

# High-frequency nasal ventilation for 21 d maintains gas exchange with lower respiratory pressures and promotes alveolarization in preterm lambs

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**BACKGROUND:** Short-term high-frequency nasal ventilation (HFNV) of preterm neonates provides acceptable gas exchange compared to endotracheal intubation and intermittent mandatory ventilation (IMV). Whether long-term HFNV will provide acceptable gas exchange is unknown. We hypothesized that HFNV for up to 21 d would lead to acceptable gas exchange at lower inspired oxygen ( $O_2$ ) levels and airway pressures compared to intubation and IMV.

**METHODS:** Preterm lambs were exposed to antenatal steroids and treated with perinatal surfactant and postnatal caffeine. Lambs were intubated and resuscitated by IMV. At ~3 h of age, half of the lambs were switched to noninvasive HFNV. Support was for 3 or 21 d. By design,  $Pao_2$  and  $Paco_2$  were not different between groups.

**RESULTS:** At 3 d ( $n = 5$ ) and 21 d ( $n = 4$ ) of HFNV, fractional inspired  $O_2$  ( $F_iO_2$ ), peak inspiratory pressure (PIP), mean airway, intratracheal, and positive end-expiratory pressures, oxygenation index, and alveolar–arterial gradient were significantly lower than matched periods of intubation and IMV.  $Pao_2/F_iO_2$  ratio was significantly higher at 3 and 21 d of HFNV compared to matched intubation and IMV. HFNV led to better alveolarization at 3 and 21 d.

**CONCLUSION:** Long-term HFNV provides acceptable gas exchange at lower inspired  $O_2$  levels and respiratory pressures compared to intubation and IMV.

**E**ndotracheal intubation and intermittent mandatory ventilation (IMV) are important risk factors for neonatal chronic lung disease (CLD), also called bronchopulmonary dysplasia (1–5). Experimental animal studies indicate that contributors to lung injury are volutrauma, atelectotrauma, and hyperoxia (6–9). Volutrauma results from cyclic overdistension of the inhomogeneously inflated parenchyma of the immature lung. Atelectotrauma occurs during reopening of collapsed lung parenchyma. Both volutrauma and atelectotrauma expose the parenchymal cells and extracellular matrix to distortion that lead to altered expression of genes involved

with lung inflammation and development (10–13). Hyperoxia sets the stage for cytotoxic reactive oxygen species that contribute to cellular injury and reprogramming of developmental processes (14). Disruptions of lung developmental processes are structurally manifest as alveolar simplification.

Early use of noninvasive respiratory support, such as nasal continuous positive airway pressure (CPAP), is associated with better outcomes. For example, nasal CPAP is associated with less use and fewer days of intubation and IMV, lower levels of inspired oxygen ( $O_2$ ) (15), and lower rates of bronchopulmonary dysplasia or bronchopulmonary dysplasia/death (1,16,17). Insights from studies using preterm experimental animal models of evolving neonatal CLD reveal possible mechanisms for better outcomes of noninvasive respiratory support. Functional studies using preterm lambs reveal lower expression of genes involved in acute-phase responses and markers of inflammation at 2 h of bubble nasal CPAP compared to intubation and IMV (18,19). Structural studies using preterm baboons demonstrate that early use of noninvasive respiratory support promotes alveolarization compared to invasive respiratory support (9,20,21). Our previous study shows that an approach to noninvasive respiratory support for 3 d leads to alveolarization compared to intubation and IMV of preterm.

Whether long-term noninvasive support will provide prolonged physiological gas exchange across the lung accompanied by alveolar formation is unknown. Therefore, we used our preterm lamb model of evolving neonatal CLD to prospectively determine the impact of prolonged high-frequency nasal ventilation (HFNV) on physiological outcomes for respiratory gas exchange and respiratory pressures, and morphological outcomes for alveolar formation. We hypothesized that HFNV for up to 21 d would lead to acceptable respiratory gas exchange at lower inspired  $O_2$  levels and airway pressures, as well as alveolar formation, compared to intubation and IMV. The principal results of our study show that HFNV out to 21 d provides acceptable respiratory gas exchange that is accompanied by alveolar formation.

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## RESULTS

We defined successful experiments as reaching the prospective 3 or 21 d of respiratory support. By this definition, overall success was ~75%. For the 3 d studies, the sample size is five. Seven preterm lambs were necessary because two male lambs developed respiratory distress secondary to uncontrolled air leaks that occurred within the first 12 h of life. Both lambs died shortly thereafter and thus were excluded. All five preterm lambs that were weaned to HFNV survived. For the 21 d studies, the sample size is four. Seven preterm lambs were necessary because three lambs (two male; one female) developed respiratory distress secondary to uncontrolled air leaks or renal failure within week of life 3, before 21 d, and therefore were excluded. The male lamb required reintubation and IMV during week of life 3 (day 15) and could not be weaned back to HFNV.

Demographic characteristics are summarized in **Table 1**. Gestational age, birth weight, and sex were the same within and between the 3 and 21 d groups. However, body weight at 3 or 21 d was significantly greater in HFNV group compared to the corresponding intubation and IMV group ( $P < 0.05$ ).

The radiographic shadow of the uncuffed true Murphy tube is visible in the midline of the head (**Figure 1a**). Postmortem, the nasal cavity was opened for all five 3 d HFNV lambs to measure the location of the tip from the nostril. The tip was ~5 cm into the nasal cavity (~10 cm long; data not shown).

A segment of an intratracheal pressure (ITP) trace in a lamb supported by HFNV is shown in **Figure 1b**. The Percussionaire ventilator was configured to provide high-frequency, low amplitude pressure fluctuations during both inspiration and expiration (**Figure 1b**). Also evident are two background low-frequency breaths (pressure limited at 17 cmH<sub>2</sub>O).

Pressure measurements at the ventilator, connector piece from the ventilator circuit to the nasal tube, in the nasopharynx, and intratracheally are summarized in **Table 2**. We tested two peak pressure settings: high peak inspiratory pressure (PIP) (~27 cmH<sub>2</sub>O) or moderate PIP (~14 cmH<sub>2</sub>O). Regardless of PIP setting, the corresponding peak and mean pressures persisted at the junction of the connector to the nasal tube. However, peak and mean pressures were ~60% less at the nasopharynx (1–2 cm cranial to the vocal cords), as well as in the trachea (~10 cm caudal to the vocal cords).

