Cardiopulmonary Function in Very Preterm Lambs during Liquid Ventilation

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Summary

Cardiopulmonary function was evaluated in very preterm lambs (106 ± 0.7 S.E. days gestation, 1.66 ± 0.12 S.E. kg birth weight) during fluorocarbon ventilation. Lambs were delivered by cesarean section after epidural anesthesia. Indwelling arterial, venous, and tracheal cannulae were placed before clamping the cord. Lambs were then mechanically ventilated with oxygenated fluorocarbon for approximately 2 h. During this period it was possible to maintain adequate gas exchange and stable cardiac function. Transpulmonary pressure, liquid flow, and tidal volume tracings enabled determination of lung compliance, $C_L = 0.58 \pm 0.12$ S.E. $\text{ml} \cdot \text{cmH}_2\text{O}^{-1} \cdot \text{kg}^{-1}$, inspiratory resistance, $R_I = 3600 \pm 604$ S.E. $\text{cmH}_2\text{O} \cdot \text{liter}^{-1} \cdot \text{sec}^{-1}$, and expiratory resistance, $R_E = 4034 \pm 2183$ S.E. $\text{cmH}_2\text{O} \cdot \text{liter}^{-1} \cdot \text{sec}^{-1}$. Lung compliance of the 106-day-old fluorocarbon-filled lung is similar to the more mature 138-143-day-old air-filled lung in preterm lambs. Based on the data presented herein we have extended the viability of the preterm lamb to the limit of pulmonary capillary development rather than that of the pulmonary surfactant system.

Abbreviations

A-a DO_2 , alveolar-arterial oxygen gradient
 C_L , lung compliance
FRC, functional residual capacity
 R_E , expiratory resistance
 R_I , inspiratory resistance
 V_T , tidal volume

The lamb has been employed extensively for studying cardiopulmonary development in the perinatal period (1, 2, 3, 12, 18, 21). But there are few data in very preterm animals because cardiopulmonary stability and viability are highly correlated with maturity. The majority of studies are those in lamb fetuses at various stages of development (14), exteriorized lambs when the ewe was anesthetized (1, 5), or in older lambs delivered by cesarean section after 125 days of gestation (12, 18, 19, 21). Although adequate oxygen transport has been demonstrated by 110 days of gestation in exteriorized preterm fetal lambs, viability of these animals is much later (approximately 140 days, term 145-147 days).

Recent studies have revealed the feasibility of ventilation with fluorocarbon liquid in preterm lambs delivered by cesarean section between 135-138 days gestation (17). In contrast to the respiratory problems that occur during mechanical gas ventilation (18, 19, 21), lambs ventilated with fluorocarbon liquid exhibited good gas exchange and stable blood gas tensions. It has been suggested that the elimination of high surface forces in the liquid-filled lung could account for improved gas exchange and respiratory stability. The first objective of this study was to quantitate baseline cardiovascular, gas exchange, acid-base balance, and lung mechanics data in very immature lambs using liquid ventilation techniques. There are also apparent differences in lung mechanics between

the gas and liquid-filled lungs. With this in mind, we sought to correlate these changes with the differences in physical properties of the respiratory medium.

MATERIALS AND METHODS

Animal preparation. Five preterm lambs of 106 ± 0.7 S.E. days gestation (70% of term gestation) and a mean birth weight of 1.66 ± 0.12 S.E. kg were studied. Before delivery, the dated ewes were restrained in a prone position, epidural anesthesia ($0.5-1.0$ mg/kg of 0.75% bupivacaine HCl) was induced and the uterus was exposed. Through a uterotomy, the head of the preterm lamb was allowed to emerge; inspiration was prevented by placing a rubber glove filled with warm ($37-39^\circ\text{C}$) saline over the head. After a local filtration of 1% lidocaine in the neck, the right carotid artery and the right jugular vein were each cannulated with appropriate sized French 5 or 8 polyvinyl catheters. An occluded cannula was inserted through a tracheotomy midway along the trachea, secured and its tip positioned proximal to the carina.

