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Development of an artificial placenta V: 70 h veno-venous extracorporeal life support after ventilatory failure in premature lambs

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Abstract

Purpose—An artificial placenta would change the paradigm of treating extremely premature infants. We hypothesized that using a veno-venous extracorporeal life support (VV-ECLS) artificial placenta after ventilatory failure would stabilize premature lambs and maintain normal fetal physiologic parameters for 70h.

Methods—A near-term neonatal lamb model (130 days; term=145) was used. The right jugular vein (drainage) and umbilical vein (reinfusion) were cannulated with 10–12 Fr cannulas. Lambs were then transitioned to an infant ventilator. After respiratory failure, the endotracheal tube was filled with amniotic fluid, and VV-ECLS total artificial placenta support (TAPS) was initiated. Lambs were maintained on TAPS for 70h.

Results—Six of seven lambs survived for 70h. Mean ventilation time was 57±22min. During ventilation, mean MAP was 51±14mmHg, compared to 44±14mmHg during TAPS (p=0.001). Mean pH and lactate during ventilation were 7.06±0.15 and 5.7±2.3mmol/L, compared to 7.33±0.07 and 2.0±1.8mmol/L during TAPS (p<0.001 for both). pO₂ and pCO₂ remained within normal fetal parameters during TAPS, and mean carotid blood flow was 25±7.5mL/kg/min. Necropsy showed a patent ductus arteriosus and no intracranial hemorrhage in all animals.

Conclusions—The artificial placenta stabilized premature lambs after ventilatory failure and maintained fetal circulation, hemodynamic stability, gas exchange, and cerebral perfusion for 70h.

Keywords

Artificial placenta; ECLS; ECMO; Neonatal; Fetal; Respiratory failure

Extremely low gestational age newborns (ELGANs), defined at birth with an estimated gestational age (EGA) <28 weeks, are at the greatest risk for increased morbidity and mortality related to prematurity [1–3]. In particular, respiratory failure in preemies contributes to significant mortality and long-term morbidity [4]. Despite advances such as surfactant replacement therapy and low-pressure ventilator strategies, iatrogenic injury to immature lungs is common [5]. Even conventional extracorporeal life support (ECLS/ECMO) risks damage to the fragile lungs, and it is generally contraindicated in the ELGANs population [6]. In theory, the most effective solution is to create an artificial placenta (AP) that maintains fetal circulation and bypasses the lungs, obviating the need for mechanical ventilation and allowing the lungs to mature.

The development of an AP to support prematurely exteriorized fetuses has been an aspiration of researchers for many decades. One of the first reports of an arterio-venous (AV)-ECLS AP was published by Callaghan and colleagues in 1963 [7]. The most successful study described 3 week AV-ECLS support of 2 goat fetuses in 1993, but these results have not been reproduced nor have there been significant advances leading to clinical translation [8]. Despite limited initial success in our laboratory with AV-ECLS AP models, we made greater strides after transitioning to a pump-driven VV (venovenous)-ECLS AP preparation [9,10]. We reported a series of 5 near-term premature sheep that maintained hemodynamic stability and fetal gas exchange parameters for 24h using a VV-ECLS AP [10]. No animal studies to date, however, have mimicked the clinical scenario of initiating AP support after ventilatory failure in a premature animal model. We hypothesized that employing a VV-ECLS AP after the onset of ventilatory failure would stabilize premature lambs and maintain normal fetal physiologic parameters for 3 days.

1. Methods

The sheep used in this study were treated in compliance with the *Guide for the Care and Use of Laboratory Animals* (US National Institutes of Health publication No. 85–23, National Academy Press, Washington DC, revised 1996), and all methods were approved by the University of Michigan Committee for the Use and Care of Animals (protocol 9001-3).

1.1. Surgery

Seven pregnant ewes with an EGA of 130–135 days (term= 145 days) were used, and all operations were conducted using standard sterile technique. General endotracheal anesthesia was induced. Central monitoring and infusion lines were placed in the ewe. A lower midline laparotomy incision was used to expose the uterus, and a hysterotomy was performed to expose the fetus. After intramuscular injection of buprenorphine and local infusion of 1% lidocaine, a central monitoring line was placed in the lamb's left carotid artery. Next, 100 U/kg of heparin was injected intravenously into the fetus, and activated clotting time (ACT) was monitored with a Hemachron machine (Model 801, International Technodyne Corp, NJ). Goal ACT was 200–250s. The right jugular vein was then cannulated with a 10 or 12 French (Fr) Bio-Medicus venous cannula (Medtronic, Minneapolis, MN), with the catheter tip resting in the right atrium. Next, one of the umbilical veins (UV) was cannulated with a 10–12 Fr Bio-Medicus arterial cannula. The fetus was then intubated with a 5.0 mm cuffed

endotracheal tube(ETT), and the remaining umbilical vessels were ligated. Maintenance IV fluids, fluid boluses, and medications were infused through the UV cannula.