Daily gas exchange and respiratory support results for the 3 d groups of ventilated preterm lambs are summarized in **Table 3** (middle columns). By design, the two groups ( $n = 5$  each) had the same targeted range for Pao<sub>2</sub> (73–85 mm Hg)

and targeted range for Paco<sub>2</sub> (44–64 mm Hg) at days of life 1, 2, and 3. The physiologically targeted Pao<sub>2</sub> range was attained using ~30% lower F<sub>i</sub>O<sub>2</sub> on day of life 3 ( $P < 0.05$ ) for the HFNV group compared to the intubation and IMV group. Likewise, the physiologically targeted Paco<sub>2</sub> range was attained using ~40% lower PIP ( $P < 0.05$ ) at each day-of-life for the HFNV group. Pressure in the airway ( $P_{aw}$ ) was ~50% lower ( $P < 0.05$ ). Positive end-expiratory pressure (PEEP) at the ventilator was the same between the two groups at each day of life. ITP measured *in situ* was ~90% lower ( $P < 0.05$ ) at each day of life for the HFNV group compared to the intubation and IMV group.

We calculated oxygenation index (OI), Alveolar–arterial gradient of O<sub>2</sub> (A-a gradient), and Pao<sub>2</sub>/F<sub>i</sub>O<sub>2</sub> (P/F) ratio (**Table 3**). Median OI was ~60% lower at each day of life ( $P < 0.05$ ) for the HFNV group compared to the intubation and IMV group. The difference was even larger when we substituted mean ITP measured *in situ* for  $P_{aw}$  in the formula for OI. Using ITP in the formula, median OI was ~80% lower at each day of life ( $P < 0.05$ ) for the HFNV group compared to the intubation and IMV group. Median A-a gradient was ~60 to 90% lower at days of life 2 and 3 for the HFNV group. The calculation of A-a gradient took into account the lower barometric pressure at Salt Lake City (~5,000 feet above sea level). Conversely, median P/F ratio was ~50% higher at day-of-life 3 for the HFNV group of lambs supported for 3 d.

Prolonged HFNV for 21 d also provided acceptable gas exchange and respiratory system pressures (**Table 3**; right-most columns). A caveat is one lamb in the HFNV group developed a blood clot in its arterial catheter. Therefore, arterial blood could not be sampled during its final week of life. Thus, sample size became 3 for just arterial blood gas values at week of life 3 for the HFNV group. We continued to measure O<sub>2</sub> saturation, which remained between 88 and 94%, and therefore was within the same range as all of the other lambs in the 21 d groups (data not shown).

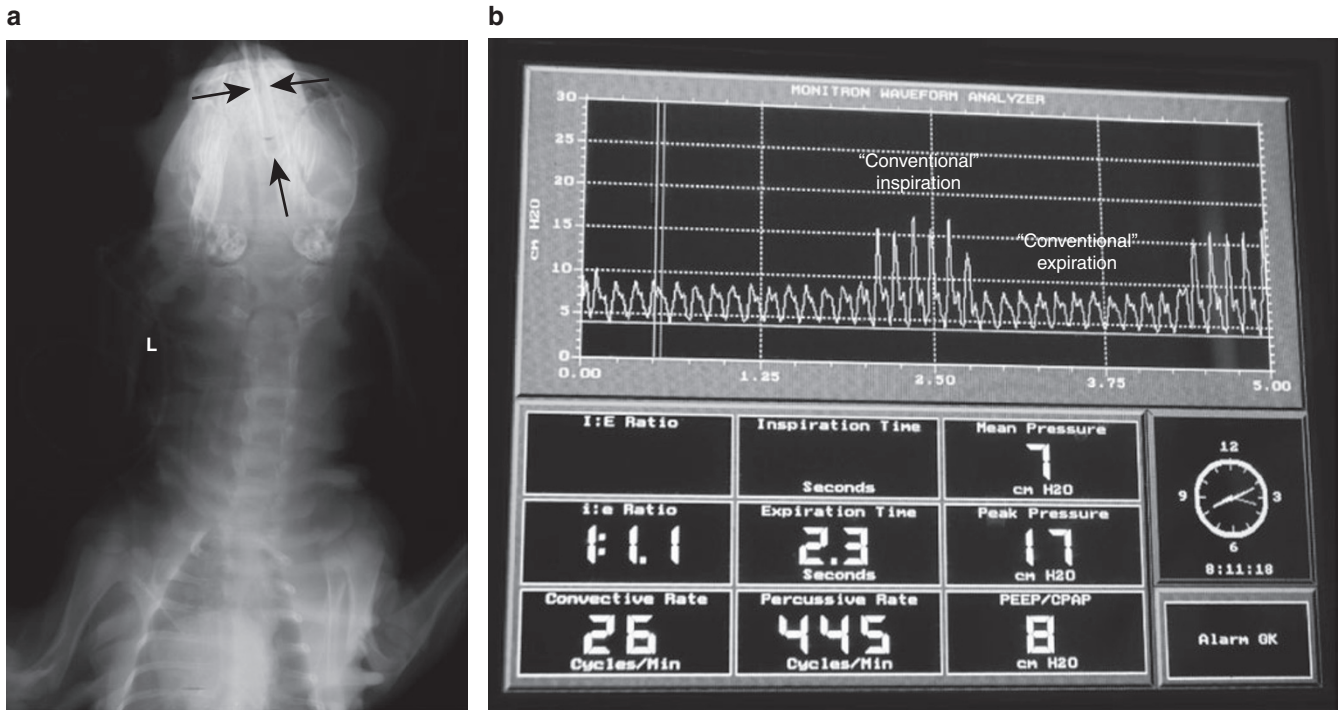
For the 21 d groups, targeted arterial Pao<sub>2</sub> range was attained using ~30% lower F<sub>i</sub>O<sub>2</sub> at weeks of life 2 and 3 ( $P < 0.05$ ) for the HFNV group compared to the same weeks for the matched intubation and IMV group. The targeted Paco<sub>2</sub> range was attained using ~75% lower respiratory system pressures for the HFNV group ( $P < 0.05$ ). Both  $P_{aw}$  and PEEP were ~50% lower at weeks of life 2 and 3 ( $P < 0.05$ ) compared to the matched weeks of life for the intubation and IMV group. At weeks of life 2 and 3, pH was significantly higher ( $P < 0.05$ ) for the HFNV group. We did not measure ITP *in situ* in the 21 d HFNV group because that

