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Authors

Vali, Payam
Gugino, Sylvia
Koenigsknecht, Carmon
et al.

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Video Article

The Perinatal Asphyxiated Lamb Model: A Model for Newborn Resuscitation

Payam Vali*[1], Sylvia Gugino*[2], Carmon Koenigsknecht[2], Justin Helman[2], Praveen Chandrasekharan[2], Munmun Rawat[2], Satyan Lakshminrusimha[1], Jayasree Nair[2]

[1]Department of Pediatrics, University of California Davis

[2]Department of Pediatrics, University at Buffalo

*These authors contributed equally

Correspondence to: Sylvia Gugino at sfgugino@buffalo.edu

Abstract

Birth asphyxia accounts for nearly one million deaths worldwide each year, and is one of the primary causes of early neonatal morbidity and mortality. Many aspects of the current neonatal resuscitation guidelines remain controversial given the difficulties in conducting randomized clinical trials owing to the infrequent and often unpredictable need for extensive resuscitation. Most studies on neonatal resuscitation stem from manikin models that fail to truly reflect physiologic changes or piglet models that have cleared their lung fluid and that have completed the transition from fetal to neonatal circulation. The present protocol provides a detailed step-by-step description on how to create a perinatal asphyxiated fetal lamb model. The proposed model has a transitioning circulation and fluid-filled lungs, which mimics human newborns following delivery, and is, therefore, an excellent animal model to study newborn physiology. An important limitation to lamb experiments is the higher associated cost.

Video Link

The video component of this article can be found at https://www.jove.com/video/57553/

Introduction

Perinatal asphyxia occurs in roughly 4 per 1,000 term births in the United States and is responsible for approximately 25% of the 4 million neonatal deaths worldwide[1,2]. Throughout the fetus's natural development, several adaptations must take place during labor and at birth to allow for a seamless transition from the intra- to the extrauterine environment when the lungs take on the role of the placenta as the organ of gas exchange. Any failure of the newborn to adequately transition at birth further compromises resuscitative efforts. Instances when fetal lung clearance is incomplete or delayed[3,4], and circumstances that result in a persistent high pulmonary vascular resistance (PVR)[5] impact the efficacy of ventilation, which remains the most important intervention in the resuscitation of the asphyxiated newborn[6]. In addition, immediate clamping of the umbilical cord and removal of the low-resistance placenta can lead to abrupt changes in cardiac output that may cause myocardial dysfunction[7,8].

Owing to the infrequent need for aggressive resuscitation (need for chest compressions and/or epinephrine administration)[1,9], there is a lack of strong evidence from large randomized clinical trials to support the current neonatal resuscitation program (NRP) guidelines. Many translational research studies in neonatal resuscitation are conducted using postnatal animal models (particularly piglets) that fail to adequately depict the transitioning fetal circulation and fluid-filled lungs inherent to the newborn in the delivery room. Given the unique challenges related to transition from fetal circulation to neonatal circulation, the perinatal asphyxiated cardiac arrest fetal lamb model is ideal to study newborn resuscitative physiology.

The studies by Joseph Barcroft on fetal lambs, as early as the 1930's, laid the foundation for fetal and neonatal physiology[10]. In the second half of the 20th century, Geoffrey Dawes' innovative and meticulous experiments on fetal lamb models, and later those by Abraham Rudolph have tremendously contributed to the knowledge of cardiovascular and pulmonary physiology in the fetus[11,12]. In recent years, studies on fetal/neonatal lamb models have provided a better understanding of the impact of ventilation on hemodynamics[13,14], the effects of oxygenation on PVR[15,16], as well as the circulatory changes that occur during cord clamping[7,17]. Finally, in the past year, the newborn lamb has served as a novel model to study the hemodynamic effects during resuscitation[18,19,20]. A step-by-step narrative of what is involved in conducting a lamb experiment, as well as a detailed description of the surgical instrumentations and the experimental methodology will be presented.

Protocol

All protocols have been approved by the Institutional Animal Care and Use Committee (IACUC) by the State University of New York Buffalo. An illustration of the methodology depicting invasive and non-invasive monitoring is shown in **Figure 1**.

