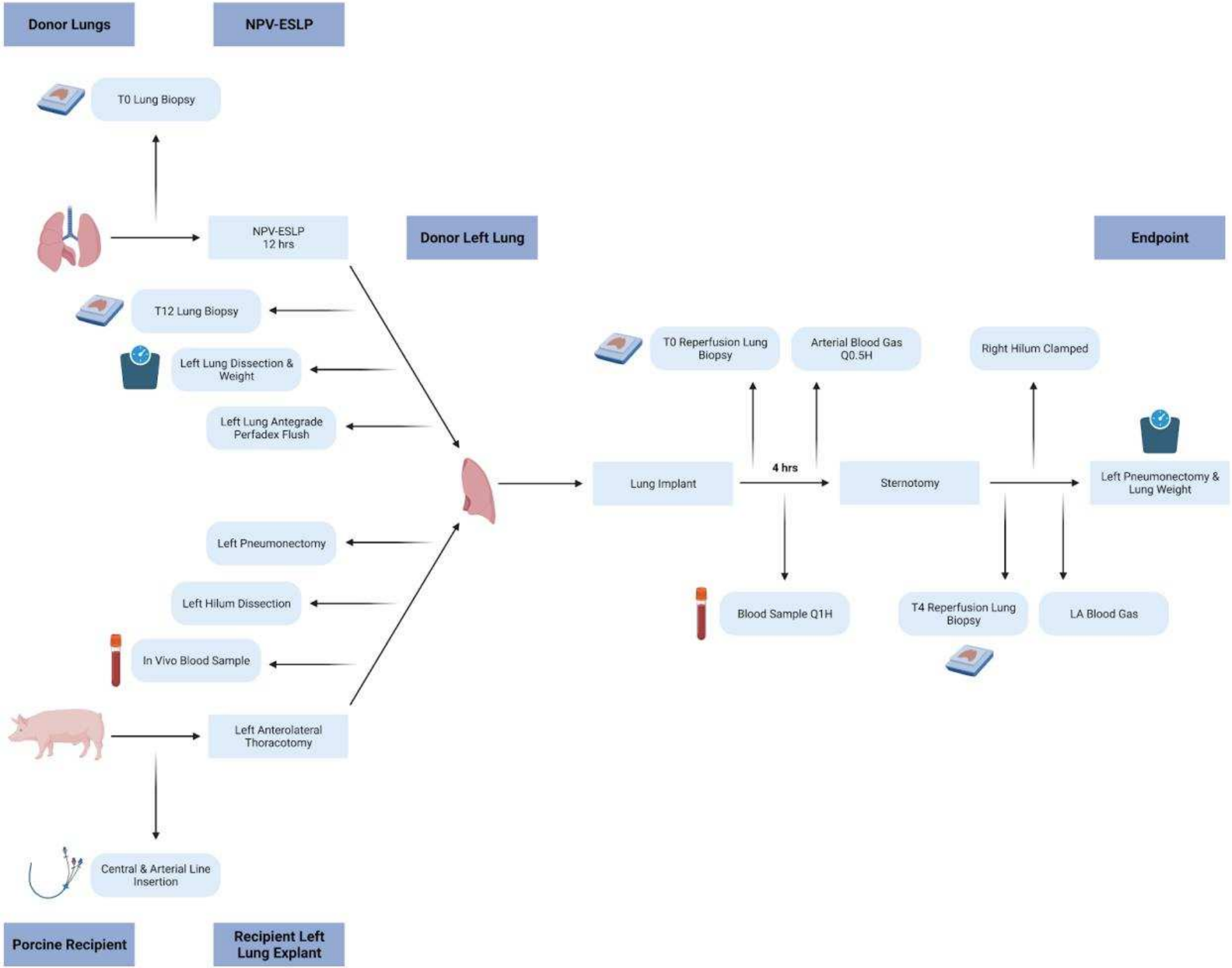

Figure 1: Schematic of Porcine Left lung Transplant Protocol. Schematic representation of 12-hour NPV-ESLP run followed by left lung transplantation in a Yorkshire pig.

Figure 2

Click here to access/download;Figure;Figure 2.psd

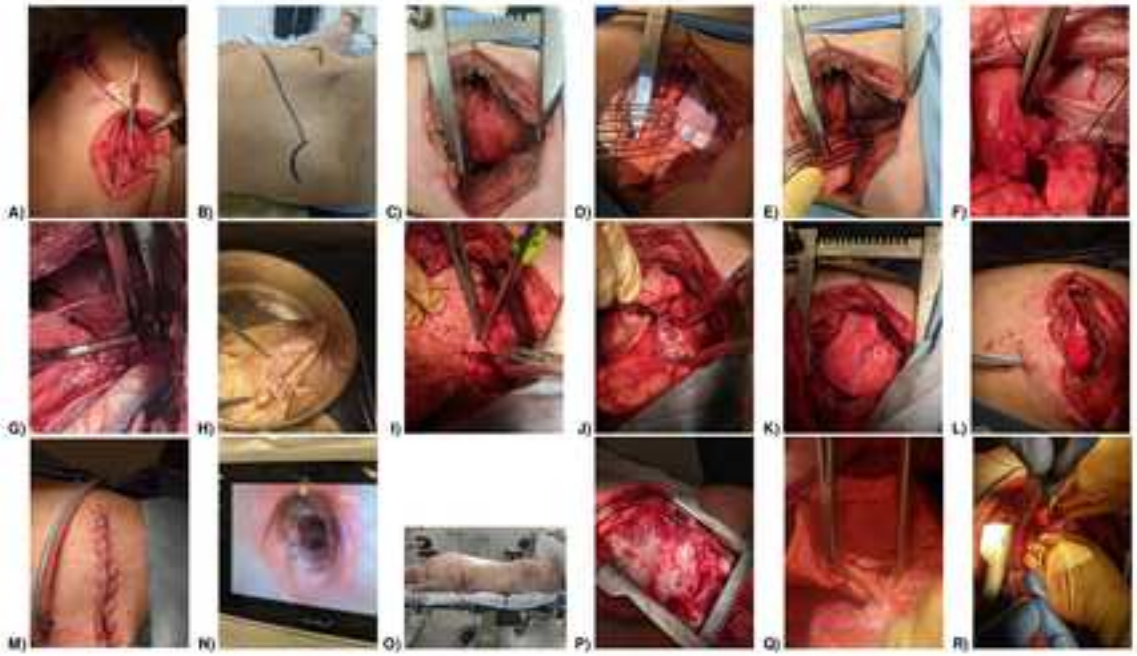

Figure 2: Photos of porcine left lung transplant surgery protocol. A) Internal Jugular and Common Carotid line placement B) Thoracotomy incision C) thoracotomy D) Left Hemi-Azygous vein E) Ligated Left Hemi-Azygous vein F) Isolation of pulmonary veins G) Clamped left atrial cuff, left bronchus, and left pulmonary artery H) left donor lung with pulmonary vein, bronchial and PA cuffs I) Pulmonary artery anastomosis J) Left lung transplanted and unclamped K) lung repositioned L) Chest tube positioned M) Thoracotomy closure N) Bronchial anastomosis O) Pig in prone position P) Sternotomy Q) Right lung clamped (not shown) and left pulmonary vein blood samples drawn from pulmonary vein anastomosis (bleeding from prior puncture site).

