

Video Article

A Swine Model of Neonatal Asphyxia

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Abstract

Annually more than 1 million neonates die worldwide as related to asphyxia. Asphyxiated neonates commonly have multi-organ failure including hypotension, perfusion deficit, hypoxic-ischemic encephalopathy, pulmonary hypertension, vasculopathic enterocolitis, renal failure and thrombo-embolic complications. Animal models are developed to help us understand the patho-physiology and pharmacology of neonatal asphyxia. In comparison to rodents and newborn lambs, the newborn piglet has been proven to be a valuable model. The newborn piglet has several advantages including similar development as that of 36-38 weeks human fetus with comparable body systems, large body size (~1.5-2 kg at birth) that allows the instrumentation and monitoring of the animal and controls the confounding variables of hypoxia and hemodynamic derangements.

We here describe an experimental protocol to simulate neonatal asphyxia and allow us to examine the systemic and regional hemodynamic changes during the asphyxiating and reoxygenation process as well as the respective effects of interventions. Further, the model has the advantage of studying multi-organ failure or dysfunction simultaneously and the interaction with various body systems. The experimental model is a non-survival procedure that involves the surgical instrumentation of newborn piglets (1-3 day-old and 1.5-2.5 kg weight, mixed breed) to allow the establishment of mechanical ventilation, vascular (arterial and central venous) access and the placement of catheters and flow probes (Transonic Inc.) for the continuously monitoring of intra-vascular pressure and blood flow across different arteries including main pulmonary, common carotid, superior mesenteric and left renal arteries. Using these surgically instrumented piglets, after stabilization for 30-60 minutes as defined by $Z < 10\%$ variation in hemodynamic parameters and normal blood gases, we commence an experimental protocol of severe hypoxemia which is induced via normocapnic alveolar hypoxia. The piglet is ventilated with 10-15% oxygen by increasing the inhaled concentration of nitrogen gas for 2h, aiming for arterial oxygen saturations of 30-40%. This degree of hypoxemia will produce clinical asphyxia with severe metabolic acidosis, systemic hypotension and cardiogenic shock with hypoperfusion to vital organs. The hypoxia is followed by reoxygenation with 100% oxygen for 0.5h and then 21% oxygen for 3.5h. Pharmacologic interventions can be introduced in due course and their effects investigated in a blinded, block-randomized fashion.

Video Link

The video component of this article can be found at <https://www.jove.com/video/3166/>

Protocol

1. Anesthesia

1. Set the flow rate of the anesthetic machine at 2L/min. Connect the exhaust to vacuum suction.
2. Charge face mask with anesthetic gas (Isoflurane) at 5% (~3 min).
3. Newborn piglets will be induced with inhaled Isoflurane 5% in 100% oxygen (~3 min).
4. Maintain anesthesia at 2-3% of Isoflurane. Fine adjustment of Isoflurane by 0.5% as appropriate, however, it may range from 0.5 to 5% depending on the condition of piglets.
5. Once the vascular access has been established, the inhalational anesthesia can be switched to intravenous anesthesia using fentanyl (5-50 mcg/kg/h) and midazolam (200-500 mcg/kg/h) infusions. Pancuronium (50-100 mcg/kg/h) may be required to control excessive muscle movements during the surgery, whilst the ability to observe animal's state is preserved for the adjustment of anesthetic medications.
6. The piglet is monitored by pulse oximetry (percutaneous oxygen saturation at 95-100%) and ECG (heart rate at 130-170 beats/min).
7. The piglet's rectal temperature is maintained at 38-40°C with heating blanket and radiant warmer.
8. The anesthetic state of piglet is being regularly evaluated throughout the experimental period using neurological (pupil size, tearing, body movements), behavioral (agitation), cardiovascular (tachycardia and hypertension) and respiratory (tachypnoea) parameters as appropriate. Minimal paralysis is given. Previous experience of anesthesia in piglets with and without paralysis would be useful for evaluation.
9. The protocol is a non-survival procedure with euthanization of the animal at the end of experiment with an overdose of pentobarbital (100 mg/kg) intravenously.