1. Animals

1. Use time-dated, Q-fever seronegative, pregnant ewes (mixed-breed, Suffolk-Dorset-Katahdin breed) with fetal lambs at 127–143 days gestation.
   NOTE: Sheep term gestation is 145 days and 127 gestation lambs behave like extreme premature infants.

2. Pre-surgical Preparation

1. Withhold food from the ewe 12 h prior to the surgery.
2. Sedate the ewe with intravenous (IV) diazepam (0.25–1.5 mg/kg) and ketamine (4 mg/kg).
3. Guide the ewe into lateral recumbency on to a lift cart.
4. Move the ewe to dorsal recumbency onto a V-top surgical table.

3. Anesthesia

NOTE: Prior to the caesarean section, the ewe is sedated with diazepam and ketamine and intubated followed by continuous inhalation of isoflurane (1–4%). Adequacy of anesthesia is monitored by evaluation of muscle tone and eye reflexes along with continuous monitoring of blood oxygen level with a pulse oximeter and the heart rate. During the instrumentation, lambs will be under the influence of maternal anesthesia and will also receive local anesthetic to sites of instrumentation.

1. **Intubate the ewe with a 10.0 mm cuffed endotracheal tube (ETT). Advance the ETT through the vocal cords 1–2 cm beyond the cuff. Inflate the ETT cuff and tie the ETT around the ewe's jaw to secure it in place.**
   1. When the palpebral response is absent, adjust the isoflurane (2–3% is usually sufficient).
   2. Provide artificial breaths by conventional mechanical ventilation (CMV) with a fraction of inspired oxygen (FIO_2) of 0.21. Set the ventilator to provide tidal volumes of 10–15 mL/kg.
   3. Monitor the oxyhemoglobin saturation continuously by a pulse oximeter placed on the ewe's tongue.
   4. Measure the end-tidal carbon dioxide ($EtCO_2$) with a capnograph.
   5. Adjust the FIO_2 and CMV to maintain oxyhemoglobin saturation between 90 - 97% and exhaled CO_2 between 35–45 mm Hg, respectively.

2. **Secure the ewe's limbs to the surgical table with surgical tie-downs.**
   1. Adjust the surgical table to reverse Trendelenburg position to relieve pressure on the fetus.

3. Place an orogastric tube to decompress and drain gastric contents.
4. Supplement IV fluid (normal saline or Lactated Ringers solution) at 10 mL/kg/h.
5. Place a rectal temperature probe to obtain continuous core temperature.

4. Surgery

NOTE: The cesarean section and fetal procedures are considered acute surgeries in which the animals are euthanized by administering sodium pentobarbital 100 mg/kg IV. Ewes are euthanized following the delivery of the lambs and the lambs are euthanized after the completion of the experiments. Euthanasia is confirmed by asystole. A secondary method of bilateral thoracotomy or exsanguination is also employed. In this case, the aseptic technique is not practiced during the surgery. Personnel still need to wear protective attire during animal contact.

1. Shave the wool from the ventral abdomen.
2. **Use the cautery to make a 15–18 cm abdominal skin-deep incision over the linea alba to expose the fascia.**
   1. Create a small opening into the abdomen using pressure with a blunt tipped hemostat.
   2. Maintain this opening while sliding a blunt tipped scissor into the abdominal wall.
   3. Use the scissors to complete the incision of the linea alba.

3. **Locate and exteriorize the fetal head within the uterus from the abdomen.**
   1. Hold the fetal head with one hand and use the cautery to make a 10 cm incision through the uterine wall (over the lamb's forehead). Ensure to avoid any cotyledons.
   2. Use Babcock forceps to clamp the layers of the uterus and placenta together at four opposing corners.
   3. Expose the lamb's head through the uterus and outside of the ewe's abdomen.
   4. Leave the Babcock forceps laying outside of the abdomen during the fetal surgery.

5. Fetal Airway

1. **Intubate the partially exposed fetal lamb with a 3.5–4.5 mm cuffed ETT. Advance the ETT through the vocal cords 1 cm beyond the cuff.**
   1. Inflate the cuff and secure the ETT by tying an umbilical tape around the tube and then around the head.
   2. Allow excess fetal lung fluid in the ETT to drain passively by tilting the head to the side. This simulates egress of lung liquid during labor.
   3. Occlude the ETT to prevent gas exchange during gasping in the asphyxial period.