Figure 3

Click here to access/download;Figure;Figure 3.eps

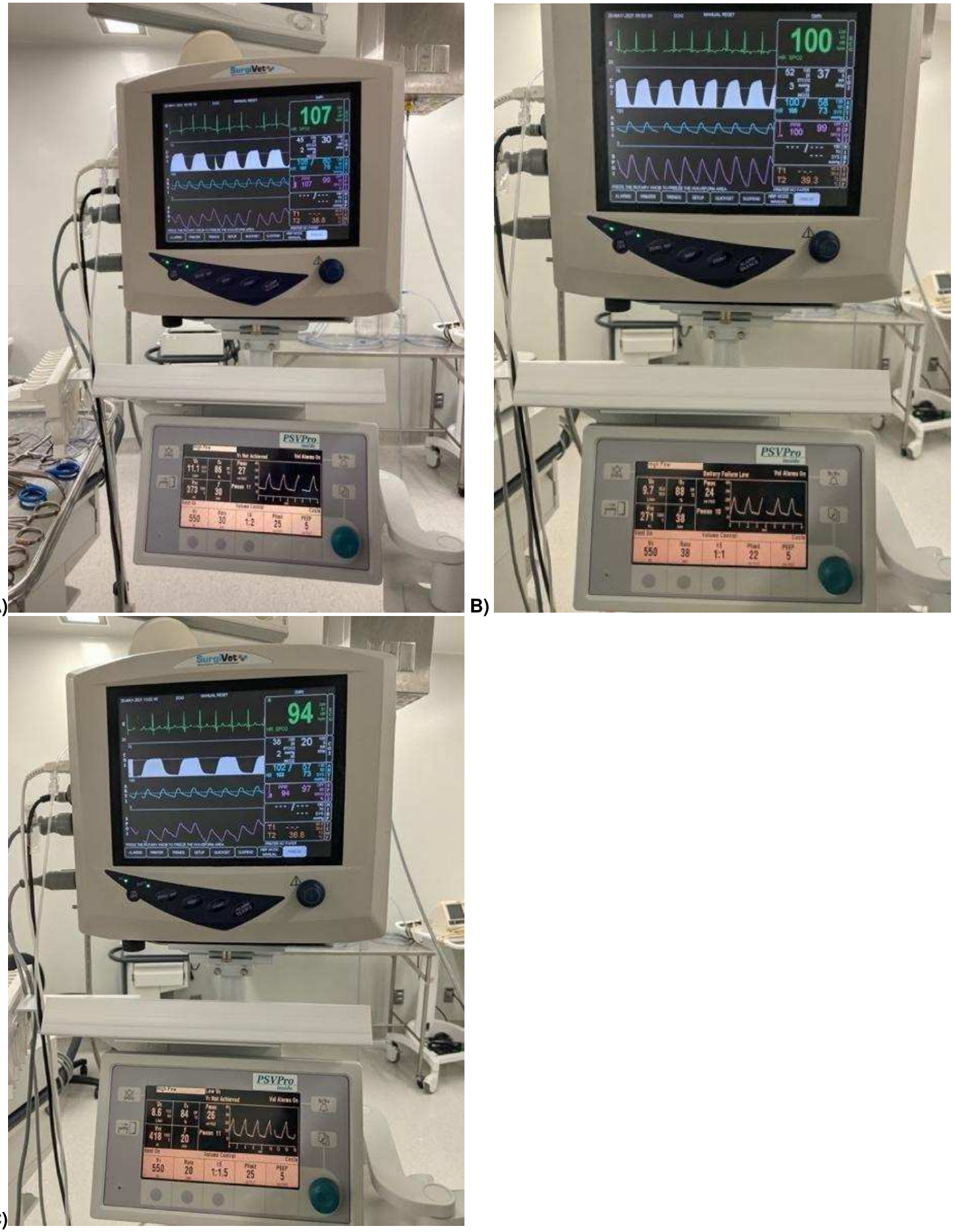

Figure 3: Photos of porcine left lung transplant surgery protocol monitor and ventilation parameters. A) Typical parameters for recipient pre-transplant B) Typical parameters at recipient left lung explant C) Typical parameters 4-hours post-left lung donor transplant.

