Pressure-limited sustained inflation vs. gradual tidal inflations for resuscitation in preterm lambs

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Tingay DG, Polglase GR, Bhatia R, Berry CA, Kopotic RJ, Kopotic CP, Song Y, Szyld E, Jobe AH, Pillow JJ. Pressure-limited sustained inflation vs. gradual tidal inflations for resuscitation in preterm lambs. J Appl Physiol 118: 890–897, 2015. First published January 29, 2015; doi:10.1152/japplphysiol.00985.2014.—Support of the mechanically complex preterm lung needs to facilitate aeration while avoiding ventilation heterogeneities: whether to achieve this gradually or quickly remains unclear. We compared the effect of gradual vs. constant tidal inflations and a pressure-limited sustained inflation (SI) at birth on gas exchange, lung mechanics, gravity-dependent lung volume distribution, and lung injury in 131-day gestation preterm lambs. Lambs were resuscitated with either 1) a 20-s, 40-cmH2O pressure-limited SI (PressSI), 2) a gradual increase in tidal volume (VT) over 5 min from 3 ml/kg to 7 ml/kg (IncrVT), or 3) 7 ml/kg VT from birth. All lambs were subsequently ventilated for 15 min with 7 ml/kg VT with the same end-expiratory pressure. Lung mechanics, gas exchange and spatial distribution of end-expiratory volume (EEV), and tidal ventilation (electrical impedance tomography) were recorded regularly. At 15 min, early mRNA tissue markers of lung injury were assessed. The IncrVT group resulted in greater alveolar-arterial oxygen difference from 10 min (P < 0.017; two-way ANOVA), higher alveolar-arterial oxygen difference from 10 min (P < 0.01), and least uniform gravity-dependent distribution of EEV. There were no other differences in lung mechanics between groups, and the PressSI and 7 ml/kg VT groups behaved similarly throughout. EEV was more uniformly distributed, but VT least so, in the PressSI group. There were no differences in mRNA markers of lung injury. A gradual increase in VT from birth resulted in less recruitment of the gravity-dependent lung with worse oxygenation. There was no benefit of a SI at birth over mechanical ventilation with 7 ml/kg VT.

resuscitation; mechanical ventilation; infant; newborn; lung recruitment; ventilation homogeneity

SUCCESSFUL RESPIRATORY TRANSITION from fetal to ex utero life is a complex process requiring the rapid aeration of the fluid-filled lung and establishment of a functional residual capacity (FRC) (13). Failure to achieve uniform lung-liquid clearance reduces FRC (34) and results in ventilation inhomogeneity (37) in preterm animal models: both reduced FRC and ventilation inhomogeneity being associated with increased lung injury. Without positive pressure ventilation, many preterm infants are unable to aerate the lung adequately, or maintain tidal ventilation for effective gas exchange. Even brief periods of positive pressure ventilation applied inappropriately to the immature lung can cause lung (15, 24) and brain inflammation and injury (25) and influence the benefits of subsequent therapies, such as surfactant (5, 37).

Beyond the use of adequate applied positive end-expiratory pressure (PEEP), how to optimally and safely support the preterm lung at birth remains unclear. An initial sustained inflation (SI) may overcome the high resistance and long-time constant of the fluid-filled respiratory system at birth (33, 34). Current newborn resuscitation guidelines (23–28), based on limited human (35) and animal model (32) data, advocate a fixed inflation pressure applied over a predetermined time period. This approach assumes a uniformity of mechanical properties in the preterm lung and is not without potential clinical and injurious consequences from both inadequate and excessive pressures and durations (5, 33). Inappropriate inflation patterns may explain the conflicting animal model data with regards to the lung protective effects of an initial SI (26, 33, 34). In addition, in many of these preterm animal studies, the applied SI attains inflating volumes that approach the reduced FRC of the structurally immature preterm lung (26, 33, 34, 36, 37).

Lung aeration is dynamic, initiated within the airways, followed by a small number of lung units, before incrementally expanding other alveoli in an exponential pattern (13, 29). Early in this process, only a few lung units will be engaged in tidal ventilation. Delivering tidal volumes (VT) intended to be protective to the fully inflated lung may inadvertently provide excessive volumes to those lung units first engaged in aeration, setting in process a pattern of inhomogeneous ventilation (15). An approach intentionally designed to dynamically, and gently, support respiratory transition through a process of careful sequential recruitment aimed at minimizing alveolar tidal stretch using increases in VT as aeration is established is not reported. We hypothesized that ventilating with such an approach at birth may prevent regional overinflation while still

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achieving similar lung fluid clearance and establishment of FRC as the currently recommended use of adequate PEEP and SI (15).

We aimed to compare the effect of gradual vs. constant tidal inflations and pressure-limited SIs (PressSI) as a resuscitation strategy to recruit the preterm lung at birth on gas exchange, lung mechanics, thoracic volumes, homogeneity of end-expiratory volume (EEV), distribution of tidal ventilation within the thorax, and upregulation of early markers of lung injury in preterm lambs.