2. Surgical placement of vascular catheters at the groin (Figure 1)

1. Make a long 2-3cm incision in the right groin.
2. Dissect 1cm of the right femoral venous and 1cm right femoral artery. Put two 3-0 strings around each vessel.
3. Right femoral venous catheterization: Ligate the distal of the vein. Insert an Argyle catheter (3.5 or 5 French, double-lumen)(Covidien, Mansfield, MA) to 15cm and this will place at the right atrium. Tie both strings to secure the catheter. The catheter can be used for maintenance fluid and medications infusion (secondary port) and central venous/right atrial pressure measurement (primary port).
4. Right femoral arterial catheterization: Ligate the distal of the artery. Lift up the proximal string to stop the blood flow. Insert an Argyle catheter (3.5 or 5 French, single-lumen) to 5cm. This will place the arterial catheter at the infra-renal aorta for continuous mean arterial pressure measurement and blood sampling. Tie both strings to secure the catheter.
5. Close the skin.

3. Establish mechanical ventilation (Figure 2)

1. Make a long 2-3cm horizontal incision in the neck.
2. Dissect and expose 1cm of the trachea. Put two 1-0 strings around the trachea.
3. Insert an endotracheal tube (3.0 or 3.5) at 1cm into the trachea. Connect to a ventilator and commence mechanical ventilation. Secure the endotracheal tube.
4. Dissect and expose the common carotid artery. Encircle the vessel with a transit time ultrasound flow probe (2SB or 2RB, Transonic Systems Inc., Ithaca, NY) to continuously measure the blood flow.

4. Placement of flow probes at superior mesenteric (Figure 3) and left renal (Figure 4) arteries

1. Extra doses of fentanyl (5-10 mcg/kg) and acepromazine (0.01-0.02 mg/kg) are required prior to skin incision.
2. Make a long subcostal-flank incision and carefully dissect muscle layers.
3. Expose the abdominal aorta.
4. Minimize vascular handling (vasospasm) and lymphatic injury.
5. Dissect 0.5-1cm superior mesenteric artery and put a Transonic flow probe (3SB) around it.
6. Dissect 0.5-1cm left renal artery and put a Transonic flow probe (2SB) around it.
7. Close the skin and secure the flow probe.

5. Placement of pulmonary artery catheter (Figure 5) and flow probe (Figure 6)

1. Extra doses of fentanyl (5-10 mcg/kg) and acepromazine (0.01-0.02 mg/kg) are required prior to skin incision.
2. Lie the animal at the right lateral position.
3. Thoracotomy at the left 4th intercostal space.
4. Watch out for the internal mammary artery and vein, ligate if needed.
5. Use a dental swab to press down the left lung and increase oxygen as needed.
6. Open the pericardium.
7. Identify the ductus arteriosus which runs from the pulmonary artery to the aorta.
8. Ductus arteriosus may be ligated by placing a clip or by a thick "3-0 silk" tie at its origin.
9. Free the main pulmonary artery and pass a vascular sling using a thick "0" tie.
10. Perform a purse string (5-0 prolene) suture at the base for the placement of pulmonary artery catheter.
11. Insert a 20G Angiocath (with 3 side holes at less 1 cm from the tip of the catheter) through the purse string to a maximum of 1 cm.
12. Check for free flow of venous blood.
13. Connect to pressure transducer, check for pulmonary artery pressure and waveform.
14. Tighten the purse string and secure the pulmonary catheter.
15. Place a Transonic flow probe (6SB) around the main pulmonary artery.
16. Place ultrasonic gel between the flow probe and artery to allow for optimal signal transduction.
17. Cover the wound with moist saline gauze.

6. Hypoxia and reoxygenation protocol

1. Decrease the inspired oxygen concentration to 10% by increasing the concentration of inhaled nitrogen gas to induce hypoxemia.
2. Adjust the inspired oxygen concentration between 10% and 15% to obtain a PaO_2 of 20-40 mmHg or SaO_2 of 30-40% for 2h.
3. Perform arterial blood analysis to assess PaCO_2 and adjust ventilator rate accordingly.
4. With the induction of hypoxemia, the first hour is dedicated to steadily inducing a tachycardic (and cardiac output) response.
5. Continue to monitor for changes in blood flow at the common carotid, superior mesenteric and left renal arteries.
6. During the second hour of hypoxia, the hypoxic stress is increased to steadily lower cardiac output to 30-40% of baseline, mean arterial pressure to 30-35 mmHg and arterial pH 6.95-7.05.
7. Hypoxic stress may be prematurely terminated or extended by 15 min as appropriate.
8. Increase inspired oxygen concentration abruptly to 100% abruptly by discontinuing nitrogen gas, while continuing pure oxygen.
9. Monitor cardiac output, mean arterial pressure and other hemodynamic parameters for rapid recovery.
10. Resuscitation with 100% oxygen can be continued for 0.5h. Following this time period, reduce the inspired oxygen concentration quickly to 21%.