Figure 4

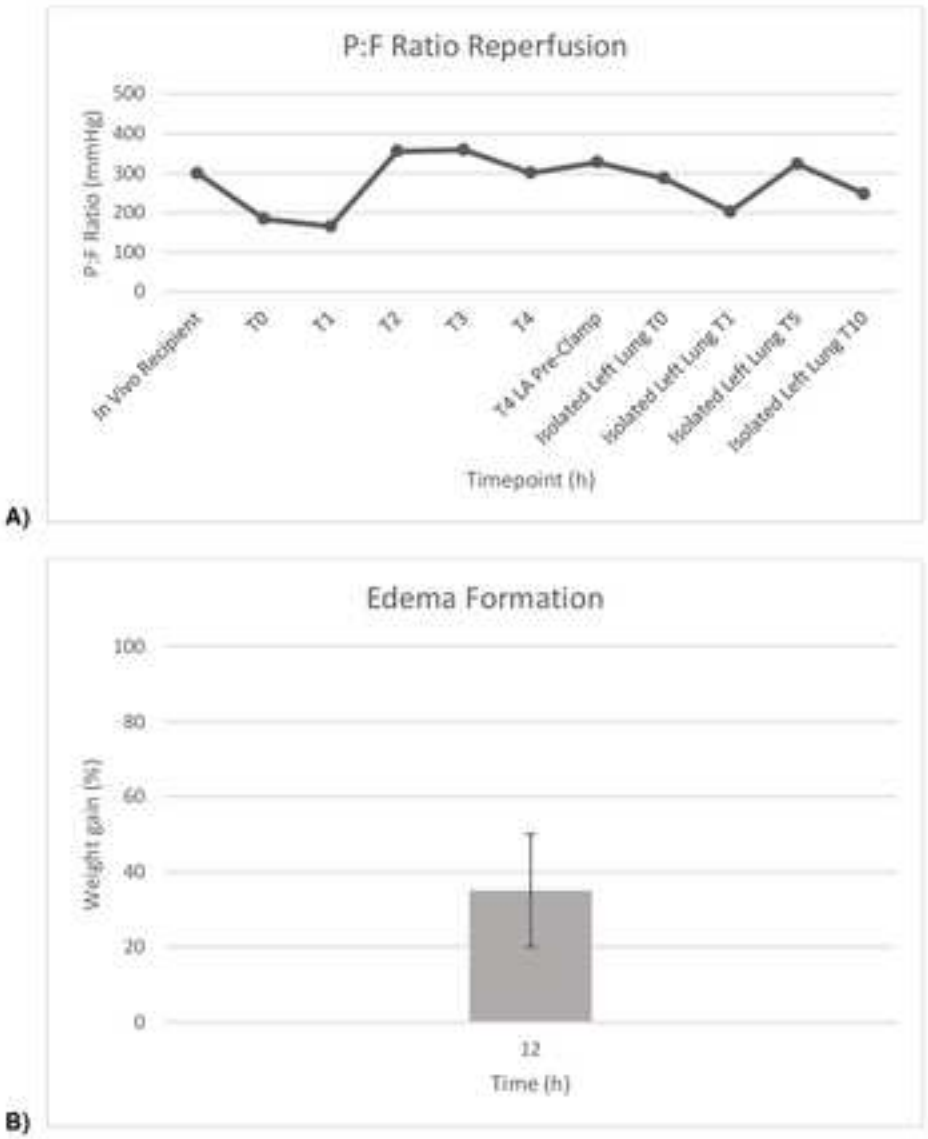

Figure 4. P:F ratio and weight gain pre-and post-transplant. A) PaO2:FiO2 ratios throughout the transplant. B) Weight gain of left lung throughout transplant after 12 h of NPV-ESLP.

Table 1

Arterial Blood Gases (100% FiO_2)	***In vivo* Recipient**	**T0 Reperfusion**	**T1 Reperfusion**	**T2 Reperfusion**	**T3 Reperfusion**
Blood Gas Values					
pH	7.402	7.327	7.284	7.402	7.421
pCO2 (mmHg)	47.7	57.3	56.4	36.9	35.3
pO2 (mmHg)	299	184	165	355	358
Oximetry Values					
Hb (g/dL)	11.2	12.5	11.3	11.6	10.3
sO2 (%)	100.1	99.2	99	99.8	99.8
Electrolyte Values					
K^+ (mmol/L)	4.5	6.2	4.4	4	4.1
Na+ (mmol/L)	141	143	140	245	145
Ca^{2+} (mmol/L)	0.99	0.88	0.81	0.74	0.66
Cl^- (mmol/L)	97	97	95	101	100
Osm (mmol/kg)	287	287.9	293.7	292.4	297.5
Metabolite values					
Glucose (mmol/L)	4,2	2.7	13.4	2.8	8.3
Lactate (mmol/L)	1.2	1.3	3.8	2.5	1.3
Acid Base status					
HCO^{-3} (mmol/L)	29	29.1	25.9	22.4	22.5

T4 Reperfusion	Isolated Left Lung Pre-clamp	Isolated Left Lung Post-clamp (0 min)	Isolated Left Lung Post-clamp (1 min)	Isolated Left Lung Post-clamp (5 min)
7.479	7.504	7.399	7.371	7.423
35.6	34.2	45.6	48.1	40.6
300	327	287	207	335
-	17.1	11.7	13.5	16.3
-	99.9	100.2	99.7	99.8
4.6	5.2	5.4	5.3	6.9
144	140	141	139	137
0.61	0.36	0.98	0.42	0.36
96	91	102	94	91
293.5	284.7	287.1	282.9	278.2
5	5.1	4.9	4.5	4.6
1.2	1.4	1.8	1.4	1.9
26.1	26.7	27.6	27.1	26.1

Isolated Left Lung Post-clamp (10 min)
7.435
36.6
249
13.8
99.9
7.4
136
0.38
94
277.1
4.2
2.7
24.1

Rebuttal Letter 2

All requested revisions have been made. Specific comments have been made in the manuscript document as replies to the editor.

Sincerely,

Dr. Keir Forgie MD, PhD candidate (2023)

Dear Dr. Forgie,

Your manuscript, JoVE62979 "Left Lung Transplantation in a Porcine Model of NPV-ESLP: Evaluation of Lung Function with Four Hour Survival.," has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.

After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually.

Your revision is due by **Jul 13, 2021**.- extended to **July 27th, 2021**

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision". Please note that the corresponding author in Editorial Manager refers to the point of contact during the review and production of the video article.

Best,

Nilanjana Saha, PhD
Review Editor
JoVE
nilanjana.saha@jove.com
617.674.1888
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Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

The manuscript has been proofread for spelling and grammar. We use the Canadian/British spelling for "centre". Sentences that were originally written in point-form have been re-written into full-sentences.

2. Please revise the following lines to avoid previously published work: 38-40, 74-75, 80-81, 188-191.

Line 38-40 has been removed from the Abstract.

Line 74-75 has been removed from the Introduction

Line 80-81 has been removed from the Introduction

Line 188-191 (now 534-605) now read: "4.3. At this point, our lab takes a tissue biopsy of the accessory lobe, divides it into three equal pieces, and place one piece into each of the following: optimum cutting temperature (OCT) gel, formalin, and snap freeze in liquid nitrogen. We then store the samples for future analysis as follows: OCT and snap frozen samples are kept in a -80 °C freezer, and formalin-stored samples are placed in a properly sealed container and stored in 4 °C refrigerator. Details of our specific ESLP protocol and tissue analysis are published elsewhere (16)."

3. This article is similar to another article submitted to JoVE by the same group (Manuscript no: 62982), though the focus of the two reports is different. Please ensure that there are no overlapping texts/results between these two articles.

I am not familiar with how to locate a manuscript published by JoVE by using the manuscript number. I am aware of an ESHP paper from our lab published by JoVE. I am also aware of a previously submitted NPV-ESLP manuscript that was submitted, but the editorial revisions were never completed; therefore, it was never published. We recently submitted a new NPV-ESLP manuscript, with zero access to the previous version, so there should be no overlap. This transplant protocol is the first manuscript submitted to JoVE

from our lab that describes a full transplantation model. Any overlapping text is inadvertent, and the results are all original. Any similarity in tables is a result of our lab's monitoring charts being similarly formatted for simplicity of collaboration. We are happy to change any specific aspects that you feel are too similar.

4. Corresponding authors are different in the main manuscript (Jayan Nagendran) and the Editorial software (Keir Forgie, where the authors provide input while uploading the manuscript). Please clarify.

Thank you for pointing this out. Dr. Jayan Nagendran MD/PhD in the primary investigator and should be the corresponding author. Dr. Keir Forgie MD is a PhD student, responsible for most of the writing and experiments. Keir will be able to provide the timeliest communication, which is why he was listed as the corresponding author in the Editorial software. If congruence is required, please use Dr Jayan Nagendran as the corresponding author.

5. Please ensure that abbreviations are defined at first usage.

We believe that all abbreviations have been defined at first usage. If there are any specific abbreviations that you would like us to address, please identify them by line. Thank you.

6. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

Reference numbers now appear as superscripts.

7. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials. Please sort the Materials Table alphabetically by the name of the material.

All commercial language has been removed. The Materials table is in alphabetical order. Names of instruments used in the text are common names, and not manufacturer commercial names.