**Table 1.** Demographic characteristics of preterm lambs managed by invasive or noninvasive respiratory support (mean ± SD)

Parameter	Preterm 3 d ( $n = 5$ each)		Preterm 21 d ( $n = 4$ each)	
	Intubation and IMV	HFNV	Intubation and IMV	HFNV
Gestational age (d)	131 ± 1	132 ± 1	132 ± 1	133 ± 1
Weight at delivery (kg)	4.3 ± 0.4	4.7 ± 0.3	4.5 ± 0.5	4.1 ± 0.9
Weight at study end (kg)	3.9 ± 0.4	4.6 ± 0.5*	3.9 ± 0.4	4.5 ± 0.4*
Sex	Three female/two male	Three female/two male	Two female/two male	Two female/two male

HFNV, high-frequency nasal ventilation; IMV, intermittent mandatory ventilation.

\*Statistically different from IMV group by unpaired t-test,  $P < 0.05$ .



**Figure 1.** High-frequency nasal ventilation (HFNV) support preparation and airway pressure tracings. (a) Head radiograph of a preterm lamb supported by HFNV for 3 d (posterior-anterior view; L, left). Black arrows highlight the uncuffed tube in the nasal cavity. The vertical black arrow identifies the tube’s tip, which is ~5 cm along the length of the nose (~10 cm). (b) View of the display panel of the Percussionaire VDR4 ventilator, showing phasic trace of intratracheal pressure and numeric data for respiratory values. The intratracheal pressure trace has continuous high-frequency percussions (jagged contours), including through “conventional” inspiration. The convective rate is 26, peak inspiratory pressure is 17 cmH<sub>2</sub>O, and positive end-expiratory pressure is 8 cmH<sub>2</sub>O. High-frequency rate is ~7 Hz (445 cycles/min) and mean pressure is 7 cmH<sub>2</sub>O.

**Table 2.** Airway pressure profile at 3 d of high-frequency nasal ventilation support of preterm lambs (mean ± SD; n = 3)

Location	High peak inspiratory pressure (cmH <sub>2</sub> O)		Moderate peak inspiratory pressure (cmH <sub>2</sub> O)	
	Peak	Mean	Peak	Mean
Ventilator	27 ± 1	9 ± 1	14 ± 1	6 ± 1
Connector	27 ± 1	9 ± 1	14 ± 1	6 ± 1
Nasopharynx	10 ± 2	4 ± 2	3 ± 2	1 ± 1
Intratracheal	10 ± 2	4 ± 2	3 ± 2	1 ± 1

would have required sedating and intubating the lambs, which could have affected the physiological results. For this reason, OI was calculated using  $P_{aw}$  for the 21 d groups. Median OI was ~20 to 60% lower at each week of life ( $P < 0.05$ ) for the HFNV group compared to the intubation and IMV group. Median A-a gradient was ~25 to 50% lower at weeks of life 2 and 3 ( $P < 0.05$ ) for the HFNV group. Median P/F ratio, while larger for the HFNV group at each week of life, was not different between the two 21 d groups. These physiological improvements for the HFNV group were not influenced by initial differences in ventilator settings during the first 3 h of IMV, before weaning to HFNV (see **Supplementary Table S1** online).

We found time effects and group-time interactions between the HFNV group and the intubation and IMV group for the 21 d study, using linear mixed effects models. Time effects were

detected overall for both PIP ( $\beta = -5.6, t(16) = -6.0, P < 0.05$ ) and  $P_{aw}$  ( $\beta = -2.4, t(16) = -5.3, P < 0.05$ ). PIP and  $P_{aw}$  decreased from week-of-life 1 to 3 for the HFNV group, whereas PIP increased slightly and  $P_{aw}$  remained constant during the same time period for the intubation and IMV group. These group differences were corroborated by group-time interactions (PIP:  $\beta = 7.5, t(16) = 6.0, P < 0.05$ ;  $P_{aw}$ : ( $\beta = 2.4, t(16) = 4.0, P < 0.05$ ). The models also showed that  $F_iO_2$  tended to decrease from week-of-life 1 to 3; however, the trend was not statistically significant ( $\beta = -0.04, t(16) = 1.8, P = 0.09$ ).

The physiologically better outcomes for the HFNV groups at 3 and 21 d were associated with better radiographic and histologic appearances of the lungs (**Figure 2**). Radiographically, the lung fields were more translucent and lung volumes appeared larger for all five preterm lambs at 3 d of HFNV and all four preterm lambs at 21 d of HFNV compared to the matched number of preterm lambs per period of intubation and IMV. Also, air bronchograms were less obvious radiographically for all of the preterm lambs in the two HFNV groups. Histologically, terminal respiratory units appeared more developed in the HFNV group than the matched intubation and IMV groups. That is, the lung’s parenchymal appearance in the HFNV groups included distal airspaces that had more uniform and homogeneous size and shape. These observations were borne out by quantitative histology (**Figure 3**). Morphometry and stereology showed that at 21 d of ventilation support, the HFNV group had greater radial alveolar

**Table 3.** Respiratory gas exchange parameters in preterm lambs managed by invasive or noninvasive respiratory support (mean ± SD, except as noted)