A bolus of 50% sodium bicarbonate solution (2.5 ml/kg) and a subsequent bolus of pancuronium bromide (0.1 mg/kg) were administered intravenously. Simultaneously, the animal was delivered, the rubber glove removed from the head and the umbilical cord clamped and tied. The animal was immediately connected to the liquid ventilating system.

During this period, blood was collected from the placenta in a heparinized container. The animal was dried and warmed under a radiant heat source. An intravenous crystalloid infusion (10% dextrose with 10 mEq sodium bicarbonate/100 ml fluid) was commenced at a rate of 3 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, and the catheters were connected to appropriate pressure transducers. Data were collected throughout the study period while the lambs under skeletal muscle paralysis (continuous administration of pancuronium bromide at 0.1 mg/kg/h) remained on the liquid ventilating system.

Liquid ventilation procedure. Liquid ventilation with fluorocarbon (RIMAR 101) was achieved using a previously described and modified liquid-breathing system (16). Briefly, the liquid ventilation system acted as a volume ventilator in which the tidal volume, frequency, I:E ratio, and liquid flow could be adjusted. Oxygenation of the liquid and removal of carbon dioxide from the liquid was accomplished with a membrane oxygenator (Sci. Med. Life Systems, Inc., Model 3500). The temperature of the liquid was maintained at 39°C using an electrically controlled temperature regulator and heater (Masterline Bath and Circulator, Model 2095). The PO_2 and PCO_2 of the liquid were sampled from the system before and during liquid ventilation of the animal. Oxygenated liquid was removed from the liquid-breathing system and placed in a suspended reservoir. A volume equivalent to the estimated (18) functional residual capacity (30 ml/kg) of the lungs was instilled from this reservoir via the tracheotomy tube into the animal's lungs. Postural and thoracic manipulations were performed to equilibrate the fluorocarbon with prenatal lung fluid.

V_T was measured by the change in the animal's weight utilizing a previously described strain gauge platform (17). This was also

necessary to monitor the FRC and was accurate to within 1 ml of fluorocarbon liquid. Based on previous experiments (7, 15) ventilation schemes were adjusted for effective carbon dioxide elimination and maintenance of physiologic arterial carbon dioxide tensions. Animals were ventilated at a mean FRC of 30 ml/kg, V_T of 17.9 ± 2.0 S.E. ml/kg, and frequency of 5.2 ± 0.3 S.E. breaths/min.

Experimental methods. Arterial blood gas tensions, pH and hematocrit were determined at 15–30-min intervals. If the lamb's hematocrit fell below 50% as a result of blood sampling, fetal blood collected from the ewe's placenta was infused into the lamb. Blood gas and pH measurements were corrected to the body temperature of the animal (10, 13) and base deficit and percent O_2 saturation were calculated. The A-a DO_2 was determined from measured arterial blood gas tensions and estimated mean alveolar oxygen tension with the assumption that alveolar $PACO_2$ was equivalent to $Paco_2$ and that the respiratory quotient was 1.0.

Cardiopulmonary measurements were conducted after the arterial blood gases and pH were stabilized (approximately 60 min. postnatally). Central venous pressure and mean arterial pressure were measured by connecting the jugular vein and carotid artery catheters to the Statham P23BC and P23A transducers, respectively. Pressures were recorded on a Grass Model 7 polygraph recorder. Heart rate was obtained from blood pressure recordings. A standard limb electrocardiogram was monitored on a Cambridge VS-III EKG machine and recorded on a Grass Model 7 polygraph.