8. Introduction: Line 95-96: Please support the statement with published References.

The introduction has been re-written. The line is now supported with references.

Line 114-115 now reads: Proper ESLP research requires the in-vivo validation of in-vitro findings; however, there is limited literature on porcine lung transplantation models for ESLP (12-15).

9. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We have reviewed the manuscript to ensure that the "how" questions are fully explained. Please let us know if there are any specific steps that require further explanation.

10. Please add more details to your protocol steps:
Step 2.1.2: Please provide details regarding how this step is done.

There are no further details that can be added to the explanation of this step. The line reads: "Divide the skin and subcutaneous fat using cautery." Steps 2.1.1 and 2.1.3 provide sufficient contextual information. Video of this step is the only way to make it clearer. If a reader is unable to follow this simple surgical instruction, they should seek out some basic surgical training before attempting any of this procedure.

Step 2.1.4: “2-0 silk ties”, Is this any commercial name? If yes, then please use the generic term instead. Please add the details in the Table of Materials.

"2-0 silk ties" refers to the recommended size and material of suture used. This is not a commercial name.

The line has been re-written for clarity: "2.1.4. Obtain proximal and distal control of the jugular vein using silk ties (size 2-0) as vessel loops."

Step 3.2.1: Please mention how the xiphoid process is identified.

The text has been updated as follows (line 471-473):
"Mark the thoracotomy incision using the following landmarks: use palpation to identify the tip of the left scapula; likewise, identify the xiphoid process inferior to the sternum with palpation. Connect the two as shown in in Figure 2b"

Step 3.2.4: "Use a mosquito to puncture…", is this phrase correct? Again, "Push the lung away," to what extent can this be done?

A "mosquito" refers to a mosquito hemostat, which is a surgical instrument. It is a common name, not a commercial name.

The lungs can very easily be pushed away using a Yankauer suction. This is a common name, not a commercial name. The video will make the task clear.

The text has been updated as follows:
"3.2.4. Use a mosquito hemostat to puncture the intercostal muscles immediately above the rib and then feel inside the chest for adhesions using a finger. Push the lung away using a Yankauer suction as you cauterize along the top edge of the rib to extend the thoracotomy. Extend the thoracotomy anteriorly until 1 inch away from the sternum. Extend the thoracotomy posteriorly to the paraspinal muscles. "

Step 3.2.6: What is the dissection tool?

The text has been updated as follows:
3.2.6. Circumferentially dissect the left hemiazygos vein using Metzenbaum scissors and a fine Lauer. Encircle the vessel with silk ties, then ligate and transect it (Figure 2E). Keep a silk tie on the proximal stump for added control.

Step 5.9/5.10/6.2: Please explain how this step is done.

The texts have been updated as follows:

"5.9. Take a reperfusion blood gas from the central line, and a tissue biopsy from the left middle lobe. To take a tissue biopsy, use a size 0-silk tie to encircle a 1 cm portion of the middle lobe apex, tie down to ensnare the tissue, then cut the isolated portion with Metzenbaum scissors."

"5.10 Perform a left lung bronchoscopy to assess the bronchial anastomosis and suction secretions. Insert a bronchoscope into the endotracheal tube using an adaptor connection. Connect the scope to suction. Advance the bronchoscope into the left bronchus. Inspect the bronchial anastomosis. Advance the scope down the bronchioles and suction and fluid. Do not allow the oxygen saturations to fall below 90%. If saturations fall below this level, remove the scope and allow the pig a few minutes of uninterrupted ventilation to recover."

"6.2. Open the left pleura using Metzenbaum scissors and take a tissue biopsy from the left lower lobe as previously described."

Step 8: Please specify the centrifugation speed, duration, and temperature.

The text has been updated as follows:
"6.10. Run the final gas analyses, centrifuge perfusate samples, and store tissue biopsies as previously described. Centrifugation settings include: 1600RPM, 9 acceleration, 9 deceleration, 4 degrees Celsius, and 15-minute duration."

11. In the software, please ensure that all button clicks and user inputs are provided throughout. Also, please ensure that the button clicks are bolded.

Any mention of the ESLP software has been removed upon suggestion of the reviewers. This manuscript exclusively focuses on the left lung transplant methodology. A separate submission to JOVE recently under

review and returned to us for minor revisions exclusively addresses the NPV-ESLP software. We originally planned to submit both protocols together in one coherent manuscript, but our initial contact at JoVE suggested we submit two separate manuscripts. This has produced some disjointedness in the text, and we have extensively revised this manuscript to act as a stand-alone text on swine left lung transplantation without details regarding our NPV-ESLP protocol.

12. Please include a one-line space between each protocol step and then highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

A one-line space has been added in-between each protocol step.

Three pages of the Protocol have been highlighted.

13. Please modify the Result section to include all the observations and conclusions you can derive from the Figures.

Thank you for your comment. We have elaborated on the results section to include additional observations and conclusions that can be derived from our figures. The following text has been added:

Line 794: During one lung ventilation, we reduce the ventilation volumes by half to protect the left lung from overinflation, and we increase the respiratory rate to target a physiologic end-tidal carbon dioxide level (Figure 3).

line 868: Although we target 10ml/kg for tidal volumes, we generally attain 6-8 ml/kg (Figure 3), and this is achieved gradually over the first 2-3 hours of reperfusion, depending on the ESLP protocol used and the quality of the implanted lung.

Line 917: A successful transplant has predictable findings at completion of the experiment (Table 1 and Figure 4). Typically, the left lung will experience a 40% weight gain; however, residual blood in the circulation contributes to this weight. PF ratios drop by approximately 100 at reperfusion as the left lung is not immediately effective at oxygenation, but this discrepancy improves by 2-3 hours. Upon isolated left lung assessment at four hours, the PF ratio will remain stable or decline slightly. Generally, the isolated left lung gas at 10 minutes will be similar to the final gas analysis post 12 hrs ESLP (Table 1). An unsuccessful transplant can be caused by clotting of the LPA, which results in an infarcted lung that does not oxygenate. Likewise, the duration of the transplant surgery can affect the quality of the reperfused lung function. An implantation surgery should take between 30-60 minutes. Longer operations expose the donor lung to damaging warm ischemic time that exacerbates ischemic reperfusion injury and can confound the results of the experimental ESLP protocol. The specific ESLP protocol of a given experiment may produce a non-functioning lung that fails to oxygenate after transplantation despite patent anastomoses. Such isolated left lung gases will be very dark in colour (deoxygenated) with low partial pressure of oxygen (PaO2).

14. Please include a title and a description of each figure and/or table. All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable). Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.

A title and a description of each figure and table is included. Measurement definitions, scale bars, and errors bars are not applicable, and have not been included. The Figure and Table Legends are included at the end of the Representative Results section.

15. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol.

We believe we have followed this instruction.

16. Please submit each figure individually as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps.).

Each figure has been submitted individually as a .eps file.

17. Please upload the Tables as xls/xlsx files instead of as PDF files.

The table has been uploaded as an .xls file.