Parameter	Preterm 3 d (n = 5 each)			Preterm 21 d (n = 4 each)		
	Day of life	Intubation and IMV	HFNV	Week of life	Intubation and IMV	HFNV
PaO <sub>2</sub> (mm Hg)	1	73 ± 17	73 ± 11	1	76 ± 13	75 ± 12
	2	85 ± 9	81 ± 10	2	76 ± 3	73 ± 14
	3	73 ± 8	77 ± 16	3	74 ± 6	68 ± 3 (n = 3)
F <sub>i</sub> O <sub>2</sub>	1	0.33 ± 0.03	0.32 ± 0.03	1	0.42 ± 0.11	0.30 ± 0.04
	2	0.35 ± 0.04	0.31 ± 0.03	2	0.36 ± 0.04	0.26 ± 0.03*
	3	0.38 ± 0.04	0.27 ± 0.03*	3	0.33 ± 0.05	0.23 ± 0.02*
PaCO <sub>2</sub> (mm Hg)	1	49 ± 7	50 ± 16	1	46 ± 7	52 ± 4
	2	44 ± 5	55 ± 8	2	49 ± 4	45 ± 5
	3	53 ± 10	64 ± 11	3	47 ± 8	42 ± 6 (n = 3)
PIP (cmH <sub>2</sub> O) <sup>a</sup>	1	22 ± 2	13 ± 4*	1	14 ± 1	18 ± 3
	2	20 ± 2	13 ± 4*	2	15 ± 2	10 ± 4
	3	25 ± 2	13 ± 2*	3	19 ± 5	4 ± 1*
ITP (cmH <sub>2</sub> O)	1	22 ± 2	2 ± 2*	1	ND	ND
	2	20 ± 2	2 ± 3*	2	ND	ND
	3	25 ± 2	2 ± 2*	3	ND	ND
P <sub>aw</sub> (cmH <sub>2</sub> O) <sup>a</sup>	1	11 ± 1	6 ± 1*	1	10 ± 1	9 ± 3
	2	12 ± 2	6 ± 1*	2	10 ± 1	5 ± 1*
	3	12 ± 1	7 ± 1*	3	10 ± 1	4 ± 1*
PEEP (cmH <sub>2</sub> O) <sup>a</sup>	1	6 ± 1	5 ± 1	1	7 ± 2	7 ± 3
	2	7 ± 1	6 ± 2	2	7 ± 1	4 ± 1*
	3	7 ± 1	6 ± 3	3	7 ± 1	4 ± 1*
pH	1	7.34 ± 0.05	7.35 ± 0.09	1	7.34 ± 0.10	7.33 ± 0.06
	2	7.43 ± 0.07	7.37 ± 0.03	2	7.30 ± 0.04	7.43 ± 0.06*
	3	7.37 ± 0.14	7.34 ± 0.08	3	7.35 ± 0.07	7.45 ± 0.04 (n = 3)
Oxygenation index (median (IQR))	1	5.7 (0.8)	2.3 (0.5) <sup>b</sup>	1	5.0 (2.7)	1.9 (0.9) <sup>b</sup>
	2	4.6 (1.8)	2.2 (0.2) <sup>b</sup>	2	4.8 (0.9)	4.0 (1.6) <sup>b</sup>
	3	5.6 (1.7)	2.5 (1.3) <sup>b</sup>	3	3.2 (0.8)	1.4 (0.1) <sup>b</sup> (n = 3)
A-a gradient (mmHg) (median (IQR))	1	65 (14)	47 (15)	1	83 (124)	33 (40)
	2	55 (24)	20 (26) <sup>b</sup>	2	70 (48)	53 (20) <sup>b</sup>
	3	90 (47)	9 (11) <sup>b</sup>	3	40 (53)	18 (10) <sup>b</sup> (n = 3)
PaO <sub>2</sub> /F <sub>i</sub> O <sub>2</sub> ratio (median (IQR))	1	209 (24)	243 (59)	1	212 (102)	261 (133)
	2	243 (58)	284 (53)	2	220 (49)	233 (50)
	3	184 (62)	262 (88) <sup>b</sup>	3	256 (121)	294 (23) (n = 3)

Oxygenation index = (P<sub>aw</sub> × F<sub>i</sub>O<sub>2</sub>)/PaO<sub>2</sub>; A-a gradient = ((F<sub>i</sub>O<sub>2</sub>) × (640–47)) – (PaCO<sub>2</sub>/0.8) – PaO<sub>2</sub>. Barometric pressure at Salt Lake City (~5,000 feet above sea level) is ~640 mm Hg. HFNV, high-frequency nasal ventilation; IMV, intermittent mandatory ventilation; IQR, interquartile range; ITP, intratracheal pressure; ND, not determined; PEEP, positive end-expiratory pressure; PIP, peak inspiratory pressure.

\*Statistically different from IMV group by unpaired t-test, P < 0.05.

<sup>a</sup>Pressure at the ventilators. <sup>b</sup>Statistically different from IMV group by Mann-Whitney U-test, P < 0.05.

count (P < 0.05) and volume density for alveolar secondary septa (P < 0.05), and narrower distal airspace walls (P < 0.05), compared to the IMV group.