1.2. Ventilation

The lambs were then transitioned to gas breathing after suctioning the airways. Bag ventilation with 100% O₂ was used to gently recruit the lungs and initiate ventilation. Lambs were then transitioned to an infant ventilator (Model IV 100B, Sechrist Industries INC, Anaheim, CA), and low-pressure ventilation was used: 100% FiO₂, PIP=20–25 cm H₂O, PEEP=5–8 cm H₂O, RR=35–50 breaths per minute. During ventilation only, prostaglandin E1 was given intravenously at a rate of 0.08 µg/kg/min to maintain ductus arteriosus and ductus venosus patency. No exogenous surfactant or other ventilator strategies were used. Mechanical ventilation was continued until lambs developed ventilatory failure (persistent pO₂ <60mmHg and/or pCO₂ >100mmHg) or hemodynamic instability (HR<100 or MAP<30mmHg). The ETT was then filled with sterile amniotic fluid and occluded, and VV-ECLS was initiated.

1.3. Circuit

The VV-ECLS circuit consisted of a low resistance 0.5m² surface area polypropylene hollow fiber oxygenator (Capiiox RX05, Terumo Cardiovascular Systems, Ann Arbor, MI), a heat exchange unit connected to the oxygenator (40°C), and a gravity-filled peristaltic roller pump (M-pump, MC3 Inc, Ann Arbor, MI) (Fig 1). The sweep gas was a mixture of 70% O₂ and 3% CO₂, with balance nitrogen. Prior to initiating total artificial placenta support (TAPS), the circuit was primed with 200mL of maternal red blood cells, 25% human albumin, tromethamine (THAM), sodium bicarbonate, heparin, and calcium gluconate. TAPS was then initiated, with deoxygenated blood draining from the right jugular vein and oxygenated blood returning to the umbilical vein, utilizing the physiologic fetal shunts (ductus venosus, foramen ovale, and ductus arteriosus).

Circuit flow was monitored with an ultrasonic tubing flow probe (Transonic Systems Inc, Ithaca, NY), and carotid blood flow was monitored with a 2.5 mm perivascular flow probe placed around the right carotid artery. A near-infrared spectroscopy (NIRS, Somanetics INVOS Cerebral Oximeter, Troy, MI) probe was placed in the center of the scalp to continuously measure cerebral regional oxygen saturation (% rSO₂). Goal carotid arterial blood gas values were pH 7.25–7.35, pO₂ 30–35mmHg, and pCO₂ 40–60mmHg. To this end, VV-ECLS circuit flow was adjusted between 50 and 100mL/kg/min, and sweep gas flow was 1–3L/min.

1.4. Incubation

Lambs were incubated on a dry heated waterbed to help maintain a core temperature of 40°C, and prophylactic broad-spectrum antibiotics were given on a scheduled basis during TAPS. Maintenance fluid was a 10% Dextrose, 0.225% NaCl infusion at 4mL/kg/h in 6 experiments and total parenteral nutrition (TPN) at 4mL/kg/h in the final experiment. Additional boluses of colloids and crystalloids were given as needed for hypotension, low circuit flow, or anemia (Hgb<10g/dL). Lambs were maintained on VV-ECLS for up to a

total of 70h of TAPS. At the completion of each experiment, a thorough necropsy was performed.

1.5. Echocardiogram

Cardiac transthoracic echocardiography (ECHO) with color flow Doppler was performed on one lamb (HP Sonos 7500) by an experienced pediatric cardiologist during TAPS to evaluate flow through the ductus arteriosus.

1.6. Wet-to-dry ratio

To estimate organ edema, wet-to-dry (WTD) ratios were calculated for the lungs and liver. After TAPS, lung and liver samples were weighed, dried in an oven for 7 days, and weighed again to calculate a WTD ratio. These were compared to tissue samples collected from control animals that were euthanized at the time of delivery.