Mechanics of breathing were studied by simultaneously monitoring transpulmonary pressure, inspiratory and expiratory flow rates, and tidal volume on a Polygraph recorder (Grass Model 7) and X-Y recorder (Hewlett Packard 7035). Intraesophageal pressure was measured with an esophageal balloon, 2.5 cm in length and 3.0 mm in diameter. The balloon was filled with 0.5 ml of air and positioned in the lower third of the esophagus. The esophageal catheter was connected to one side of a Statham PM131TC differential strain gauge transducer with a low volume adapter inserted at the inlet. Tracheal pressure was transmitted by connecting the other side of the same transducer to the side tap on the tracheal tube. The recorded signal represented an estimate of transpulmonary pressure. V_T was determined from changes in weight as measured by the strain gauge platform. Liquid flow was derived by differentiating V_T signals with a general purpose polygraph differentiator (Grass 7P20).

Values of respiratory rate were ascertained directly from the polygraph record and dynamic lung compliance was determined graphically by the method of Neergard and Wirz (9). Lung resistance was computed by dividing the difference in transpulmonary pressure at midvolume points during inspiration and expiration by the algebraic difference in airflow at those instants. Each resistance and compliance determination was based on the average of at least three breaths. A constant tidal volume, lung volume, and frequency history preceeded all measurements.

Oxygen consumption was determined by a closed circuit spirometry method. The animal was ventilated from a liquid-filled circuit which consisted of: a spirometer to supply oxygen and to record its volume change, a Baralyme CO_2 absorber to remove expired CO_2 , and a volume controlled liquid ventilation system (20). The system was found leak-free when tested for periods up to 2 h. Oxygen consumption recordings were made using a wide scale strip-chart recorder (Varian, Model 9176).

The animal's rectal, esophageal, body surface, and tracheal temperatures were monitored with a digital centigrade thermometer and thermistors (Cole-Parmer, 8502-25) throughout the duration of the experiment. The source of the radiant heat was adjusted to maintain the body core temperature within a physiologic range (37 – $39^\circ C$).

RESULTS

Liquid ventilation experiments were successfully completed in five very preterm lambs. Mean values $\pm$ S.E.M. for prenatal

carotid blood gas tensions ($Pao_2 = 25.3 \pm 1.9$ mmHg, $Paco_2 = 20.4 \pm 2.7$ mmHg) and acid base status ($pH = 7.29 \pm 0.02$, $HCO_3^- = 13.3 \pm 4.3$ mEq/liter) fell within the range previously reported in fetuses of similar gestational age (14). It has been recognized that exteriorization of the fetus leads to some differences in placental gas exchange (5).

Figure 1 illustrates sequential Pao_2 , $Paco_2$, pH and, rectal temperature data in lamb #50681 after exteriorization delivery, and during 165 min of liquid ventilation. As shown, it took at least 30 min for appropriate adjustment of ventilation parameters to stabilize arterial oxygen tension and 60 min to overcome the initial hypothermia associated with delivery. Summarized arterial blood chemistry data for individual lambs after 1 h of ventilation are presented in Table 1 along with mean $\pm$ S.E.M. values. The arterial pH, similar to the prenatal value, was 7.29 ± 0.2 during liquid ventilation. A base deficit of 10.9 ± 0.9 mEq/liter persisted in spite of a low dose bicarbonate infusion. The gaseous exchange was significantly increased with mean oxygen saturation of over 99%; the mean $\pm$ S.E. arterial Pao_2 was 242.5 ± 49.4 mmHg and was associated with an A-a DO_2 gradient of 415.5 ± 50.0 mm Hg. Based on the scheme of ventilation used, $Paco_2$ was maintained at 30.8 ± 1.0 mmHg. The oxygen consumption was measured to range from 5.4 – 8.3 ml $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ between 30–90 min postnatal age.

Representative tracings of cardiopulmonary data after one hour of liquid ventilation are illustrated in Figure 2. Mean arterial

Fig. 1. Sequential values of arterial oxygen tension Pao_2 , carbon dioxide tension $Paco_2$, pH, and rectal temperature in lamb #50681 after exteriorization, delivery, during 165 min of liquid ventilation.