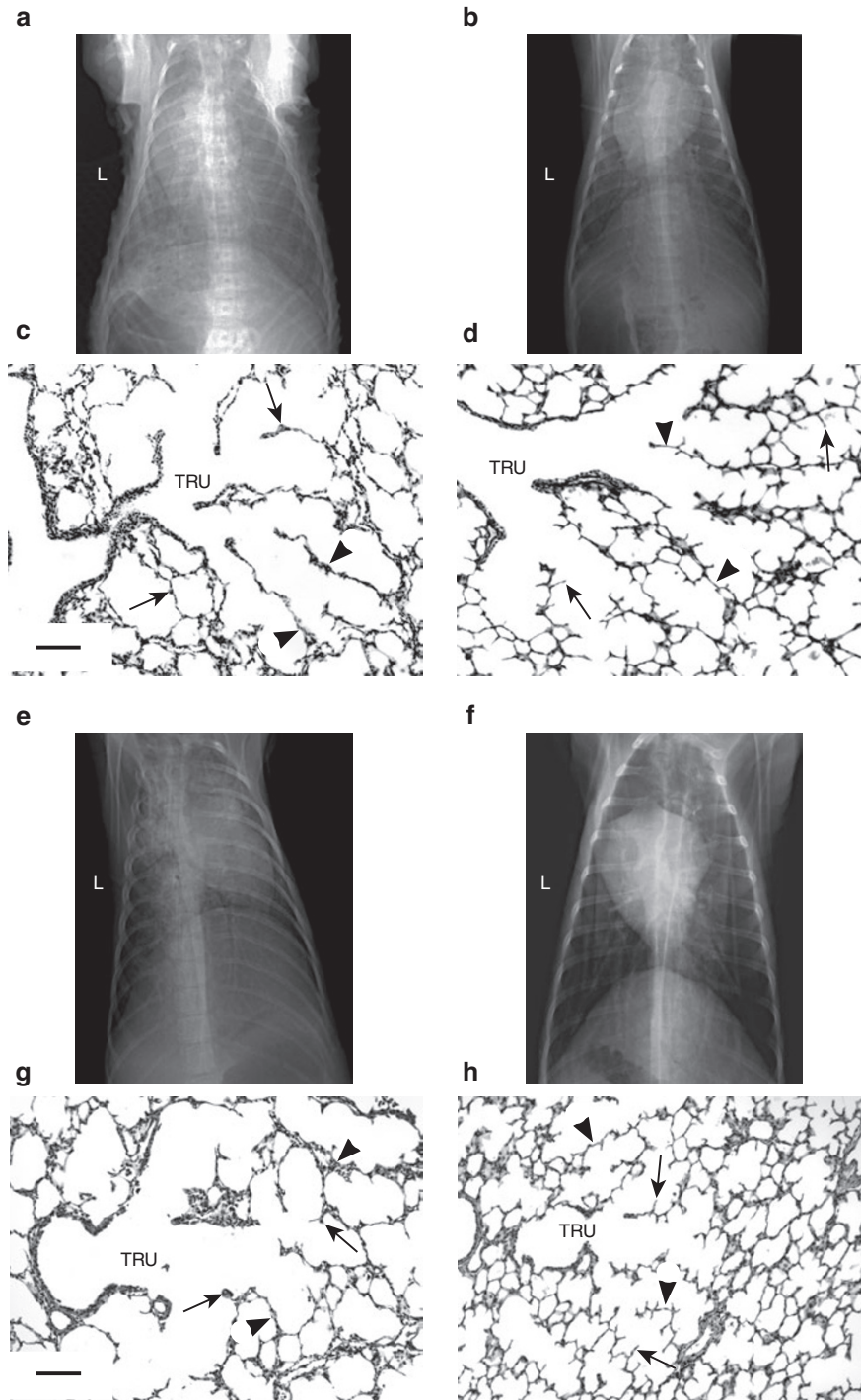
We compared the feeding volume between HFNV and IMV groups because of the potential for differences in nutrition to influence lung development. For the 3 d study, the daily volume of colostrum was the same between the HFNV group

and the intubation and IMV group (Table 4). For the 21 d study, by comparison, the weekly volume of colostrum/milk increased for the HFNV group, whereas the weekly volume decreased for the intubation and IMV group (Table 4). At week of life 3, the volume of milk delivered was 3.5-fold larger for the HFNV group. Nonetheless, plasma glucose remained physiological in between HFNV and IMV groups (Table 4).



We also compared the dosages of pentobarbital and buprenorphine that were given to the HFNV and IMV groups (Table 4). For the 3 d study, the daily dosages of pentobarbital and buprenorphine were comparable between the

HFNV group and the intubation and IMV group. However, for the 21 d study, weekly dosages of pentobarbital and buprenorphine were significantly lower for the HFNV group ( $P < 0.05$ ).



**Figure 2.** Chest radiographic and histologic appearance of lungs. Invasive support is endotracheal intubation and intermittent mandatory ventilation (IMV) (left column). Noninvasive support is high-frequency nasal ventilation (HFNV) (right column). The top two rows juxtapose radiographic and histologic images for the 3 d groups. The bottom two rows juxtapose radiographic and histologic images for the 21 d groups. Panels **a** vs. **b**; and **e** vs. **f**: chest radiographs show better aeration and larger lung volume in the HFNV groups compared to the corresponding intubation and IMV groups (posterior-anterior view; L, left). Panels **c** vs. **d**; and **g** vs. **h**: terminal respiratory units (TRU) have more uniform distal airspaces, thinner distal airspace walls (arrowheads), and more and thinner alveolar secondary septa (arrows) in the HFNV groups compared to corresponding intubation and IMV groups (the scale bar is 100  $\mu$ m in length).

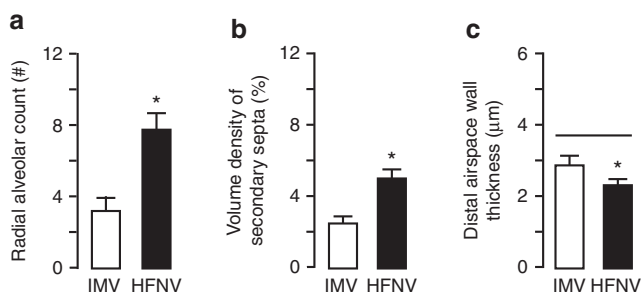
## DISCUSSION

Our study has two principal results for chronically ventilated preterm lambs. The first principal result is physiological arterial blood gas values are maintained for 21 d of HFNV, with significantly lower applied O<sub>2</sub> and respiratory system pressures, compared to intubation and IMV. Secondly, alveolar formation progressed during 21 d of HFNV, whereas alveolar simplification occurred during 21 d of intubation and IMV. Together, these principal results show that noninvasive HFNV leads to prolonged improvement in functional and structural development of the lung of preterm lambs. Conversely, prolonged invasive ventilation evolves into neonatal CLD through persistent disruption of both functional and structural development of the parenchyma of the immature lung.

The driver for noninvasive respiratory support was a Percussionaire ventilator (Sand Point, ID). This ventilator allows for a combined low-frequency conventional/

high-frequency percussive approach, whereby a conventional pressure-limited, time-cycled breath provided at low background frequency is coupled with an underlying high-frequency pattern of small, percussive pressures that deliver subphysiological tidal volumes (22). Thus, both conventional and high-frequency gas exchange principles were configured. This combination of principles is similar to combining conventional pressure-limited breaths with high-amplitude bubble nasal CPAP, which augments volume delivery and reduces work of breathing (23). Coupling creates “biologic noise” (also called stochastic resonance) in the total waveform that is postulated to contribute to recruitment of lung volume (24–26). The Percussionaire ventilator, when configured to provide low-frequency conventional breaths, is unique in its ability to continuously provide subphysiologic tidal volume, high-frequency breaths throughout both inspiratory and expiratory phases of respiration.