11. Continue reoxygenation with 21% oxygen for the remaining period of experiment. The inspired oxygen concentration can be titrated to 25% if needed.
12. Fluid boluses of 10 ml/kg Ringer's lactate solution may be needed as appropriate during the experimental period. Its use has to be protocolized.

7. Representative Results:

The induction of hypoxemia in the newborn piglet over the first hour of hypoxia should increase the cardiac output (pulmonary arterial flow) to 120%-130% of baseline (**Figure 7A**) and heart rate (**Figure 7B**). Typically, cardiac output should reach its peak compensation between the first 0.5h and 1h of hypoxia. Further, blood flow should become centralized resulting in decreased mesenteric and renal perfusion but a preserved or increased common carotid arterial flow (**Figure 8**). During the second hour of hypoxia, there is a steady decrease of cardiac output, development of hypotension (**Figure 9A**), slowing of heart rate with or without arrhythmia occurred. Hypoxia should induce pulmonary hypertension with increased pulmonary artery pressure (**Figure 9B**), which may sometimes lower in the final 30 min of hypoxia as the cardiac output decreases.

Upon resuscitation, all hemodynamic parameters will immediately recover to normoxic baseline, except for the renal blood flow which gradually recovers over the first hour of reoxygenation. However, the hemodynamic parameters especially for the cardiac output and mean arterial pressure will gradually deteriorate over the first 2 hour of the reoxygenation to about 70-75% of normoxic baseline and 35-45 mmHg, respectively. This cardiovascular dysfunction is at least in part for myocardial stunning and warrants cardiovascular supportive therapies such as vasoactive and inotropic agents.

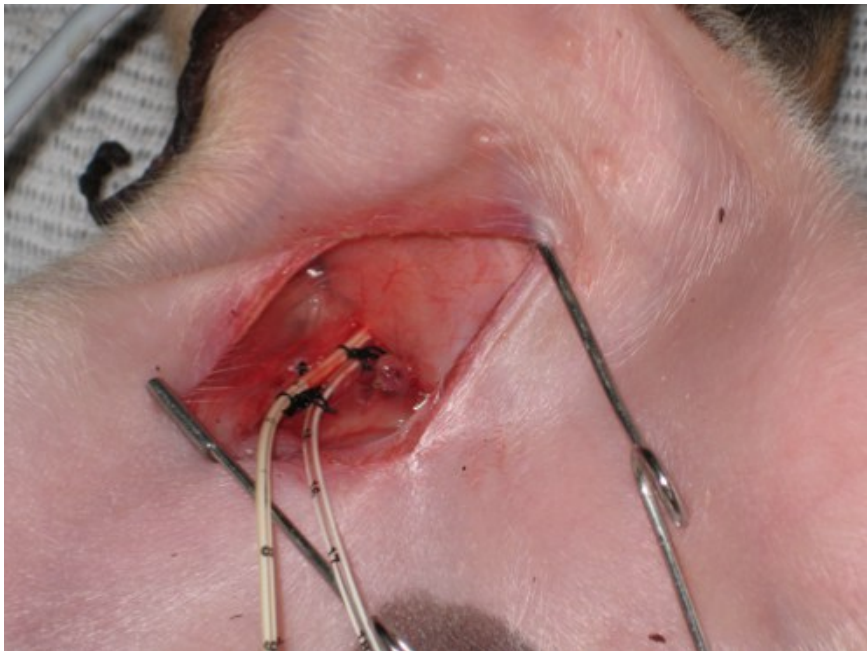


Figure 1: Groin incision with the placement of femoral arterial and venous catheters