Table 1. Arterial blood chemistry data

Lamb no.	P _O ₂ (mmHg)	P _{CO} ₂ (mmHg)	pH	Base deficit (meq/liter)	PiO ₂ (mmHg)	A-aDO ₂ (mmHg)	Saturation (%)
52281	145	32	7.31	9.4	650	505	98.7
51281	314	29	7.27	12.6	710	396	99.6
22380	340	33	7.25	12.3	622	282	99.7
50681	171	29	7.34	9.2	650	479	99.1
Mean	242.5	30.8	7.29	10.9	658	415.5	99.3
±S.E.	±49.4	±1.0	±0.02	±0.9	±18	±50.0	±0.2

Fig. 2. Representative tracing of cardiopulmonary data during liquid ventilation in lamb #50681 at 60 min after birth.

Table 2. Liquid ventilation data

Lamb no.	Tidal volume (ml)	Frequency (breath/min)	Flow (ml/sec) I/E	Tracheal pressure (mmHg) I/E	I/E Ratio
52281	11.6	5.0	3.6/2.3	31.5/-2.7	0.61
52081	23.1	5.0	5.1/5.1	36.9/-2.8	1.0
51281	21.0	4.0	4.7/4.7	22.0/-15.0	1.0
22380	16.0	6.0	8.0/3.2	34.0/-25	0.40
50681	17.6	6.0	10.0/6.7	15.0/-5.5	0.67
Mean	17.9	5.2	6.3/4.4	27.9/-10.2	0.74
±S.E.	±2.0	±0.3	±1.1/±0.8	±4.1/±4.3	±0.12

pressure was 58 mmHg with a gradual decrease during the course of the experiment. The central venous pressure and heart rate remained relatively stable during the experiments with mean values of 7.9 mmHg and 156.5 beat/min, respectively.

Steady state ventilation data for individual animals are shown in Table 2 and are associated with the blood chemistry data in Table 1. It is noteworthy that inspiratory and expiratory tracheal pressures were only 27.9 and -10.2 mmHg, respectively, for the

given ventilation schemes. Dynamic pressure-volume loops were obtained in three lambs and a typical curve (lamb #50681) is shown in Figure 3. Characteristic hysteresis is described during inspiration and expiration. The values of dynamic compliance, inspiratory, and expiratory resistance are tabulated in Table 3.

Gross postmortem examination following elective termination of the experiment were conducted on all lambs. These did not exhibit any evidence of fluid or air leaks in the pleural cavity, pulmonary hemorrhage, or non-homogeneous ventilation.

DISCUSSION

Stable gas exchange, acid base status, and pulmonary function have been previously demonstrated in distressed preterm lambs (135–138 days of gestation) ventilated with oxygenated fluorocarbon liquid (17). The applicability of this procedure has been extended in the present study to include very preterm lambs of 106 days gestation. Unlike the previous studies, the fetal lambs were delivered without breathing gas and the respiratory tract was filled with warm, oxygenated fluorocarbon liquid. Hypoxemia and cold stress were thus minimized during the stage of transitional neonatal circulation.

Born *et al.* (1) investigated gas transport and pulmonary circulation in exteriorized preterm lambs at various stages of gestation and in isolated preterm lung preparations. These studies demonstrated that pulmonary blood flow is not sufficient to maintain adequate gas exchange in lambs of less than 105–110 days gestation. In addition they suggested that the pulmonary capillaries are sufficiently developed by 110 days gestation to provide total oxygen transport at a developmental age when the lungs remain unable to sustain normal gas ventilation. In the present study we were able to experimentally confirm Born's speculation and establish effective pulmonary oxygen transport for approximately 2 h in 106-day-old preterm lambs that were ventilated with oxygenated fluorocarbon liquid. It is noteworthy that these lambs did not receive augmented umbilical circulation (5) during the liquid ventilation procedure and that total oxygen and carbon dioxide

transport were due to pulmonary gas exchange.