We used low-frequency conventional and high-frequency percussive configuration because preterm lambs that were supported by bubble nasal CPAP for more than 3–4 h had progressive deterioration of respiratory gas exchange (9). We initially tried bubble nasal CPAP because it is associated with less abundance of proinflammatory mediators (18) and more production of surfactant in the lung (27). However, we found that during prolonged bubble nasal CPAP, PaCO<sub>2</sub> and pH became unphysiological even though the lambs were treated with antenatal corticosteroids to promote lung development, exogenous surfactant to enhance lung compliance, and caffeine citrate to stimulate respiratory drive. While the cause of progressive deterioration of respiratory gas exchange remains unknown, we suspect that the long nose and neck of lambs, which increase anatomic dead space, may be contributory. Our results suggest that long-term use of HFNV as applied in this study may be a more effective approach to non-invasive respiratory support than either nasal CPAP or nasal IMV (28–31).



**Figure 3.** Summary of morphometric measurements (mean  $\pm$  SD) made in lung tissue sections from preterm lambs managed by intermittent mandatory ventilation (IMV; white bars) or high-frequency nasal ventilation (HFNV; black bars) ( $n = 4$ /group). Radial alveolar count (panel **a**) and volume density of secondary septa (panel **b**) were significantly greater, whereas distal airspace wall thickness (panel **c**) was significantly narrower, in the HFNV group than in the IMV group. \*Different from the corresponding IMV group by unpaired  $t$ -test ( $P < 0.05$ ).

**Table 4.** Feeding and sedation parameters in preterm lambs managed by invasive or noninvasive respiratory support (mean  $\pm$  SD)

Parameter	Preterm 3 d ( $n = 5$ each)			Preterm 21 d ( $n = 4$ each)		
	Day of life	Intubation and IMV	HFNV	Week of life	Intubation and IMV	HFNV
Plasma glucose (gm/dl)	1	101 $\pm$ 75	118 $\pm$ 46	1	84 $\pm$ 20	95 $\pm$ 10
	2	96 $\pm$ 32	109 $\pm$ 30	2	81 $\pm$ 20	105 $\pm$ 7
	3	83 $\pm$ 25	93 $\pm$ 13	3	84 $\pm$ 7	99 $\pm$ 6
Enteral feeding (ml/kg/d) <sup>a</sup>	1	13 $\pm$ 9	9 $\pm$ 7	1	106 $\pm$ 10	107 $\pm$ 10
	2	35 $\pm$ 22	24 $\pm$ 15	2	81 $\pm$ 55	149 $\pm$ 59
	3	51 $\pm$ 35	52 $\pm$ 31	3	53 $\pm$ 23	188 $\pm$ 70*
Pentobarbital (mg/kg/d)	1	7 $\pm$ 4	4 $\pm$ 4	1	24 $\pm$ 20	1 $\pm$ 1*
	2	2 $\pm$ 2	1 $\pm$ 1	2	29 $\pm$ 31	1 $\pm$ 1
	3	4 $\pm$ 2	2 $\pm$ 1	3	73 $\pm$ 64	1 $\pm$ 1*
Buprenorphine (mcg/kg/d)	1	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	1	0.06 $\pm$ 0.02	0.03 $\pm$ 0.03*
	2	0.04 $\pm$ 0.03	0.05 $\pm$ 0.01	2	0.06 $\pm$ 0.01	0.03 $\pm$ 0.03*
	3	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	3	0.05 $\pm$ 0.01	0.01 $\pm$ 0.01*

HFNV, high-frequency nasal ventilation; IMV, intermittent mandatory ventilation.

<sup>a</sup>Ewe's colostrum (days of life 1–3) followed by ewe's maturing milk (day of life 4 and thereafter).

\*Statistically different from IMV group by unpaired  $t$ -test,  $P < 0.05$ .

Use of short-term noninvasive ventilation support, with supraphysiological respiratory frequency through a nasopharyngeal tube, is reported for preterm infants. Two studies used an Infant Star HFV ventilator in its HFV mode. Van der Hoeven *et al.* (32) used nasal HFV in 21 premature infants within the first 20 d of life; 72% of infants were started on nasal HFV within 36 h of age. Nearly 80% of the infants showed improved ventilation and oxygenation, with a median of 36 h support via nasal HFV. Colaizy *et al.* (33) used the same type of ventilator to support 14 stable preterm infants for 2 h. Their results showed that nasal HFV significantly reduced  $P_{aCO_2}$  and raised pH. However, the Infant Star ventilator is no longer made. Furthermore, the Infant Star ventilator was limited by maximum amplitude that it could generate, which limited the tidal volume that was delivered. A third study used a Dräger Babylog 8000 ventilator for 24 h after extubation of a 760 gm preterm infant. Hoehn and Krause (34) showed that the infant's  $P_{aCO_2}$  remained in physiological range, thereby avoiding reintubation. A fourth study used the VDR3 Percussionaire, which is what we used. Dumas De La Roque *et al.* (35) used HFNV in a prospective, unmasked, randomized, controlled clinical trial for 46 eligible newborn infants who were hospitalized for transient tachypnea of the newborn. HFNV reduced the period of transient tachypnea by 50%. However, those studies did not test long-term efficacy. Our experimental animal model's results show that 21 d of HFNV, as we used it, facilitates physiological arterial blood gas values at lower  $F_{iO_2}$  and respiratory pressures. Whether our results in preterm lambs are translatable will require testing HFNV in high-risk preterm infants. This test will be important because of anatomic differences of the nose and neck between lambs and infants.

Only one of the lambs failed to reach 21 d of HFNV. Failure was related to progressive respiratory failure. Another observation of our study is that air accumulated in the stomach of some of the lambs that were supported by HFNV. This observation, also common among preterm infants on nasal ventilation, was easily managed by vented orogastric tube and did not impair feeding tolerance.

An important physiological point made by our study is that ITP is very low during HFNV. The *in situ* measurements show that ITP during HFNV of spontaneously breathing preterm lambs is about 1/10th that during intubation and IMV (Table 3). The location where pressure is attenuated is after the connector piece, in the nasopharynx. Attenuation is ~60% of the pressure setting at the ventilator (Table 2). Peak and mean airway pressures were respectively constant within the nasopharynx and trachea. The *in situ* measurements also reveal that HFNV maintains positive mean ITP of 1–2 cmH<sub>2</sub>O, therefore low PEEP. In our model, substantial opportunity exists for leak from the nasal-oropharyngeal cavity. In the presence of substantial leak, others also demonstrated, through direct or indirect measurements, more significant reduction in distal airway pressure than expected based on the measured or set pressure at the device interface (36, 37). During intubation and IMV support, by comparison, airway pressures reflected the pressure settings at the ventilator (Table 3). The latter finding

is consistent with the results of a study showing that ITP during invasive mechanical ventilation is comparable to ventilator pressure (38).