6. Neck Vessel Instrumentation

1. **Shave the wool and infiltrate 1–2 mL of subcutaneous bupivacaine hydrochloride (0.25%) at all incision sites.**
   1. Make two 3 cm skin incisions on each side of the neck (approximately 6 cm distal to the head) adjacent to the trachea. The incisions should transverse the neck.
   2. Perform the incision using the cautery while tenting the skin, to avoid lacerating the deep tissue.
2. Separate the fascia using curved Kelly or mosquito forceps.
3. **Isolate the right internal jugular vein and right common carotid artery.**
   1. Place two 20 cm, 0 silk sutures under both vessels.
   2. Allow a (proximal to distal) space of 1 cm between the silk sutures.
   3. Gently lift each suture that is around the vessel, superficially, to restrict blood flow and make a 1–2 mm transverse cut into the vessel using Iris surgical scissors.
4. **For the carotid artery, insert one pre-flushed catheter (15–17 G) in the right carotid artery caudally towards the aortic arch for blood pressure monitoring and blood sampling.**
   1. Use the proximal tie to ligate the artery completely.
   2. Repeat steps 6.3.2 - 6.3.3.
   3. Insert the pre-flushed carotid arterial catheter and use the distal silk suture to tie around both the artery and catheter with 3 knots.
5. Use a similar technique, to insert one pre-flushed catheter (14–16 G) into the jugular vein, repeat steps 6.3.2–6.3.3, advance 7–10 cm caudally to rest within the thoracic inlet (towards the right atrium). This is used for fluid and medication administration as well as venous pressure measurements.
   1. Check the catheter for patency with heparinized saline and then complete the suture tie around the vessel and catheter (with the proximal suture only) using 2–3 knots.
   2. Insert the second catheter (14–16 G) approximately 5 cm rostrally to collect blood from the cerebral circulation. Use the same cut for the placement of both catheters.
   3. Repeat step 6.5.1 as it pertains to the distal suture and catheter and then tie the silk sutures around the opposing catheter lines to secure them in place to prevent twisting.
6. **Extend the previously made incision into a T-shape (1–2 cm) on the left side.**
   1. Elevate the left carotid artery using opened curved hemostats.
   2. Place a 2 mm perivascular flow-probe around the vessel to measure the blood flow.
   3. Cover the flow-probe with a 1-cm flexible polymer sleeve to stabilize the probe in position.
   4. Place the flow-probe cable line into the T-shaped incision, allowing the cable to run parallel with the vessel.
   5. Close the skin incision. Tie around the cable, and around a loop of the cable, to avoid restricting the flow if pulled.

7. Great Vessel Instrumentation

1. Extract the fetal lamb to expose the chest, and cover the exposed skin with polyethylene wrap to prevent heat loss.
2. Place the lamb in right lateral recumbency.
3. Position the left forelimb around the neck to expose the surgical site.
4. **Infiltrate 3 mL of bupivacaine hydrochloride (0.25%) along the 4th intercostal space and make a 6 cm skin incision using the cautery.**
   1. Carefully pierce the intercostal muscle with blunt curved forceps, circle under the 3rd rib, and come out between the next intercostal space. Make sure not to injure the lung or the heart.
   2. Open the clamp for a pre-cut piece of 0 silk and bring it through to encircle the rib.
   3. Repeat steps 7.4.1–7.4.2 to secure the suture under the 4th rib.
   4. Clamp the ties around each rib. Gently pull up and insert a cotton-tipped applicator into the chest under the 4th intercostal space.
   5. Use the applicator stick to protect the chest contents while using the cautery to open the intercostal space in 1 cm for a final opening of 6 - 8 cm. Ensure that the lung is not caught in the rib ties.
   6. Place a rib spreader into the chest-opening. Opening the chest will reveal the left upper lobes of the lung, the main pulmonary artery (PA) and the ductus arteriosus.
5. **Use 2 x 2 inch gauzes to envelop the lung and gently push any exposed tissue away from the surgical area.**
   1. Use a small manual retractor to improve the field of view (if necessary).
   2. Place an umbilical tape (1/4 inch width, 6 inches length) pre-soaked in amnion around the great vessels. Soaking the umbilical tape decreases friction (and injury) onto the vessel walls.