1.7. Data collection and analysis

Fetal blood gases were analyzed (ABL 505, Radiometer America, Cincinnati, OH) from the carotid artery and pre and post-oxygenator ports. Basic physiologic parameters, hemodynamics, temperature, and ACTs were recorded at regular intervals during TAPS. A comprehensive metabolic panel and complete blood count were drawn at the beginning and end of each experiment, and fetal blood cultures were sent after TAPS. Basic statistics were performed using SPSS version 17 software package (IBM, Chicago, IL), using the Paired t-test for Equality of Means and the Pearson Chi-Square test. Significance was defined as $p < 0.05$.

2. Results

2.1. Survival

Six of 7 fetal lambs survived for at least 70h on TAPS, with support terminated as a planned end to the experiment in 5 cases. The last lamb was allowed to continue on TAPS until its death at 92h resulting from an early cannulation injury that caused a large abdominal bleed. Hematoma in the lesser omentum taponaded the injury for a time, but the animal eventually suffered multisystem organ failure. The single early death occurred after 30 min of TAPS from hepatic injury caused by the jugular cannula. Mean ($\pm$ S.D) final weight in the experimental lambs was 4.3 ± 0.6 kg (range 3.5–5.1 kg).

2.2. Ventilation

Ventilation time in the survivors ranged from 20 to 80min, with a mean of 57 ± 22 min. All but one lamb achieved a pO_2 of > 110 mmHg during mechanical ventilation, with the final one reaching a maximum of 20 mmHg. Despite attaining high pO_2 values, 4 of 5 lambs had worsening oxygenation at the time of ventilatory failure (median 55.3 mmHg). Every subject but one failed mechanical ventilation due to worsening hypercapnia, despite adjustments to ventilator settings. At the end of ventilation, pO_2/pCO_2 values in the lambs were: 23/71 mmHg, 65/142 mmHg, 64/163 mmHg, 448/78 mmHg, 20/73 mmHg, and 46/80 mmHg, respectively. The fourth lamb was hemodynamically unstable, despite acceptable blood gas values. Mean pH, pCO_2 , and lactic acid level at the end of ventilation were 7.05 ± 0.11 ,

101±40mmHg, and 6.1± 2.7 mmol/L, respectively. Two animals required boluses of intravenous epinephrine during ventilation due to hemodynamic instability.

2.3. TAPS

There were no complications during the transition to VV-ECLS. Mean circuit flow was 73±17mL/kg/min. Within the first hour on TAPS, carotid artery pO₂ and pCO₂ stabilized within our goal fetal values (Fig 2A). Arterial pH also stabilized within the first hour, but it took 13h for the mean serum lactate level to fall to 2mmol/L (Fig 2B). Serum lactate at hour 70 was 1.0, 2.9, 1.3, 0.8, 1.8, and 12.4mmol/L in the 6 lambs, respectively (mean 3.8±4.9, median 1.6mmol/L). The lamb with the lactate of 12.4 was hypotensive at hour 70, with a MAP of 10mmHg. Fig. 2C shows mean MAP and HR over time in the 6 survivors. Of note, mean pH was significantly higher ($p<0.001$) and lactic acid was lower during TAPS than ventilation ($p<0.001$). MAP was significantly higher during ventilation ($p=0.001$).

2.4. Brain perfusion and echocardiography

Right carotid artery flow data were collected in 5 of 6 animals. Mean flow during ventilation was 30±8mL/kg/min vs. 25±8mL/kg/min during TAPS($p=0.02$). Mean baseline carotid artery flow in our experience is 27±5mL/kg/min. Cerebral rSO₂ measured by NIRS was 75%±27% during ventilation and 52%±11% on TAPS ($p=0.01$). All lambs moved spontaneously throughout support and localized to painful stimuli. ECHO performed on day 2 of TAPS in 1 subject revealed a patent ductus arteriosus with predominantly right to left ductal flow (Fig. 3). The ductus venosus and foramen ovale were not well imaged.

2.5. Laboratory values, necropsy, and histology

Laboratory values were gathered at baseline and after 70h in all lambs. Mean serum BUN, creatinine, and WBC count were similar to baseline. Total bilirubin and serum cortisol significantly increased, and mean platelet count decreased (Fig 4). Blood cultures at the conclusion of TAPS revealed pseudomonas and citrobacter growth in the first 4 subjects. No bacteria grew from the last 2 lambs after appropriate changes in prophylactic antibiotics.