Low peak and mean ITPs during HFNV, combined with more uniform inflation seen radiographically, may contribute to the better structural development of the lung's parenchyma. Indeed, studies using chronically ventilated preterm baboons demonstrate that early nasal CPAP promotes alveolar formation compared to intubation and IMV (20,21). We recently reported that progression of alveolar formation at the end of 3 d of HFNV is associated with more apoptosis, and less proliferation, among mesenchymal cells in the walls of the distal airspaces compared to intubation and IMV (9). This level of balance between apoptosis and proliferation of mesenchymal cells is related to thinning of the distal air-space walls, which is necessary for efficient respiratory gas exchange. We are in the process of quantifying apoptosis and proliferation of mesenchymal cells in preterm lambs that were supported by HFNV vs. IMV for 21 d, and will report the results separately.

A potential confounding element of our study design is that all of the preterm lambs were endotracheally intubated and supported by IMV during the ~3 h transition period leading to HFNV support. However, no differences in the initial ventilator settings or respiratory physiological results occurred (refer to **Supplementary Table S1** online), suggesting that the 3 h transition period of IMV did not confound the outcomes for the HFNV groups.

Better pulmonary outcomes at 21 d of HFNV may be related to better enteral feeding and weight gain (Table 4) (39,40). Better enteral feeding and weight gain, in turn, may be impacted by less sedation (Table 4) because sedation decreases gastrointestinal motility and absorption, as well as growth and development of the lung (41). In new studies, we are determining the separate effects of enteral feeding vs. sedation on lung structural and functional outcomes. We are assessing the impact of matched-feeding (ml (calories)/kg/d) vs. matched-sedation (mg/kg/d) in separate groups of preterm lambs on HFNV. Matching is done at the daily level for intubated preterm lambs on IMV. Initial results suggest that only matched enteral feeding shifts lung outcomes toward the poorer outcomes that occur in intubated lambs on IMV.

We conclude that early noninvasive respiratory support via HFNV as we applied using a high-frequency flow interrupter ventilator, coupled with maternal antenatal steroids, prophylactic surfactant replacement, and postnatal caffeine therapy, effectively maintains gas exchange at lower O<sub>2</sub> levels and respiratory pressures out to 21 d. In addition, the quantitative histological results indicate that HFNV out to 21 d improves alveolarization. Our study's results raise the possibility that long-term HFNV may be an alternative approach to acceptable respiratory support for preterm infants. Our observation that lung development is occurring raises the possibility that HFNV may reduce neonatal CLD (17). Translating our results from preterm lambs to preterm infants will require clinical studies.



## METHODS

Protocols adhered to American Physiological Society/US National Institutes of Health guidelines for humane use of animals for research and were prospectively approved by the Institutional Animal Care and Use Committee at the University of Utah Health Sciences Center.

### Surgical Preparation

The methods for chronically ventilating preterm lambs are reported (9,42,43). Briefly, time-pregnant ewes that carried one fetus or twin fetuses at  $132 \pm 2$  d of gestation (term  $\sim 150$  d gestation) were used. The pregnant ewes were given an intramuscular injection of dexamethasone phosphate (6 mg; Vedco, St Joseph, MO),  $\sim 36$  h before operative delivery. At delivery, we intubated all fetal lambs with a cuffed endotracheal tube (3.5 to 4.0 French), through which 10 ml of lung liquid was aspirated and replaced with Survanta (2.5 ml; NDC 0074-1040-08, Ross Products Division, Abbott Laboratories, Columbus, OH).

### Additional Surgical Step for HFNV

We prospectively and randomly assigned a subset of all of the intubated preterm lambs to be weaned to HFNV within  $\sim 3$  h of life (see the Randomization subsection). This subset had an uncuffed oral/nasal true Murphy tube (3.0 French; 13 cm length) inserted  $\sim 5$  cm through a nostril. We used a Murphy tube because preliminary studies revealed that preterm lambs did not tolerate nasal prongs or a nasal mask.

### Initial Resuscitation of all Preterm Lambs

All lambs were intubated and managed by IMV, with warmed and humidified  $O_2$  (Bird VIP ventilator; model 15215, Palm Springs, CA). Initial settings were: respiratory rate 60 breaths/min, inspiratory time 0.3 s, PEEP 7  $cmH_2O$ , flow 8 l/min of 50%  $O_2$ , and expiratory tidal volume 5–7 ml/kg. Within 30 min of delivery, the lambs were given an intravenous loading dose of caffeine citrate (15 mg/kg, given over 2 h, Mead Johnson & Company, Evansville, IN) to stimulate ventilatory drive. Caffeine citrate was given every 24 h (5 mg/kg). Arterial blood gases, pH, electrolytes, and glucose were measured hourly, as was  $O_2$  saturation by pulse oximetry. Vascular pressures and heart rate were recorded continuously (model V6400; SurgVet, Waukesha, WI). Dextrose was infused intravenously to maintain plasma glucose level between 60 and 90 mg/dl. Plasma concentrations of total protein and hematocrit were measured at 6 h intervals.

Arterial partial pressure of oxygen ( $P_{aO_2}$ ) was targeted between 70 and 90 mm Hg by adjusting fractional inspired  $O_2$  ( $F_{iO_2}$ ). Arterial partial pressure of carbon dioxide ( $P_{aCO_2}$ ) was targeted between 40 and 60 mm Hg, and pH between 7.25–7.45, by adjusting PIP. Mean  $P_{aw}$  also was measured. We calculated OI ( $(P_{aw} \times F_{iO_2})/P_{aO_2}$ ), Alveolar-arterial (A-a) gradient ( $((F_{iO_2}/100) \times (640-47)) - (P_{aCO_2}/0.8) - P_{aO_2}$ ), and  $P_{aO_2}/F_{iO_2}$  (P/F) ratio.

### Randomization

Randomization to invasive vs. noninvasive respiratory support for 3 or 21 d was done using a block randomization approach. We studied two preterm lambs concurrently.