6. Using forceps, lift the pericardium and cut along the main pulmonary artery, taking care not to cut the vagal nerve. Use an applicator stick to keep the right atrium from interfering with a lateral cut of the pericardium along the vagal nerve.
7. **Using Gemini forceps, move the forceps slowly from the ductal/PA notch around and behind the left PA to "encircle" and emerge on the other side of the left PA. Many delicate tissues run under this area and proceed only if there is no resistance.**
   1. When the tip of the forceps is observed, open only far enough to grab one end of the pre-moistened umbilical tape. Clamp with only 1 position clicked in place. It is possible that surrounding tissue could be caught in the forceps too, and if tightly clamped that tissue might be caught with the umbilical tape resulting in vessel injury.
   2. Gently back out the forceps to pull the tape into position. This serves to open a space for the flow probe and helps to direct the insertion of the flow probes (**Figure 2**). Care to protect the notch portion of any vessel is imperative as this is the most fragile part of the tissue.

8. **Place a 4–6 mm perivascular flow-probe by gently lifting the umbilical tape and guiding the (L-bracket) metal portion of the probe around the vessel along the established opening.**
   1. Direct the tape toward the back of the probe to help seat the vessel in place and to visualize the end of the L-bracket thus allowing the sliding closure to be secured.
   2. Make sure the vessel is > 75% of the probe size to ensure a tight fit and accurate flow measurements.
   3. Carefully cut the end of the umbilical tape close to the probe and gently pull the other end to remove the tape from the vessel. The tape will interfere with measurements if not removed.
   4. Apply ultrasound gel and adjust the probe and cable position to improve signal intensity and quality.

9. Repeat steps 7.6–7.7.4 as it pertains to the placement of a 6 - 8 mm perivascular flow-probe around the ductus arteriosus.
10. Remove any protective 2 x 2 gauze from the chest and allow the lung to re-position.
11. Place a new 2 x 2 gauze around each metal bracket of the flow probe to protect the lung.
12. Close the chest in layers using a tapered needle and 2.0 synthetic monofilament suture. Secure the cables of the flow-probes to the outer skin with a loop using a cutting needle and 2.0 silk suture.

8. Non-invasive Measurement

1. Attach EKG leads at the right axilla, left axilla, and right inguinal area (three-lead EKG). Pre- and post-ductal arterial oxyhemoglobin saturation (SpO_2) is monitored with a pulse oximeter placed on the right forelimb and either hindlimb, respectively. Secure a near-infrared spectroscope (NIRS) monitor over the forehead with sutures and an opaque dressing.

9. Data Collection

1. Collect and record data using a data acquisition software. **Figure 1** illustrates invasive and non-invasive parameters including: arterial and central venous blood pressure, left common carotid, left pulmonary and ductal arterial blood flow, EKG, SpO_2, NIRS, $ETCO_2$.
   NOTE: The software and equipment set-up for data collection can vary and is beyond the scope of this article.

10. Experimental Protocol

1. Occlude and cut the umbilical cord, and move the lamb from the maternal abdomen to a radiant warmer.
2. During the asphyxial period, insert an umbilical venous catheter for epinephrine administration (0.01–0.03 mg/kg IV, per NRP recommendations).
3. Insert an umbilical arterial catheter to collect post-ductal arterial blood gases.
4. Monitor the lamb for impending asystole, which can be defined as the absence of detected carotid flow and pressure.
5. Ensure that adequate personnel is available and dedicate each person to the following tasks: (1) manage ventilation and the airway, (2) perform chest compressions, (3) administer medication, (4) draw blood samples, (5) lead the code.
6. Begin resuscitation by providing positive pressure ventilation (PPV) with a T-piece resuscitator at pressures of 35/5 cm H_2O. Initiate the 20 min timer for experimental resuscitation protocol.
7. **After 30 s of ventilation, begin chest compressions by placing the thumb and fingers of one hand on opposite sides of the chest.**
   1. Provide chest compressions by squeezing the fingers and thumb together to a depth of approximately 1/3.