Necropsy revealed a patent ductus arteriosus, foramen ovale, and sinus venosus in all cases. There were no gross or microscopic signs of intracerebral or intraventricular hemorrhage in any animal. Lambs had mild to moderate pleural effusions and ascites. The lungs were grossly dark and slightly dense in 4 animals, normal in 1, and ecchymotic in another. There was no gross or microscopic evidence of barotrauma or hyaline membranes. Histologic slides from 4 out of 6 animals showed normal neonatal structure with mild inflammation around the alveoli and bronchioles and small areas of intra-alveolar hemorrhage (Fig 5). Another animal had significant acute inflammatory changes resembling bronchoalveolar pneumonia, and slides of the ecchymotic lungs showed diffuse hemorrhage and edema. The hemorrhagic lungs were from the animal that died after 92h on TAPS. Other findings on necropsy included a section of dusky small bowel and an ischemic renal lesion in one animal and a necrotic liver lesion in another. The animal that died after 92h had a subcapsular hematoma on the liver's caudate lobe that bled into the lesser omentum. This was likely from a cannula injury. The mean WTD ratio from experimental lung tissue measured

7.1±2.2, compared to 8.7±1.4 from control animals (p=0.12). Mean liver WTD ratio from AP animals was 4.8±0.8 vs. 4.9±0.5 in controls (p=0.64).

3. Discussion

The purpose of this study was to evaluate the efficacy of a VV-ECLS artificial placenta to support premature lambs after the onset of ventilatory failure. This is the first animal study that mirrors the clinical scenario of using AP support after failure of mechanical ventilation. We found that the AP stabilized hemodynamics and gas exchange, maintained fetal circulation, and provided cerebral perfusion for over 70h of support. This is a significant step forward from our previous series of five 24h survivors on TAPS and another advance towards clinical translation of this technology [10].

AP research started in the 1950s as a strategy to treat neonatal respiratory distress. The first report was a study by Westin and Enhörning from 1958 that placed previable human fetuses on an AV-ECLS circuit through the umbilical vessels [11]. Several groups took up the research in animal studies through the 1960s and 1970s, but the work was largely abandoned by 1980 due to the successes of conventional ECMO in the treatment of neonatal respiratory failure [7,12–15]. These early studies reported mostly survival time but did not evaluate the physiologic alterations. AP research was then resumed in the late 1980s largely in Japan, with Unno and colleagues reporting long-term incubation of 2 goat fetuses in an AV-ECLS AP in 1993 [8]. Japanese groups went further to demonstrate lung growth and maturation during AV-ECLS support in 4 goat fetuses in 1997 [16].

Our group attempted to transition AV-ECLS to a pumpless system that would decrease priming volumes and make the use of an AP widely available for more than just large ECMO centers [9]. Due to difficulties with umbilical artery vasospasm and circuit resistance that overwhelmed the fetal cardiovascular system after a few hours on TAPS, we added a pump to our AV-ECLS system, but we were not able to reproduce our Japanese colleagues' results. Despite aortic cannulation to avoid umbilical artery spasm and other adjustments, we could not overcome the problem of persistent hypotension and hypoperfusion (unpublished data).

The challenges with AV-ECLS led to our studies using VV-ECLS, which has several potential advantages. It eliminates the use of spasm-prone umbilical arteries, and it can reduce circuit cavitation, as blood passively drains from the right atrium. A major advantage relates to the drainage cannula itself. Although it is feasible to advance a large cannula (10–12 Fr) through the umbilical artery into the aorta in preterm sheep, human infants are smaller than sheep and have a more tortuous arterial path to the aorta, making placement of a large umbilical artery cannula impossible. On the other hand, a 6–8 Fr cannula can easily be inserted into the right internal jugular vein in premature humans. Kato and colleagues demonstrated successful jugular drainage with umbilical vein reinfusion to treat human neonates as an alternative to traditional VV-ECMO support [17]. Finally, a VV-ECLS AP operates in parallel with systemic circulation, using an external pump for the gas exchange circuit. Similar to traditional VV-ECMO circuits, the fetal heart should experience little increased resistance and afterload from the parallel circuit [6]. In contrast, an AV-ECLS

system with an oxygenator placed in series has the potential to increase stress on the fetal heart and contribute to cardiac failure [18].