_____

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This is a very good paper that provides technical procedures and insights for juvenile porcine orthotopic left lung transplantation. The paper provides key factors that will aid lung transplant investigation.

Thank you for your positive comments.

Unfortunately, the paper provides a very disjointed abstract and Introduction that needs major revision to allow for ease of comprehension.

You are correct. The abstract and introduction have been revised for content and flow. We have also revised the manuscript to focus specifically on transplantation. We have eliminated any reference to NPV-ESLP as a protocol. We have also removed any suggestion that this procedure would only work for NPV-ESLP, when in fact, it would work for positive pressure ventilation (PPV)-ESLP as well.

My original impression based upon the Title, Abstarct and Introduction was that this paper would describe the in house ESLP device and NPV-ESLP in detail in a juvenile porcine orthotopic left lung transplantation model. However, the authors provide no methodology for NPV-ESLP or PPV-ESLP nor do they describe the ESLP device other than what is described in lines 114-116 and 181-185.

It is only in the final paragraph of the Introduction, Line 93 that we find out that "The objective of the present study is to develop a porcine model of orthotopic left lung allotransplantation…" . The authors go on to state …"with NPV-ESLP, paying particular focus on anesthetic and surgical techniques, along with troubleshooting." - but no ESLP is provided.

Thank you for your comment, and we apologize for the confusion. Our original proposal upon invitation by JoVE was to produce a manuscript that included both protocols: NPV-ESLP and lung Tx; however, the recommendation was made to split the procedures, hence this manuscript is disjointed without its partner paper. The request from JoVE was to submit both papers simultaneously, which we did. We thought the papers might be reviewed together, although this was not the case. We make reference to our NPV-ESLP protocol: "1.0 Pre-surgical Preparations/Preparations and Anesthesia: Please refer to our protocol "Normothermic Negative Pressure Ventilation Ex-Situ Lung Perfusion: Evaluation of Lung Function and Metabolism"; however, it is currently being reviewed by others at JoVE. It is understandable that this particular transplant paper would seem rather confusing without the NPV-ESLP protocol. Moving forward, we have changed both manuscripts to be entirely independent protocols/papers. As such, we have extensively revised this manuscript. Reference 16 of the revised manuscript does provide details as to our original NPV-ESLP protocol, if you are interested. The up-to-date version was recently sent back to us by JoVE for minor revisions.

My suggestion would be to describe the surgery and suggest it as a protocol for use in ESLP in general and for possible studies comparing CST duraion and NVP and PVP and other interventions with graft function.

This is a very good suggestion, and we have revised the manuscript to reflect this.

Major Concerns:

Title: The title should reflect that this is a procedure for juvenile porcine orthotopic left lung transplantation.

You are correct. The title has been changed and now reads: "Left Lung Orthotopic Transplantation in a Juvenile Porcine Model for ESLP: Evaluation of Lung Function with Four Hour Assessment."

NVP-ESLP is not described, and the surgical procedure can be used in both PPV- and NVP-ESLP. The authors include but provide no technical information other than a misplaced note.

You are correct. As mentioned above, we originally intended for this manuscript to be reviewed along with our other manuscript that explains our NPV-ESLP protocol; however, this did not happen. This manuscript has been revised to read as an independent paper.

Summary: This is what the authors describe.

NPV has been removed from this section. It now reads: " This paper describes a juvenile porcine model of orthotopic left lung allotransplantation designed for use with ESLP research. Focus is made to anesthetic and surgical techniques, as well as critical steps and troubleshooting."

Abstract: The authors should revise this abstract in total and comment on the procedure for juvenile porcine orthotopic left lung transplantation and their development of a check sheet and insights to allow for success.

Thank you for your comment. We have revised the abstract accordingly. We have included a note regarding our check sheet and insights for success.

Lines 40-42 now read: " Herein, we describe our lab's porcine model of orthotopic left lung allotransplantation. This includes our anesthetic and surgical techniques, our customized surgical checklist, troubleshooting and modifications, as well as the benefits and limitations of our approach."

Introduction: The authors should delete lines 68-93.

Thank you for your feedback. Lines 68-93 have been deleted.

Protocol: The protocol is confusing due to the inappropriate positioning of ESLP and the lack of any description. The protocol jumps from 3.0 Left Lung Procurement to 4.0 Terminate ESLP, Divide Left Lung and Flush with Perfadex.

You are correct, it is confusing without the accompanying paper on our NPV-ESLP protocol. We have revised the manuscript to eliminate any reliance on the partner paper as described above.

The statement on lines 114-116 "Please refer to our protocol "Normothermic Negative Pressure Ventilation Ex-Situ Lung Perfusion: Evaluation of Lung Function and Metabolism". Needs a reference and should be moved to a new section following 3.0.
A brief description of the ESLP methods should be provided. I would suggest at a minimum the addition of cannulation requirements and any insights that may be important.
Line 181-185: As there is no description of ESLP it will be difficult for readers to act on the instructions "On the "Setting" page, click "Shutdown Server" and "Remove the lid from the chamber". Disconnect the PA adapter from the PA cannula.

Thank you for your comment. We have revised the manuscript to avoid any direct reference or reliance on ESLP, specifically any reliance on our device of NPV-ESLP and accompanying software. We have a separate paper outlining the specifics of our up-to-date protocol that was recently reviewed by JoVE and returned for minor revisions. Reference 16 of this revised transplant manuscript details our original NOV-ESLP protocol if you are interested.

Discussion: The authors need to put some of these notes in the abstract and Introduction. I would suggest at least, the inclusion of the 'Check List".

Thank you for your comment. We agree and are happy to make this suggested improvement.

The following text has been added to the abstract (lines 39-42): " Herein, we describe our lab's porcine model of orthotopic left lung allotransplantation. This includes our anesthetic and surgical techniques, our customized surgical checklist, troubleshooting and modifications, as well as the benefits and limitations of our approach."

The following text has been added to the introduction (lines 121-124): " The objective of the present study is to describe a porcine model of orthotopic left lung allotransplantation for use with ESLP. We include descriptions of our anesthetic and surgical techniques, a custom surgical checklist, and details regarding our troubleshooting experience and protocol modifications. We also address the limitations and benefits of our left lung porcine transplantation model."

Minor Concerns:
Lines 398-401: The authors appear to suggest that this protocol would only work with NPV-ESLP. I would suggest they just use ESLP as a descriptor.

You are correct. This transplant protocol would work for any form of ventilation ESLP; therefore, we have implemented your suggestion throughout the paper.

The specific lines you refer to (1082-1084) have been edited and now read: " The model has only been assessed with a four-hour period, which only serves to assess the transplanted lung function in the acute post-operative period following twelve hours of ESLP."

Reviewer #2:
Manuscript Summary:
A manuscript by Forgie et al describing Left Lung Transplantation in a Porcine Model of NPV-ESLP: Evaluation of Lung Function with Four Hour Survival. The authors should be commended for their Negative Pressure Ventilation applied to EVLP. However regarding their lung transplant model I do have some major and minor concerns.