8. Administer epinephrine at 3-min intervals.
9. Continue resuscitation efforts until return of spontaneous circulation or up to 20 min.
10. At return of spontaneous circulation, continuous IV fluids (5 cc/kg/hr) and fentanyl (0.5-2.0 mcg/kg/hr; titrated to effect), are given through the previously established jugular venous catheter. Following the 20 min resuscitation period, the animal is euthanized. If lambs do not achieve ROSC by 20 min, proceed with euthanasia.

Representative Results

Following instrumentation of the fetal lambs, hemodynamic variables can be recorded (**Figure 3** and **Figure 4**), then analyzed and interpreted (**Figure 5**). Frequent blood samples can be collected, and **Figure 6** shows the pH and $PaCO_2$ data from one of the experiments. Occasionally, catheters or flow probes may malfunction or get dislodged and data can, therefore, not be collected and used for analysis, as is shown in **Figure 7**.
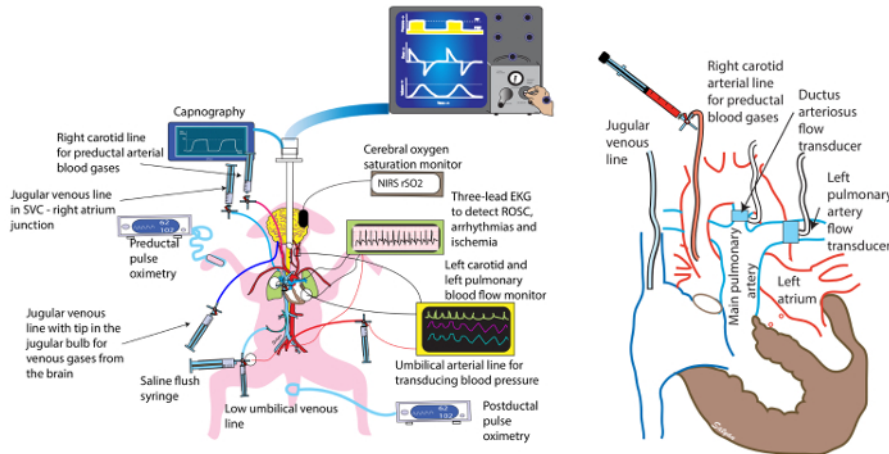
Figure 1: Invasive and non-invasive instrumentation in a lamb model. Illustration depicting invasive instrumentation (venous and arterial catheter insertion for blood sampling and pressure monitoring as well as vessel flow probe placements for blood flow measurement), and placement of non-invasive equipment (pulse oximeter, ECG leads, near-infrared spectroscopy, capnography). Please click here to view a larger version of this figure.
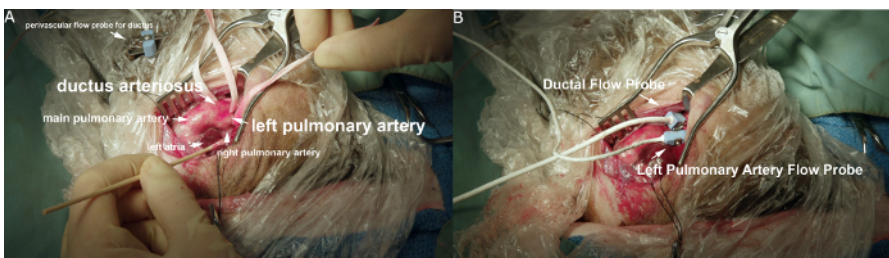

Figure 2: Great vessel isolation. Gentle separation of connective tissue creates a space for wet umbilical tape to be placed around vessels (**A**) with subsequent placement of flow probes for ultrasound measurements of blood flow inside each vessel (**B**). Please click here to view a larger version of this figure.
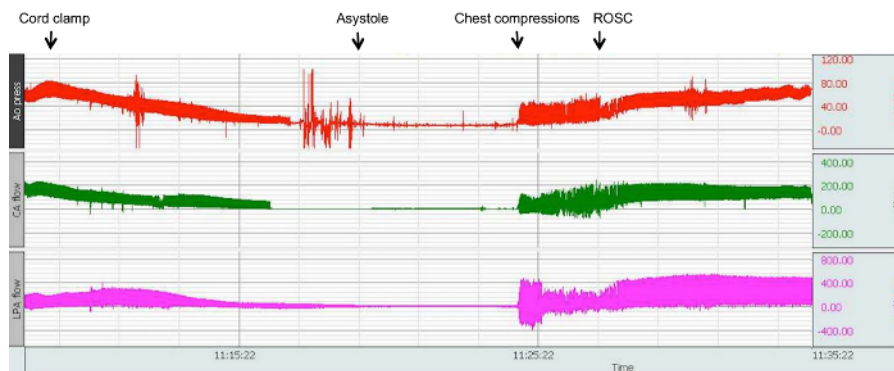