Our initial study using VV-ECLS showed the feasibility of supporting preterm neonatal sheep for at least 24h, but we encountered problems with sepsis near the end of some experiments due to the unsterile nature of the operation and the unsterile water bath that incubated the lambs. Thus, this study attempted to mimic the clinical scenario with a sterile operation, attempts at mechanical ventilation, and incubation outside of a water bath. In order to further refine our VV-ECLS AP model on larger preemies, near-term (130 days EGA, term=145 days) lambs that are developmentally equivalent to humans at 32–34 weeks EGA were used before proceeding to extremely premature lambs. The lambs in this series universally failed mechanical ventilation within 80min, mostly due to hypercapnia and progressive respiratory acidosis. All but one animal was able to attain but not sustain adequate pO₂ levels, likely due to insufficient endogenous surfactant. Unfortunately, we did not have a reliable surfactant assay available at the time, so we cannot report our subjects' surfactant levels. We did find, however, that serum cortisol levels were relatively low at birth, rising significantly after 70h. Cortisol is known to be one of the stimuli for organ maturation prior to birth, importantly affecting the lungs and surfactant production [19,20]. The low cortisol levels we observed could be associated with insufficient surfactant, leading to early ventilatory failure. Similarly, the increased cortisol we found after TAPS could be related to endogenous attempts at organ maturation, or it could represent a systemic stress response. An obvious limitation of our ventilatory strategy is the lack of exogenous surfactant and HFOV prior to TAPS. These would help us create a more accurate ventilation control and will be employed in future studies.

After the initiation of TAPS, the 6 long-term survivors all had normal and stable fetal hemodynamics for at least 70h except one lamb that became hypotensive in the last several hours of support. In addition, TAPS caused animals with worsening pH and lactate values on the ventilator to improve to normal values within the first hour. This stabilization of gas exchange allowed the animals to gradually clear lactate to normal levels. It should be noted that we kept the mean pO₂ during TAPS higher than normal fetal values of 25–30mmHg, using a goal of 30–35mmHg. We did this to account for the fact that 200 mL of maternal red blood cells was used to prime the circuit. This effectively decreased the total percentage of fetal hemoglobin, thereby decreasing the oxygen carrying capacity of blood in the circuit [21].

During TAPS, fetal flow through the ductus arteriosus was documented in one animal that had an ECHO demonstrating right to left ductus flow on day 2 of support (Fig 3). Combined with findings of a patent ductus at necropsy in all lambs, this evidence helps us conclude that fetal flow was maintained during perfusion. This is likely due to maintaining low arterial pO₂ (<50mmHg) and low intra-thoracic pressure during TAPS. Additionally, the prostaglandin E1 given during ventilation likely helped to preserve ductus patency in times of high arterial pO₂, allowing for resumption of fetal circulation. Unfortunately, we were not able to perform an ECHO during ventilation or the transition to TAPS, but we believe that this will be invaluable information to collect in the future and represents a limitation of this

study. Understanding and controlling the resumption of fetal circulation will be critical for clinical translation.

Despite ligating one carotid artery for a monitoring line, cerebral blood flow was maintained during TAPS at near baseline levels. This mirrors work in neonatal lambs on ECMO that found no decrease in cerebral blood flow after initiation of VV-ECMO [22]. Additionally, cerebral rSO₂ measured by NIRS remained in a range that appeared to be adequate for a fetus, but regrettably little work has been done to determine normal rSO₂ values in fetal sheep and humans. One study of cerebral NIRS on humans during birth quoted values from 21% to 72%, but no literature in unstressed late-term fetuses is available [23]. The real question is how changes in cerebral flow affected neurologic activity and brain maturation. While we observed spontaneous movement in all neonates in this study, we plan to employ EEG technology to further investigate the effect of the AP on brain activity.

Laboratory values revealed a significant decrease in platelet counts and a trend towards lower white blood cell count during perfusion. These findings are common among neonatal ECMO patients [6,24–26]. Circuit tubing, cannulas, and oxygenator surfaces cause activation of the complement system and platelets, often resulting in leukopenia and thrombocytopenia [24–26]. To address these issues, there is ongoing research in the development of nonthrombogenic and biocompatible surfaces for ECLS circuits.

It was encouraging to find that 4 sets of lungs from this study appeared fairly normal microscopically. We would expect to see mild inflammatory changes after 3 days of ventilatory failure in a preemie [27]. Only one animal had intense pulmonary inflammation most likely from meconium in the amniotic fluid. We have since added a large particle filtration process after collecting amniotic fluid to remove meconium and other contaminants. Finally, the ecchymotic, edematous lungs from the lamb that died at 92h are typical for critically ill infants on ECMO in the late stages of organ failure.