Major Concerns:
1. I read the manuscript with great interest, since good porcine models are rare. I was in particular interested in negative pressure ventilation; however, this is only mentioned as 1.0 Pre-surgical Preparations/Preparations and Anesthesia: Please refer to our protocol "Normothermic Negative Pressure Ventilation Ex-Situ Lung Perfusion: Evaluation of Lung Function and Metabolism". This manuscript outlines the retrieval process of porcine lungs in 35-50 kg Yorkshire pigs and the establishment and termination of NPV-ESLP using a custom-built device. Unfortunately, I was unable to find any reference to this protocol in the manuscript. I tried to search for the protocol on pubmed and other sites - but without luck. Please add a reference to the protocol referred to.

You are correct that the NPV-ESLP manuscript is not yet available. Our original proposal upon invitation by JoVE was to produce a manuscript that included both protocols: NPV-ESLP and lung Tx; however, the recommendation was made to split the procedures, hence this manuscript is disjointed without its partner paper. The paper you were looking for was under review and has been returned by JoVE with minor revisions required. The request from JoVE was to submit both papers simultaneously, which we did. We thought the papers might be reviewed together, although this was not the case. It is understandable that this transplant paper would seem rather confusing without the NPV-ESLP protocol. Moving forward, we will change both manuscripts to be entirely independent protocols/papers. As such, we have extensively revised this manuscript. Reference 16 of this revised manuscript contains our original NPV-ESLP protocol. The up-to-date version for JoVE will be re-submitted with minor revisions soon.

2. I would recommend leaving out the NPV-ESLP as it is not described in the current protocol and does not add any valuable information of the technology. I would recommend focusing on the main aim of the manuscript - as stated in the summery by the authors: paying particular focus to anesthetic and surgical techniques. I believe this refers to the lungtx rather than the NPV-ESLP - since the later is not describes in the manuscript.

We appreciate your honest feedback and agree. As mentioned above, our original proposal upon invitation by JoVE was to produce a single manuscript outlining NPV-ESLP and lung Tx; however, it was suggested that due to length and content, the procedures should be split. Hence, this manuscript is disjointed without its partner paper. This manuscript has been revised to describe lung transplant as an entirely independent protocol that can be applied to any ESLP strategy, not exclusively NPV-ESLP.

3. I was surprised by the terminology ESLP, especially in the introduction where the ESLP is clearly used for describing EVLP (line 68-76). I believe the authors do use the term ESLP regarding their own technology of applying negative pressure to the EVLP chamber, however this becomes highly confusing for the reader. Please keep to the well know and accepted terminology - at least when referring to other authors that clearly has used EVLP.

Thank you for making this distinction. The terminology ESLP is encouraged in place of the traditional EVLP due to the ethical argument that the organ being treated did not come from a living donor, but one that had (experimentally-induced) brain or circulatory death; therefore, should not be referred to as "vivo". Instead,

"ex-situ" is more accurate. ESLP is the more common terminology in recently published studies (clinical and basic research) and will become the norm.

4. Insert Central Line and Arterial Line - the incision is describing as midline incision centered over the trachea and extend cranially from the sternal notch. Why did the authors choose to place the incision in the midline? To place the incision approximately 2-3 cm lateral from the midline is more convenient (and common, and also advised in the pig surgery textbooks) and get a better access. Both the arterial and the venous line can then be placed in the vessel branches, and you may then get in multiple catheters in several branches if you would like to put in a PA catheter and a dialysis catheter for multiple surveillance of the pig. The pig does have multiple branches of both arteries and veins on the neck that the humans lack.

Thank you for sharing your perspective. You are correct that an incision 2-3 cm lateral from the midline is more common; however, a midline incision is convenient and practical for our purposes. A midline incision is easy to perform and is our standard approach during lung procurement operations due to the ability to continue the incision to the xiphoid for sternotomy (16). Also, in the setting of a procurement, it saves time by fully exposing the trachea for clamping at the time of pneumonectomy. We could perform an incision as you describe for our recipient pig, but there is no immediate advantage for our current experimental needs. For example, we do not require the insertion of additional lines, such as a dialysis catheter or a PA catheter, although your point is well-taken. If we are unable to cannulate the internal jugular vein or the carotid artery on one side, a midline incision allows for immediate access to the contralateral vessels (this occurs occasionally while teaching medical students). Furthermore, the tissue plane at the level of the trachea is easily dissected bluntly to access the carotid sheath, enabling an exposure in a matter of seconds. Previously, we have used an incision as you described, but we simply prefer a midline approach for the above reasons.

5. Multiple injections of Heparin was given based on the fear of thrombosis. Pigs are thrombogenic and heparins is needed, however the dose seems to be extremely exaggerated compared to other pig ltx models for example: Pig lung transplant survival model Andrea Mariscal, Lindsay Caldarone, Jussi Tikkanen, Daisuke Nakajima, Manyin Chen, Jonathan Yeung, Marcelo Cypel, Mingyao Liu & Shaf Keshavjee, Nature Protocols volume 13, pages1814-1828 (2018). Please provide a rational behind this extremely large dose. Did the authors use any laboratory guidance as ACT or hematocrome to adjust or justify the dose?

Thank you for the comment. Yes, we agree that the dose of heparin as described is large. We initially followed the advice of Mariscal et al (2018) and administered 100 units/kg (IV bolus) 5 minutes pre-PA clamping and again 5 minutes pre-PA unclamping; however, two of our first 10 pig transplants experienced PA obstruction due to clotting. We decided to increase our heparin dosing accordingly. We do not have access to laboratory guided ACT, which would be helpful. We initially increased our dosing to 100 units/kg IV bolus 5 minutes pre-PA clamping, and then repeating every hour. This equates to four additional doses, which is a total heparin dose of 25 000, which is perhaps high for lung transplantation, but certainly not for cardiac surgery or ESLP. Since submitting this manuscript, we have continued to adjust our heparin administration. Currently we administer 100 units/kg IV bolus pre and post PA clamping, then 1000 units IV bolus every hour for three doses, which totals 13 000 units. This has also proved adequate. Our motivation for reducing the dose was to save on materials. We experienced no bleeding complications from a total dose of 25 000 units over a 5-hour period, which served our objective of a 4-hour assessment post-transplant.

The heparin protocol with this manuscript has been updated to reflect our current strategy. The following text has been added at lines 1071-1079: " Initially, we administered 5000 units of heparin IV 5 minutes prior to PA clamping and an additional 5000 units 5 minutes prior to PA unclamping. Dosing frequency was increased to include 5000 units every hour after PA unclamping, and there have not been any issues with bleeding or PA clotting since adopting this approach. We have since adopted a strategy that utilizes less heparin. We dose 5000 units IV heparin 5 minutes prior to PA clamping and again 5 minutes prior to partial PA unclamping. This is followed by 1000-unit IV heparin boluses every hour for the remainder of the case. We do not have access to ACT analysis, which would be the most accurate means of accessing adequacy of heparinization."