Figure 3: Acquisition software snapshot capturing hemodynamics of a perinatal asphyxiated lamb successfully resuscitated. Ao press: right carotid blood pressure; CA flow: left carotid blood flow; LPA flow: left pulmonary artery blood flow. Please click here to view a larger version of this figure.
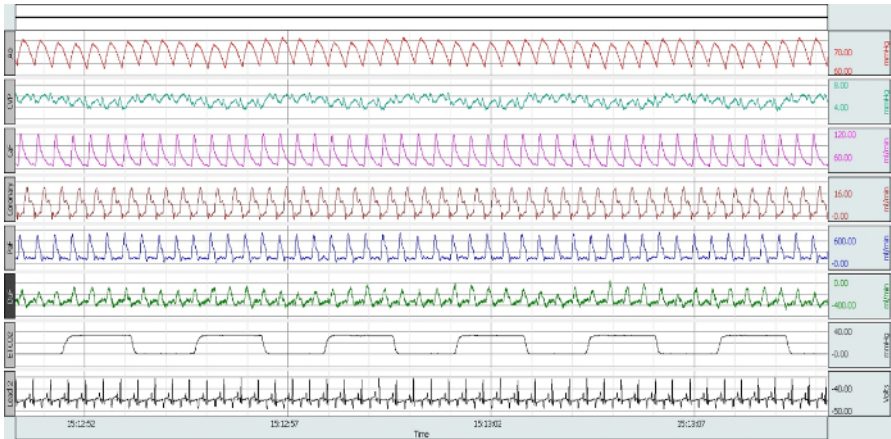
Figure 4: Acquisition software snapshot depicting invasive and non-invasive physiologic measurements. Ao: right carotid blood pressure; CVP: central venous pressure; CaF: left carotid blood flow; Coronary: left descending coronary blood flow; PaF: left pulmonary artery blood flow; DuF: ductus arteriosus blood flow; ETCO2: end-tidal CO_2; Lead 2: EKG lead 2 recording. Please click here to view a larger version of this figure.
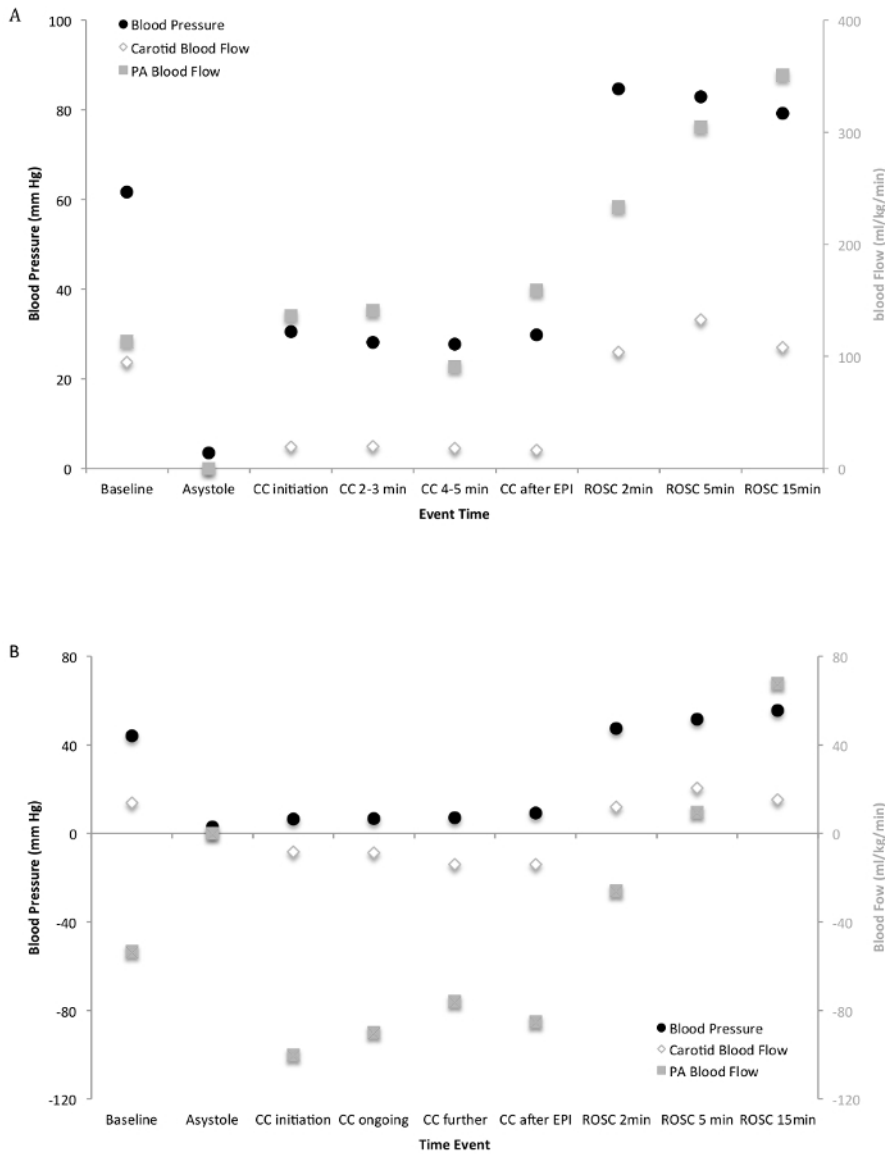