In this study, we found no signs of gross or microscopic intraventricular hemorrhage (IVH) despite using heparin. While this finding is encouraging, IVH is one of the most feared complications found in human preemies supported by ECLS. We recognize that clinical application of an artificial placenta for treatment of extremely premature infants will require a novel solution to substantially limit the risk of IVH. Our plan for future experiments with extremely premature lambs (90 days) is to first limit ACT to low levels (160–180s) and then transition to the use of non-thrombogenic surfaces, such as nitric oxide releasing polymers that are being developed in our laboratory [28,29]. Additionally, we plan to bubble nitric oxide through the oxygenator to inhibit thrombosis. Unique to the artificial placenta, the lack of positive pressure ventilation over long periods of time and hemodynamic stability may help prevent IVH by limiting fluctuations in intracranial pressure [30–32].

Although we have shown the efficacy of the AP in supporting late-term premature lambs for at least 3 days after ventilator failure, there are several limitations to this study, and further work is required prior to clinical application. First, we have not shown that the VV-ECLS AP is beneficial in extremely premature infants (90 day EGA in sheep; 24–25 week EGA in humans) that are at the highest risk for complications of prematurity. After demonstrating

full support for 24h, our next goal was to demonstrate stability for 70h after ventilatory failure. We recognize that clinical application will require several weeks of support, and subsequent studies will assess the efficacy of the AP over a period of 2 weeks while addressing the myriad issues of extreme prematurity in a 90 day model. In treating ventilated animals, we also need to refine our ventilator techniques to include modern treatment modalities and wean lambs on TAPS to mechanical ventilation and then spontaneous ventilation. Additionally, the physiologic changes in the transitions between these modalities of support should be fully elaborated. Finally, in addition to testing TAPS on smaller lambs, we need to optimize nutritional support, address complications of extreme prematurity, use coated non-thrombogenic surfaces, and demonstrate continued lung growth and development. After the AP is refined in the lamb model, clinical prognostic criteria must be optimized prior to clinical application. The CRIB II and SNAPPE II mortality prediction tools have been validated in the ELGANs population to identify those with the highest mortality risk in first 12h of life [33–35]. These offer the most reasonable criteria for early intervention with novel therapies like the AP to decrease morbidity and mortality in ELGANs.

In summary, the VV-ECLS artificial placenta stabilized premature lambs after ventilatory failure and maintained fetal circulation, hemodynamic stability, gas exchange, and cerebral perfusion for 70h. Clinical translation of this emerging technology has the potential to radically change the paradigm of treating prematurity.

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Fig. 1. Veno-venous extracorporeal life support (VV-ECLS) artificial placenta circuit. (A) Right jugular vein drainage (B) rotational pump (C) membrane lung (D) IVF/TPN (E) 70% O₂–3% CO₂ sweep gas (F) heat exchanger (G) umbilical vein inflow.

Fig. 2.

Mean carotid artery blood gas (A), acid/base (B), and hemodynamic (C) values in 6 fetal lambs over 70 h of artificial placenta support after ventilatory failure. Error bars indicate standard deviation. TAPS=total artificial placenta support, HR=heart rate, MAP= mean arterial pressure.

Fig. 3.

Representative image of color Doppler transthoracic echocardiography showing a patent ductus arteriosus with predominantly right to left flow (blue color represents flow from PA to Ao) in a later subject that was not included in this series. PA=main pulmonary artery, Ao=ascending aorta, DA=ductus arteriosus.

Mean Laboratory Values at Baseline and Hour 70 of Artificial Placenta Support

	Baseline	Hour 70	<i>P</i>
BUN (mg/dL)	18.8±2.7	18.7±12.2	0.97
Creatinine (mg/dL)	1.4±0.4	1.6±1.2	0.76
Total Bilirubin (mg/dL)	0.5±0.4	6.6±3.0	0.002*
WBC (K/ μ L)	2.0±1.2	1.1±0.7	0.23
Hemoglobin (g/dL)	9.8±1.7	10.1±2.0	0.82
Platelets (K/ μ L)	214±152	97±128	0.041*
Serum Cortisol (μ g/dL)	2.3±1.5	8.6±2.7	0.009*

Mean $\pm$ standard deviation. **P* < .05 indicates significance

Fig. 4.

Mean laboratory values at baseline and hour 70 of artificial placenta support.

Fig. 5.
H&E slides of lung tissue at 200 $\times$ magnification from a control fetus that was never on artificial placenta support (A) and from an experimental subject after 70h of support (B).