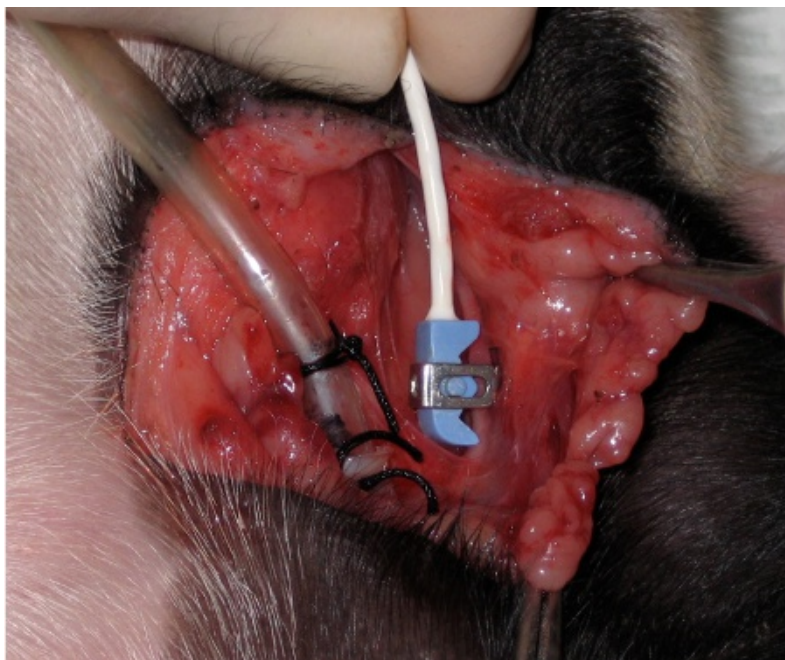


Figure 2: Neck incision with the placement of an endotracheal tube and a flow probe around the common carotid artery