Figure 5: Hemodynamics of a perinatal asphyxiated lamb successfully resuscitated. Changes in blood pressure, left carotid blood flow, and left pulmonary blood flow are shown during the compression phase of chest compressions (**A**) and during the recoil phase (**B**). Left pulmonary and carotid blood flow are plotted on the secondary axis. CC: chest compression; EPI: epinephrine; PA: pulmonary artery; ROSC: return of spontaneous circulation. Please click here to view a larger version of this figure.
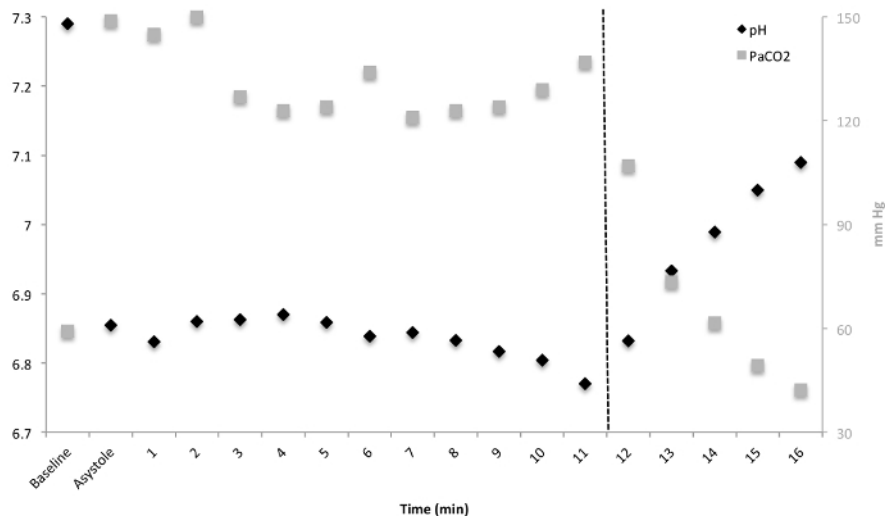
Figure 6: Blood gas analysis during an experimental study. Dashed line represents time of return of spontaneous circulation. PaCO$_2$ is plotted on the secondary axis. Please click here to view a larger version of this figure.
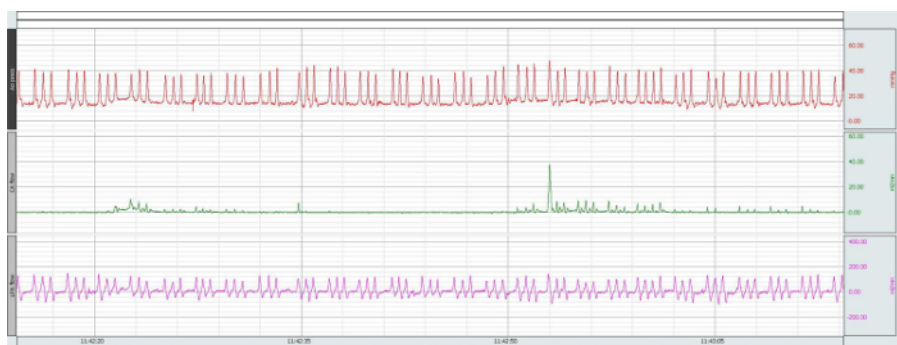