The onset of neonatal circulation has also been associated with an increase in oxygen consumption (4). Our lambs were maintained in a neutral thermal environment with the aid of external heat (lamp) and internal heat (warmed fluorocarbon liquid). Monitoring of the core temperature through the esophagus, trachea, and rectum indicated body temperature was maintained about 38°C. The oxygen consumption ranged from 5.4–8.3 ml·kg⁻¹·min⁻¹ and is higher than *in utero* fetal levels of 4–5 ml·kg⁻¹·min⁻¹ at comparable gestational age (2, 3). Postnatal rise in O₂ consumption has been observed in newborn lambs with active muscle tone secondary to spontaneous ventilation and ranges from 6–8.5 ml·kg⁻¹·min⁻¹ (2). In spite of the adequate oxygen consumption we observed an ongoing mild metabolic acidosis. This degree of metabolic acidosis has been previously observed both in preterm lambs and neonates with respiratory distress (12, 18, 21).

In the past it has been extremely difficult to deliver and keep animals alive at this early stage of gestation because both the anatomical development of alveoli and biochemical development of the surfactant system are incomplete. Using the presently described ventilation technique it appears possible to effectively maintain stable pulmonary gas exchange in an immature animal model. A more striking feature was the finding that gas exchange and lung compliance in the very preterm lambs (106 days gestation) during liquid ventilation is very similar to that in the more mature lambs (132–138 days gestation) during conventional gas ventilation (18).

The effectiveness of liquid ventilation in the very preterm lambs could involve several mechanisms. The air-liquid alveolar interface is abolished in the liquid-filled lung, thereby reducing high surface forces and possibly eliminating nonuniform ventilation which exists in the premature gas ventilated lung. Studies have demonstrated that pulmonary blood flow in the liquid-filled lung is more evenly distributed than in the gas-filled lung (22). This may be particularly significant in distressed preterm lambs that exhibit extensive intrapulmonary shunting and elevated pulmonary vascular resistance (23).

Early investigators (1) found it necessary to inflate the lungs of mature living fetuses to pressures of 20–40 mmHg before adequate ventilation was achieved. In preterm lambs of less than 110 days gestational age, inflation was even more difficult (approximately 4-fold increase in peak inflation pressure) typically resulting in extra-alveolar leaks. In contrast to gas ventilation, the mean peak tracheal pressure required for adequate liquid ventilation was low (27.9 mmHg). These low pressures were accomplished in spite of elevated airway resistance (increased by the viscosity and density of fluorocarbon liquid) by utilizing slow breathing and flow rates.

In conclusion, this study demonstrates that it is possible to maintain good gas exchange in very preterm lambs during normothermic liquid ventilation. Furthermore, the secondary effects of improved ventilation and pulmonary blood flow could, in the long run, facilitate surfactant synthesis. One might question whether this form of ventilation may have a long-term deleterious effect on the developing newborn lung, but we cannot directly address these issues. It is noteworthy that long-term experiments in adult dogs (6, 8, 11) indicated scant morphologic, biochemical, and/or histologic evidence of toxicity after ventilation with fluorocarbon liquid.

Fig. 3. Dynamic pressure-volume loop during liquid ventilation. ($\dot{V}$, liquid flow rate).

Table 3. Pulmonary mechanics on liquid ventilation

Lamb no.	FRC ¹ (ml/kg)	Compliance (ml·cmH ₂ O ⁻¹ ·kg ⁻¹)	Inspiratory resistance (cmH ₂ O·liter ⁻¹ ·sec ⁻¹)	Expiratory resistance (cmH ₂ O·liter ⁻¹ ·sec ⁻¹)
52281	30	0.36	4140	8340
51281	30	0.61	4268	1255
50681	30	0.78	2394	2507
Mean		0.58	3600	4034
±S.E.		±0.12	±604	±2183

¹ FRC, functional residual capacity.

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