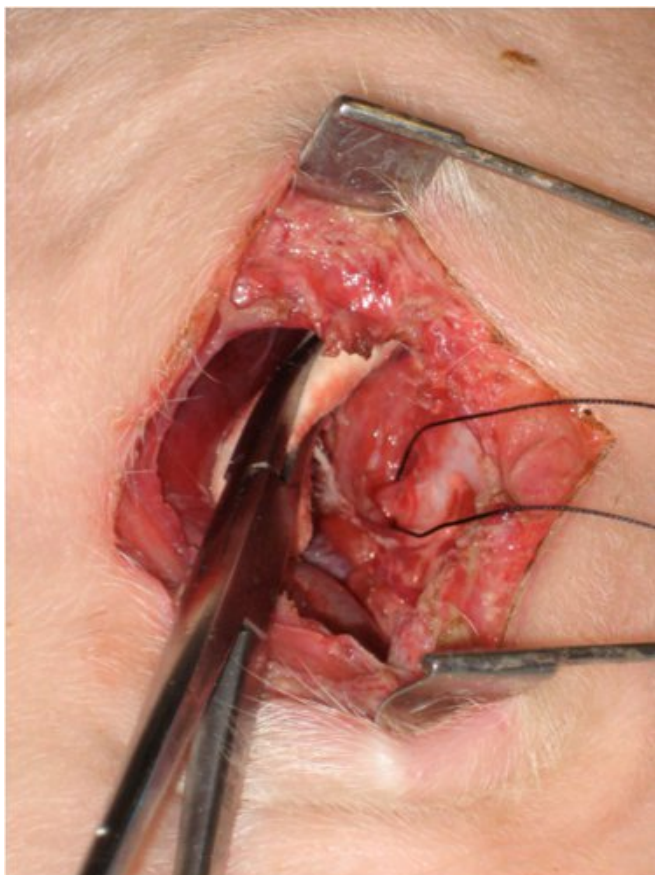


Figure 3: Flank incision with the isolation of superior mesenteric artery

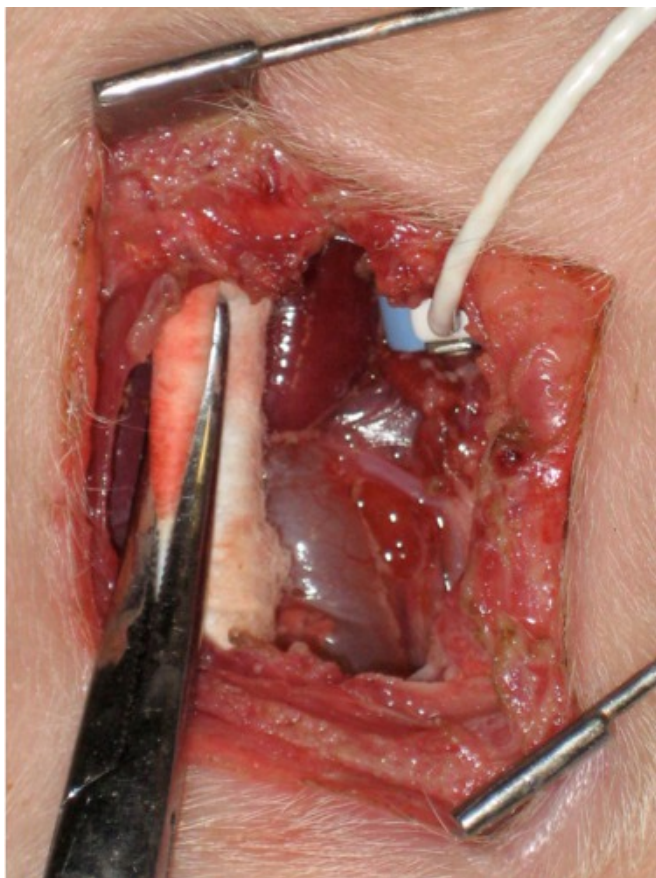


Figure 4: Flank incision with the isolation of left renal artery

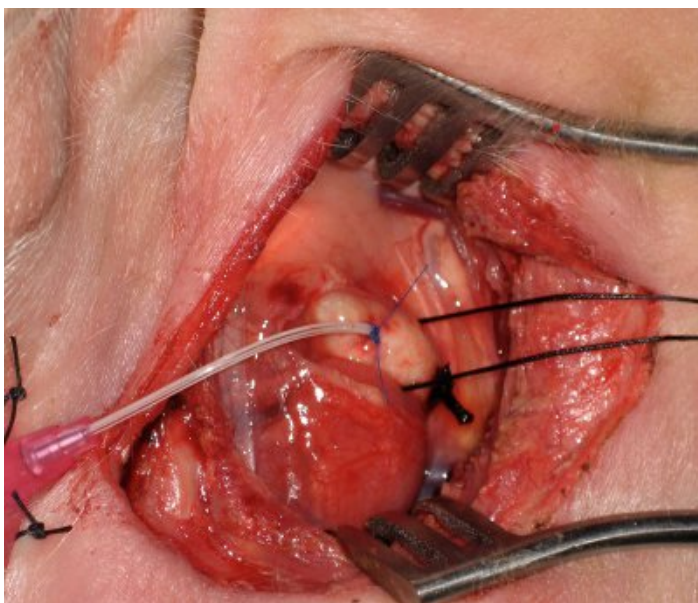


Figure 5: Thoracotomy with the placement of pulmonary artery catheter