6. The main drawback of the current model is the decision to reduce the pulmonary vein anastomosis. Given the right technique anastomosis of both upper and lower pulmonary vein many of the downstream problems the authors are facing could be avoided.

You make an excellent point. We were actively adjusting our approach to this at the time this manuscript was submitted. Our approach has been adjusted to include anastomoses for both sets of pulmonary veins, and the left upper lobe appears far healthier at exsanguination, although the final lung function did not

change. The superior pulmonary veins branches are approximately 0.5 cm in diameter, which is significantly smaller than a coronary artery and likely would clot if a direct end-to-end anastomosis were performed between donor and recipient. The common SPV trunk is of variable length and occasionally absent, so it is not a reliable vessel when developing an easily reproducible model. Instead, we have opted to sew the donor LA cuff, which includes left sided inferior and superior pulmonary veins onto the recipient LA cuff that is formed by extending the ostia of the inferior left pulmonary vein. This single anastomosis allows for adequate drainage of the entire left donor lung.

The following text has been added at lines 1115-1120: "Our most recent protocol improvement pertains to our superior pulmonary vein (SPV) anastomoses. Originally, we simply oversewed the recipient SPVs due to their small caliber and propensity to clot, but the donor upper lobe would occasionally suffer congestion as the amount of collateral drainage was variable between pigs. Now, we incorporate the donor SPV and IPVs into our recipient IPV/LA anastomosis and this has eliminated any issue with venous drainage and lung congestion."

7. The ventilatory strategy is very limited described and should be extended (included). Why was a harmful ventilation of 10 ml/kg used? The authors describe that the ventilation was set to 5 ml/kg during OLV. Whys was not a protective ventilation of 5-6 ml/kg used during the whole process? How was the ventilation adjusted according to RR, PEEP and PIP (- not only during OLV also during DLV)?

Thank you for your comment. In our experience a ventilation of 10 mL/kg is not harmful. Typically, we target a tidal volume of 10mL/kg during lung procurement and 6-10 mL/kg during NPV-ESLP. While targeting at tidal volume of 10mL/kg, the recipient pig typically has recorded tidal volumes of 6-8 mL/kg. During one lung ventilation, we reduce the ventilation volumes by half to protect the left lung, and we increase the respiratory rate to target a physiologic end-tidal carbon dioxide level. We are continuing to develop our protocol and may experiment with lower ventilation volumes of 6-8 in the future; however, our goal is maximal recruitment to achieve the greatest oxygen diffusion and the highest PF ratios possible, which is why we target higher TVs.

We have added the following text to lines 418-420: "1.9. The ventilator settings are set at: respiratory rate of 12-30 breaths/minute, TV 6-10 ml/kg, PEEP 5 cmH2O, Peak Pressure 20 cmH2O. Of note, although we target TVs as high as 10 ml/kg, we typically achieve TVs of 6-8 ml/kg."

And lines 816-818: "During one lung ventilation, we reduce the ventilation volumes by half to protect the left lung from overinflation, and we increase the respiratory rate to target a physiologic end-tidal carbon dioxide level."

As well as lines 889-892: " At the time of reperfusion, we notice the following trends. Once the bronchus is unclamped and TVs are increased back to 10ml/kg, the left lung will begin to inflate. Although we target 10ml/kg for tidal volumes, we generally attain 6-8 ml/kg, and this is achieved gradually over the first 2-3 hours of reperfusion, depending on the ESLP protocol used and the quality of the implanted lung."

8. The authors state that they in particular focus on the anesthetic of the lungtx, however very little is described. The section could be significantly described in detail.

Thank you for your comment. We believe that there is adequate description of the anesthetic technique to reproduce the procedure. You are correct that our description could be more comprehensive; however, the aim of this paper is not to educate on proper porcine anesthetic care, but simply describe how we perform the procedure, so it can be successfully replicated. Your comment has made us reconsider our wording. To better align our paper with the abstract and introduction, we have changed some key wording:

The abstract now reads: "Herein, we describe our lab's porcine model of orthotopic left lung allotransplantation. This includes our anesthetic and surgical techniques, our customized surgical checklist, troubleshooting and modifications, as well as the benefits and limitations of our approach."

The introduction now reads: " The objective of the present study is to describe a porcine model of orthotopic left lung allotransplantation for use with ESLP. We include descriptions of our anesthetic and surgical techniques, a custom surgical checklist, and details regarding our troubleshooting experience and protocol modifications. We also address the limitations and benefits of our left lung porcine transplantation model."

9. Why did one strive to have a heart rate of 100-120?

This heart rate was targeted based on the advice of our in-house veterinarian and our clinical experience.

We also reference the following text: "The Laboratory Swine: A Volume in The Laboratory Animal Pocket Reference Series" by Bollen, Hansen, and Rasumssen. On page 15, Table 1.5 Respiratory and Cardiovascular Function (mean +/- SD) of conscious swine, and miniature swine with adult weights of 35-55 kg and 70-90 kg, the HR is listed as 105 +/- 10.6.

In our experience, the pigs do well with a HR of 100-120, but occasionally we have pigs that perform well with slightly lower heart rates, in the high 80s. This is a clinical judgement that we've made based on our collective experience.

10. The authors describe the following: "Upon clamping the right hilum, the pig becomes tachycardic and 100% of the cardiac output is diverted to the left lung. Tidal volumes are not decreased at this time as the entire process takes approximately 5 minutes. The pig remains stable, but we have not tested how long the pig will survive with this situation". What is tachycardia referred to? 150? Why was not a pulmectomy preferred /done (including the accessory lobe) instead of clamping? Could the downstream problem with tachycardia be due to hypovolemia or atrial fibrillation instead of the clamping itself?

Thank you for your comments. This section now reads: " Upon clamping the right hilum, the pig becomes sinus tachycardic (120-140 bpm) and 100% of the cardiac output is diverted to the left lung. Tidal volumes are not decreased at this time as the entire process takes 10 minutes. The pig remains stable up to the 5-minute mark, but the heart develops ventricular fibrillation between 5-10 minutes and manual compressions are required to continue perfusing the left lung."

A pulmectomy is not preferred/done for two reasons: 1) considerable added surgical complexity without clinical/experimental benefit compared to clamping — clamping sufficiently isolates the right lung and accessory lobe in a fraction of the time 2) clamps can be easily applied and adjusted if the pig become acutely unstable, and a period of recovery is possible. Since submitting this manuscript to JoVE, we have altered our approach to include clamping the accessory lobe and eliminating the possibility of blood mixing entirely. The manuscript has been updated to reflect this. Other published approaches involve ligating the right pulmonary artery; however, there will still be a degree of passive pulmonary vein drainage into the left atrium that can confound results of an attempt at left lung isolated gas analysis due to mixing. That approach is superior for recovery models that keep the pig alive for days after surgery, but is inferior to our approach in terms of the accuracy of the blood gases collected due to mixing of right and left venous drainage.