Figure 7: Acquisition software picture showing malfunctioning flow probe. Carotid blood flow (green tracing) recording is lost despite adequate blood pressure (red tracing) and left pulmonary artery blood flow (purple tracing). Please click here to view a larger version of this figure.

Discussion

The lamb model is comparable in size to human newborns and allows easy instrumentation to measure invasive hemodynamic variables. The fetal/newborn lamb model has been an invaluable research tool that has richly contributed to understanding the transitioning circulation, as well as the newborn's pulmonary and cardiovascular physiology. Several experimental lamb models have been established over the years to study optimal ventilation strategies in preterm lambs[13,14,21], pulmonary hemodynamics in a persistent pulmonary hypertension ductal ligation model[22,23,24], pulmonary vascular reactivity in a meconium aspiration model[25,26,27], as well as surgical models of fetal myelomeningocele[28,29], congenital diaphragmatic hernia[30,31], and congenital heart defect[32,33], which all have had a considerable impact on clinical management.

During fetal instrumentation, care needs to be taken to avoid any pressure on the umbilical cord, as this could cause undue stress on the lamb and could affect delivery of anesthesia to the lamb. Flow probes and catheters need to be carefully sutured to the skin of the lamb to protect the vessels from injury if inadvertent traction is placed on the catheters. Surgical instrumentation of the fetal heart poses an increased risk for devastating complications (death from exsanguination) unless meticulously performed. The thoracotomy needs to be completely sutured in layers because an incomplete seal may influence chest compression efficiency. Following intubation of the lambs, the lung liquid should be drained passively by tilting the fetal head to the side. On average, 15 - 20 mL/kg of lung liquid can be passively drained. The remaining lung liquid in the fetal lamb, then, approximates the estimated lung liquid in human newborns. The ETT needs to be occluded prior to asphyxiation by cord clamping to prevent gas exchange during gasps. A 5-min period of cardiac arrest is observed following pulselessness, as return of spontaneous circulation, otherwise, occurs rapidly following positive pressure ventilation. Owing that fetal baseline arterial gases do not demonstrate significant acidosis, fetal instrumentation just prior to experiments does not appear to cause considerable stress on the lamb. Gestation is usually determined prior to experiments by ultrasound confirmation, but can also be assessed by careful examination of the ewe in experienced hands. In the event of multiple fetuses, each lamb is instrumented sequentially. An important consideration in conducting lamb experiments is the significantly higher cost.

Until recently, the lamb model has not been used to study newborn resuscitation. Since the current NRP recommendations are primarily extrapolated from the adult literature or from manikin[34,35] and post-natal animal models[36] that do not adequately depict the newborn physiology in the delivery room, the protocol described in this article demonstrates the promising contributions that the perinatal asphyxiated lamb model can provide to improve resuscitative efforts of newborns that fail to adequately transition to the extrauterine environment. **Figure 5** shows blood pressure and flows in a newborn lamb that has been successfully resuscitated. Comparing blood pressure and flows (with the advantage to

measure ductus arteriosus flow in this model) in experiments assessing different compression-to-ventilation ratios and/or vasoactive medications in a model with transitioning circulation will provide a better understanding of neonatal hemodynamics during resuscitation. Experiments on this model will improve the understanding of optimal chest compression ratio, timing and dosing of administered medications, impact of inspired oxygen during neonatal resuscitation, as well as address many other hypotheses.

Disclosures

The authors have no conflicts of interest to declare

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