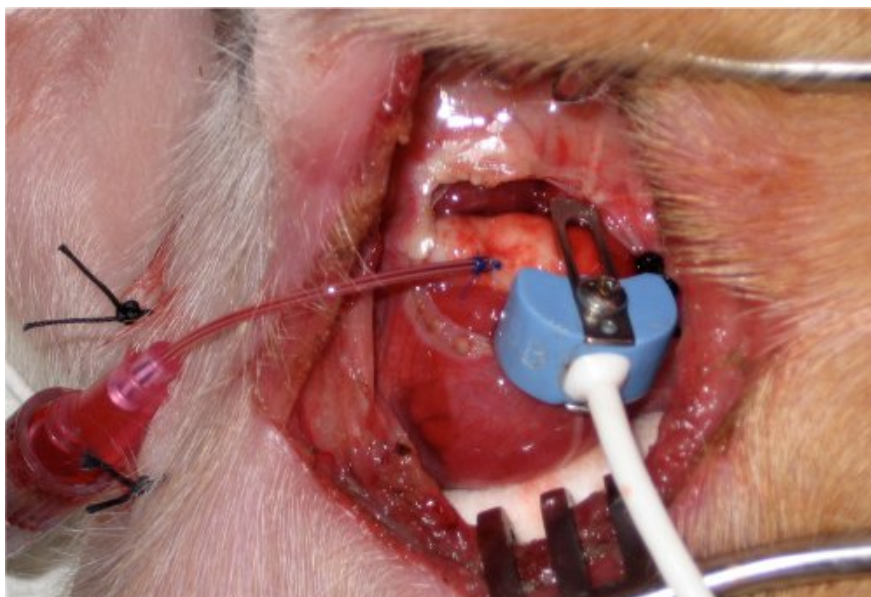


Figure 6: Thoracotomy with the placement of a Transonic flow probe around main pulmonary artery

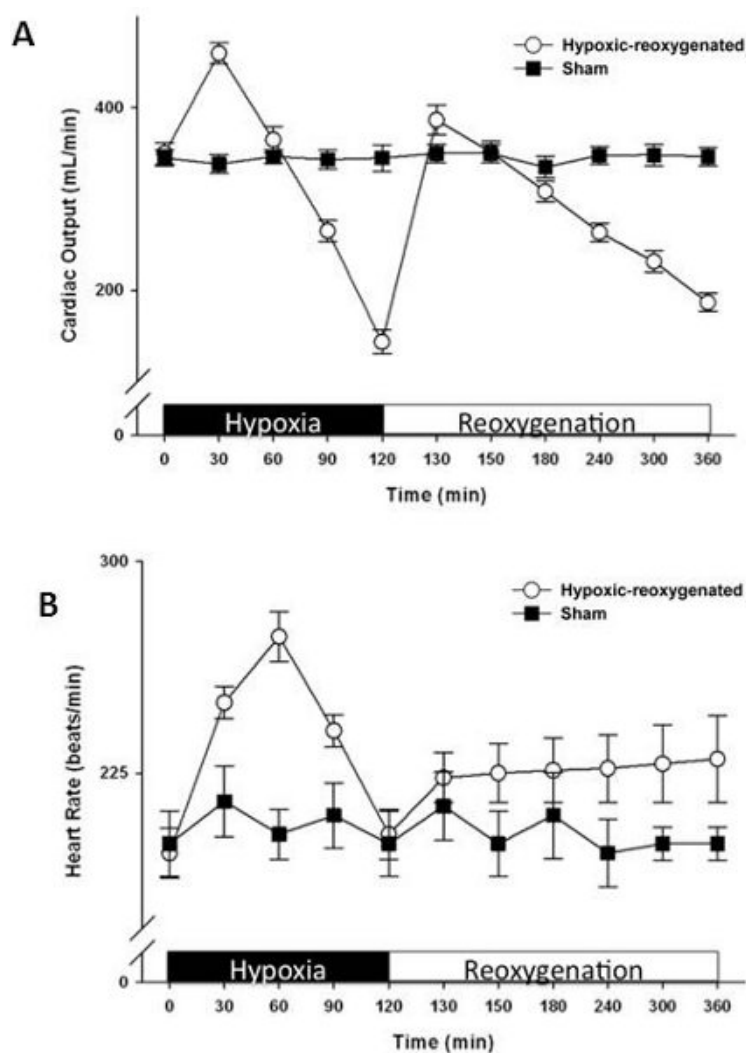


Figure 7: Temporal changes in (A) cardiac output (pulmonary arterial flow) and (B) heart rate during hypoxia and reoxygenation