Minor Concerns:
1. The title indicates survival; however, 4 hours of reperfusion is could hardly be referred to as survival. Please delete survival from the manuscript as this gives the wrong impression of the protocol described.

Thank you for your suggestion. Your point is valid, and we have changed the title and manuscript accordingly. The title now reads: "Left Lung Orthotopic Transplantation in a Juvenile Porcine Model of ESLP: Evaluation of Lung Function with Four Hour Assessment." Any description of "survival" has been removed from the manuscript.

2. In line 337 the terminology EVLP is suddenly used. Please be consistent.

Thank you for pointing this out. The line now reads: "There is often fluid within the donor lung airway after 12 hrs of ESLP and transplant."

Swine Left Lung Transplant Surgical Safety Checklist (Recipient Operation)

Before Induction of Anesthesia

Supplies/Equipment:

- ☐ Change CO_2 crystals
- ☐ Fill Isoflurane
- ☐ O_2 on, Ensure adequate O_2
- ☐ 2 suctions rtg, plus 3rd spare rtg
- ☐ Tool table, saw, drapes, cautery, lap sponges, silk ties, triple lumen catheter
- ☐ Tray for dissection
- ☐ Surgivet Monitor, incl ART line BP
- ☐ Table plugged in, heat on
- ☐ ETT tray
- ☐ Defibrillator rtg
- ☐ IV pump
- ☐ Fluid bags- Main bag on IV pump, Bolus bag on slam bag
- ☐ Syringe pumps set up, dose rates to be inputted confirmed, know dose range
- ☐ Container of Saline w/ 60cc syringe for flush
- ☐ Hep/Saline Bottle/Bag
- ☐ Various needles/syringes
- ☐ Blood gas syringes x6 rtg
- ☐ Cadaver bags x2
- ☐ Perfadex Flush rtg
- ☐ Blake Chest tube
- ☐ Bronchoscope w/ ETT adapter, Fog off, Flush
- ☐ 7Fr double lumen in Carotid

Animal Prep:

- ☐ Prepare Anesthesia sheets (Donor/Recipient)
- ☐ Ensure enough drug, check expiry
- ☐ Intubate with 8.5mm or larger ETT
- ☐ Clean/ iodine spray
- ☐ Sternotomy vs Thoracotomy
- ☐ Drape animal
- ☐ Jugular IV line (2ml/kg/hr)
- ☐ ART line BP (carotid or heart)

Premed Doses:

- ☐ Ketamine 17mg/kg IM
  - ☐ 3mg/kg IV q 1hr (range 1-3mg/kg)
- ☐ Hydromorphone 0.05mg/kg q2hr
- ☐ Atropine 0.04mg/kg IM
- ☐ Naloxone 0.01mg/kg IV if needed

MGMT Parameters:

Vitals

- ☐ MAP 60-70 mmHg
- ☐ HR 90-110
- ☐ Temp 37.5-39

Ventilation:

- ☐ RR: 15-20
- ☐ TV: 6-10 mL/kg
- ☐ I/E: 1:2
- ☐ Peak Pressure: 20-30 cm H_20

Before Skin Incision

Anticipated Critical Events:

Predraw:

- ☐ Dextrose 100mL
- ☐ Insulin R 10 units
- ☐ Calcium Gluconate
- ☐ Cephazolin and Methyl Pred.
- ☐ Bupivacaine 10ml 0.5%
- ☐ Furosemide 40mg

To Surgeon:

- ☐ What are the critical or non-routine steps?
- ☐ How long will the case take?
- ☐ What is the anticipated blood loss?

To Veterinary Technicians:

- ☐ Any specific concerns?

Have Abx and Steroids been administered after central line insertion?

- ☐ Cefazolin 1 g IV
- ☐ Methlyprednisone 500mg IV

Intercostal nerve block prior to skin incision

- ☐ Bupivacaine 5 mL – incision
- ☐ Bupivacaine 5 mL – Intercostal block

Heparin 5000 units IV

1) ☐ Prior to PA clamp
2) ☐ Prior to PA unclamp + 40mg Lasix

Bronchus Clamp

- ☐ decrease TV to 5 ml/kg
- ☐ increase RR to 30
- ☐ increase peak pressure to 25-30
- ☐ maintain end-tidal CO_2 35-55 (40) mmHg
- ☐ I:E = 1:1

Prone Pig

- ☐ T1 after reperfusion with good ABG
- ☐ Close chest
- ☐ ECG leads

PA Unclamp

- ☐ Lasix 40mg IV

Infusion Rates: MAP<60, HR<90

- ☐ **Total IV fluid rate:** 70-100 ml/hr
- ☐ Phenylephrine 2-10 ml/min
- ☐ Dobutamine 1.5-5 mcg/kg/min
- ☐ Norepinephrine 0.01-0.3mcg/kg/min
- ☐ Vasopressin 0.5-2 ml/hr (0.1-0.4 u/hr)

Before Team Leaves Operating Room

Technician Verbally Confirms:

- ☐ Review Equipment Problems
- ☐ Review New Protocol and any planned changes
- ☐ Debrief:
  - What went well?
  - What can be improved?
- ☐ Return Checklist to Surgeon

ABGs

- ☐ arterial access x1
- ☐ at reperfusion
- ☐ q30minutes after reperfusion
- ☐ Isolated Pulm. Vein sample at T4

10cc blood samples

- ☐ Invivo with arterial access
- ☐ T0 at reperfusion
- ☐ T1
- ☐ T2
- ☐ T3
- ☐ T4

Tissue Samples

- ☐ Invivo – auto transplant only.
- ☐ T0 Reperfusion
- ☐ T4 Reperfusion

Weigh Lungs

- ☐ Explant/pre ESLP
- ☐ Post ESLP
- ☐ Left lung pre-implant: _____
- ☐ Left lung post exsanguination: _____

Common Troubleshooting Algorithm:

- ☐ Rapid Afib – 50mg Amiodarone IV bolus, repeat PRN
- ☐ Tachycardia
  - ☐ assess pain
  - ☐ assess depth of anesthetic
  - ☐ assess drips
- ☐ Hypotension MAP <60 – 250 ml fluid bolus
  - ☐ HR <90 – Dob or NE
  - ☐ HR >90 – Phenyl or Vaso
- ☐ Hyperkalemia K>5.0-5.5
  - ☐ Furosemide 40mg IV bolus
  - ☐ 100 ml D25 with 10u Insulin
- ☐ Hyperkalemia K>5.5 -6.0
  - ☐ Furosemide 60mg IV bolus
  - ☐ 100 ml D25 with 10u Insulin
- ☐ Hyperkalemia K>6.0 – 6.5
  - ☐ Furosemide 80mg IV bolus
  - ☐ 100 ml D25 with 10u Insulin
  - ☐ 1-gram Calcium

Figure 5: Surgical Safety Checklist for Left lung transplantation. Rtg, ready to go; hr, hour.