### Two Modes of Respiratory Support

Invasive respiratory support was endotracheal intubation and IMV. Noninvasive respiratory support was a unique approach of nasal ventilation with a high-frequency component created by a high-frequency, flow-interruption ventilator (model VDR4; Percussionaire, Sand Point, ID). We call this mode HFNV. The Percussionaire is a flow-regulated, time-cycled ventilator that provides controlled-pressure delivery of low-frequency breathing cycles with a series of high-frequency, subphysiologic tidal volumes (22).

Weaning from IMV, begun at 2 h of life, was successful when the lambs consistently breathed spontaneously while the ventilator circuit to the Bird VIP ventilator was disconnected from the endotracheal tube (9). At that time, the endotracheal tube was withdrawn and the ventilator circuit from the Percussionaire ventilator was attached to the tube in the nose. Initial ventilator settings were background convective breaths at 5–10 breaths per min, PIP of 20–25  $cmH_2O$ , PEEP of 5–7  $cmH_2O$ , and high-frequency rate at 10 Hz. The ventilator circuit included a gas humidifier. Adjustments were made to the ventilator settings to reach the target ranges for  $P_{aO_2}$  and

$P_{aCO_2}$ . Because a leak-free seal was not possible during HFNV, lung volumes could not be directly measured.

### Measurement of Airway Pressures *In Situ*

Phasic and mean pressures from the ventilator to the trachea were measured *in situ*, using a pressure-tipped wire (Samba Sensor model Preclin 420 LP, Samba Sensors SE, Västra Frölunda, Sweden). The wire was inserted through a rubber-covered side port of the connector piece to the endotracheal tube or nasal tube.

### Management of Preterm Lambs

Lambs were kept prone in a veterinary sling mounted on a radiantly heated bed. Antibiotics were given daily (potassium penicillin, 100,000 U/kg, Pfizer (Groton, CT); and gentamicin, 2.5 mg/kg/d, Hospira, Lake Forest, IL). Gentamicin was discontinued at 10 d. Sedation was provided, using pentobarbital sodium and buprenorphine hydrochloride. Lambs that were randomized to intubation and IMV received 3–5 mg/kg of pentobarbital sodium (Abbott Laboratories, North Chicago, IL) as needed to minimize discomfort and agitation associated with endotracheal intubation. Lambs that were randomized to HFNV received only 1–2 mg/kg of pentobarbital as needed to permit spontaneous breathing. All lambs were initially given buprenorphine hydrochloride (5 mcg/kg every 3 h, Reckitt & Colman Pharmaceuticals, Richmond, VA) for the 3 d study. For the 21 d study, buprenorphine dosage was reduced at 1 wk of life and thereafter for the HFNV group to minimize respiratory depression.

An orogastric feeding tube was used for enteral feedings, using ewe's colostrum, beginning at 4 h of life (3 ml/kg every 2 h) and continued for 3 d. The volume was increased gradually by 3–5 ml increments, as tolerated, to attain a goal of 60–80 kcal/kg/d of total energy substrate, based on daily weight. After 3 d, ewe's milk was given. Volume was increased as tolerated. We monitored total fluid intake (saline, dextrose, and milk) and output (withdrawn blood, urine, and stool), and made adjustments to maintain fluid homeostasis, as indicated by urine output ( $>1$ –2 ml/kg/h) and mean arterial blood pressure ( $>45$  mm Hg). None of the lambs required pressors. Chest radiographs were taken on days of life 1, 2, and 3 of all of the 3 d ( $n = 5$ /group) and 21 d lambs ( $n = 4$ /group), and weekly thereafter for the 21 d lambs, to assess lung inflation volume and to identify atelectasis. Indices of infection were monitored by daily leukocyte total and differential cell counts, and rectal temperature.

### Terminal Collection of Tissue

Blood samples were collected before the lambs were given heparin (1,000 U, intravenously) followed by 5 mg/kg of pentobarbital (9,43). Both HFNV groups were intubated and reconnected to the Percussionaire ventilator to maintain lung inflation when the chest was opened to remove the lungs. All lambs were given 60 mg/kg pentobarbital sodium solution intravenously (Beuthanasia solution, Ovation Pharmaceuticals, Deerfield, IL). The chest was opened, the trachea was ligated at end-inspiration (to minimize atelectasis), and the lungs and heart were removed. The whole left lung was insufflated with 10% buffered neutral formalin (static pressure of 25  $cmH_2O$ ). Fixed-lung displacement volume was measured by suspension in formalin before the lung was stored in fixative (4 °C, 24 h). Paraffin-embedded tissue blocks were prepared for histology.

### Quantification of Alveolar Secondary Septation and Distal Airspace Wall Thickness

We used quantitative histology (morphometry and stereology) to statistically compare alveolar secondary septation (radial alveolar count and secondary septal volume density) and distal airspace wall thickness, using methods previously reported by our group (9).

### Data Analysis

Continuous variables that were approximately normally distributed are summarized by mean  $\pm$  SD. These results were compared by unpaired *t*-test. Continuous variables that were not approximately normally distributed are summarized by the median and interquartile range. These results were analyzed by Mann-Whitney *U*-test. Linear mixed effects models were used to test whether  $F_{iO_2}$ , PIP, and  $P_{aw}$  changed differently over time (time effects) during intubation and IMV vs. HFNV, and whether the two respiratory support groups



changed differently over time (group-time interaction). We used a simple random intercept model, with a variance-covariance structure that assumed compound symmetry across time points. Statistical analyses were done using StatView5 (SAS Institute, Cary, NC) or R v2.15.0. Statistical significance was assessed at  $P < 0.05$ .

#### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

#### ACKNOWLEDGMENTS

We thank the large number of undergraduate students and medical students who tended the chronically ventilated preterm lambs. We also thank Ronald S. Bloom for suggestions.

#### STATEMENT OF FINANCIAL SUPPORT

This study was supported by National Institutes of Health (NIH) grants HL110002, HL062875, and HL007744, the Division of Neonatology, and the Children's Health Research Center in the Department of Pediatrics, and the University of Utah Study Design and Biostatistics Center, with funding in part from the National Center for Research Resources and the National Center for Advancing Translational Sciences, NIH grant 8UL1 TR000105 (formerly UL1 RR025764).

Disclosure: The authors have no financial ties to products used in this study.

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