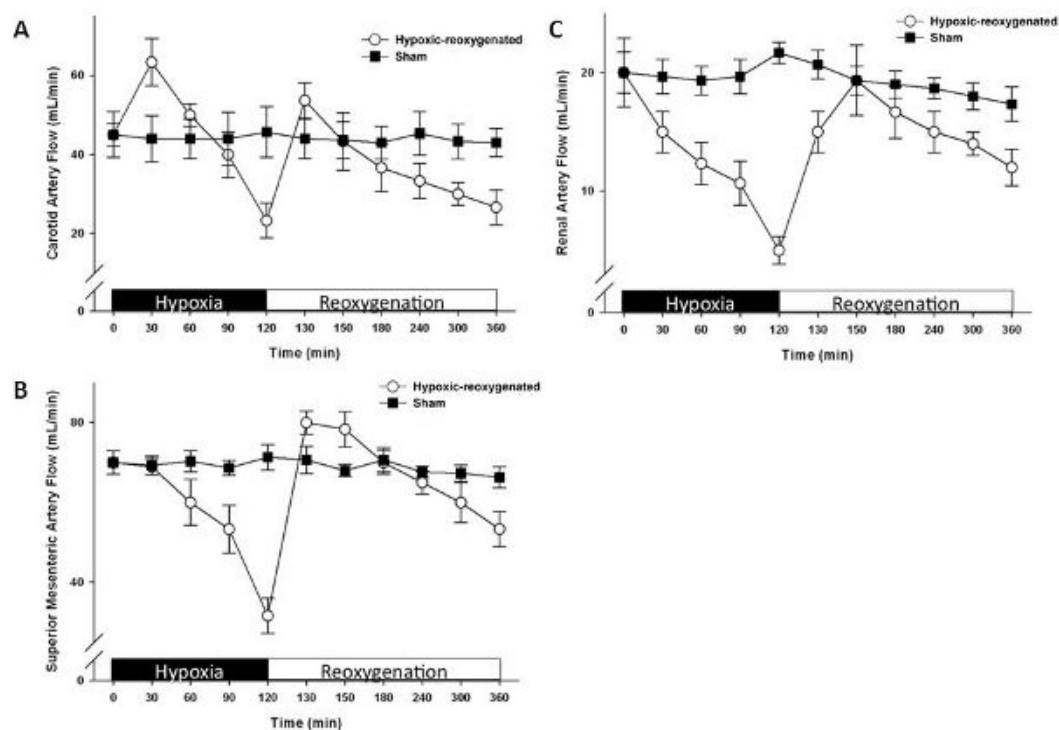


Figure 8: Temporal changes in blood flow at (A) common carotid, (B) superior mesenteric and (C) left renal arteries during hypoxia and reoxygenation

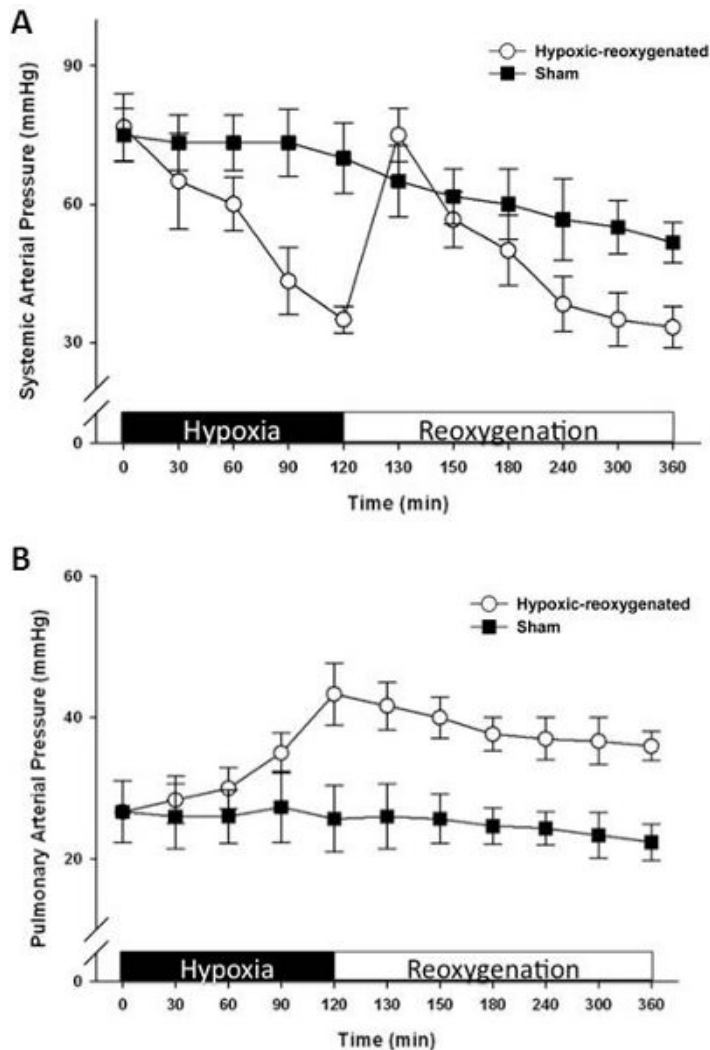


Figure 9: Temporal changes in (A) mean arterial pressure and (B) pulmonary artery pressure during hypoxia and reoxygenation

Discussion

The current experimental protocol has an advantage to examine the systemic and regional hemodynamic changes in neonatal subjects during the hypoxia and reoxygenation process. We can also examine the respective effect of interventions used to improve the cardiovascular function during recovery. We and others have reported the experience and findings in the study of neonatal asphyxia regarding the effects in cardiovascular¹, pulmonary², neurologic³, gastrointestinal⁴, hepatic⁵, renal⁶, adrenal⁷ and hematologic⁸ systems. While it is important to understand the cardiovascular function with information based on continuous data measurements, it is technically challenging if not impossible to surgically instrument small-sized animals such as rodents or guinea pigs. Recent advancement in technologies such as ultrasonography and real-time imaging may however overcome some of these challenges. Nonetheless, large-sized animals also allow the simultaneous collection of biological samples including plasma and tissue samples during the experimental period. This additional biological sampling will allow biochemical assays and histologic examination which help the understanding of the patho-physiology and pharmacology of hypoxia and reoxygenation. While the primary objective of *in vivo* animal models may be the study of patho-physiologic function of a single body system, it is important to understand it in the context of organ-organ interaction. For example, the interaction between the cardiac function and pulmonary hypertension or hepatic dysfunction is important in a multi-organ dysfunction as that of neonatal asphyxia⁹. The newborn lamb is an alternative to swine in the common animal models used to study neonatal asphyxia. The precocious development and limited litter size of newborn lambs may however restrict a more generalized use than newborn piglets, which correspond to that of 38 weeks gestation human fetus and have approximately 10 per litter^{10,11}. Nonetheless, newborn piglets are the most frequently used animals after rodents in the study of neonatal asphyxia.

However, there are limitations of this swine model of neonatal asphyxia, in addition to the challenge related to the translation of findings generated from animal studies to human. The effect of anesthesia and surgical stress as to the acute setting may be minimized with an adequate stabilizing period, appropriate use of anesthetic medications, refined surgical techniques as well as the inclusion of sham-operated control animals for comparison. Prolonging the experimental period beyond days is needed to investigate if any acute hemodynamic effect will persist in the long term. Indeed, we have been successful in modifying the experimental protocol to extended subacute (e.g. 48-72 hours)¹², survival (5-7 days)¹³ as well as chronically instrumented studies. In these prolonged protocols, careful hypoxia and intensive medical and nursing care are important to minimize the mortality and morbidity. Furthermore, the ligation of patent Ductus Arteriosus is important to our use of pulmonary arterial flow as a surrogate of cardiac output although there is minimal flow across the Ductus during hypoxia and reoxygenation

in these newborn piglets. The accuracy of estimating pulmonary vascular resistance will improve with the cannulation of left atrium for the simultaneous measurement of the left atrial pressure. In comparison with our hypoxia protocol, combining hypercapnia with severe hypoxia will better simulate clinical asphyxia. Apart from alveolar hypoxia as in the current protocol, other approaches to induce hypoxia include the creation of pneumothorax¹⁴, halting mechanical ventilation¹⁵ and the addition of carotid artery occlusion for cerebral ischemia. We attempt to make the hypoxia and reoxygenation clinically relevant. The experiment includes 2h of hypoxia which is approximate to the duration required for emergency cesarean section for fetal distress without clinical bleeding based on personal observation. The resuscitation is initiated with 100% oxygen for 30 min, instead of 60 min in our previous studies. This is to limit the hyperoxia which remains a common practice in many community hospitals prior to the arrival of neonatal transport team. Initial reoxygenation with 21% oxygen will follow the recently updated guideline on the use of supplemental oxygen in neonatal resuscitation¹⁶.

Disclosures

No conflicts of interest declared.

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