METHODS

All techniques and procedures were approved by The University of Western Australia animal ethics committee, in accordance with current National Health and Medical Research Council (Australia) guidelines (23a).

Surgical preparation. Anesthetized date-mated pregnant ewes underwent hysterotomy at 131 ± 1 days gestation (term ~147 days). The fetal carotid artery and jugular vein were cannulated on externalization of the head and neck. The trachea was intubated orally (4.5 mm inner diameter cuffed tracheal tube, Portex Ltd, UK), and fetal lung fluid suctioned. The fetal chest was exteriorized and dried, and 16 custom-built electrical impedance tomography (EIT; Goe-MF II EIT System, CareFusion, Hoechberg, Germany) needle electrodes were evenly spaced circumferentially around the chest at the level of the axillae and secured in place with Coban self-adhesive bandage (3M, St. Paul, MN) (36).

Lambs were delivered, weighed, placed prone, and ventilated with humidified gas using the FlexiVent ventilator (Scireq, Montreal, Quebec, Canada) programmed to deliver the assigned resuscitation and ventilation protocol (see below). Anesthesia and analgesia were maintained with continuous infusions of propofol (0.1 mg·kg⁻¹·min⁻¹, Repose, Norbrook Laboratories) and remifentanil (0.05 µg·kg⁻¹·min⁻¹, Ultiva, Glaxo Smith Kline) with resultant suppression of spontaneous breathing.

Measurements. Heart rate, preductal peripheral oxygen saturation (SpO₂; Nellcor OxiMax N65, Tyco Healthcare, Australia), and arterial and central venous pressure were recorded continuously from birth. Pressure and flow were measured at the airway opening using a Florian respiratory monitor sampling at 200 Hz (Acutronic Medical Systems, Hirzel, Switzerland). Gas exchange and acid balance were determined by arterial blood-gas analysis (Rapidlab 1265, Siemens Healthcare Diagnostics, Vic., Australia) at 5-min intervals throughout the study, and the alveolar-arterial difference in oxygen calculated. Change in regional thoracic impedance was determined by EIT sampling at 25 Hz using the manufacturer’s proprietary software (SCEIT, CareFusion, Hoechberg, Germany) to determine change in global thoracic volume from birth, homogeneity of EEV, and distribution of ventilation (36, 37, 40). A 10-s EIT measurement of the unaerated thorax was made immediately before commencing ventilation for use as the reference state for image reconstruction (36, 37). Partitioned measurements of respiratory mechanics were obtained with the FlexiVent using the low-frequency oscillation technique (17, 21) at 5-min intervals, immediately following blood-gas measurements.

Table 1. Birth characteristics and fetal umbilical arterial blood-gas variables at delivery

<table>
<thead>
<tr>
<th></th>
<th>PressSI</th>
<th>7 ml/kg VT</th>
<th>IncrVT</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>5 (62)</td>
<td>4 (50)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>First twin, n (%)</td>
<td>4 (50)</td>
<td>6 (75)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.04 (0.35)</td>
<td>3.04 (0.32)</td>
<td>3.17 (0.25)</td>
</tr>
<tr>
<td>pHₐ</td>
<td>7.12 (0.05)*</td>
<td>7.24 (0.08)*</td>
<td>7.17 (0.10)</td>
</tr>
<tr>
<td>PaCO₂, Torr</td>
<td>68.4 (10.0)</td>
<td>55.7 (4.2)</td>
<td>65.0 (14.1)</td>
</tr>
<tr>
<td>PaO₂, Torr</td>
<td>13.8 (6.9)</td>
<td>16.0 (5.3)</td>
<td>14.8 (3.2)</td>
</tr>
<tr>
<td>Hct, %</td>
<td>35.9 (4.7)</td>
<td>35.6 (2.9)</td>
<td>35.8 (2.7)</td>
</tr>
</tbody>
</table>

Values are means (SD); n, no. of lambs. PressSI, 20-s, 40-cmH₂O pressure-limited sustained inflation; VT, tidal volume; IncrVT, gradual increase in VT over 5 min from 3 to 7 ml/kg; pHₐ, arterial pH; PaCO₂ and PaO₂, partial pressure of arterial carbon dioxide and oxygen, respectively; Hct, hematocrit. *Significant difference (P = 0.029; one-way ANOVA with Tukey posttest).

Fig. 1. Alveolar-arterial oxygen difference (A-aDO₂; A), peripheral oxygen saturation (SpO₂; B), and arterial partial pressure of CO₂ (PaCO₂; C) for 20-s, 40-cmH₂O pressure-limited sustained inflation (PressSI, ●), 7 ml/kg tidal volume (VT; •), and gradual increase in VT over 5 min from 3 to 7 ml/kg (IncrVT; ○) groups. All data are means (SD). Significant difference between †IncrVT and both PressSI and 7 ml/kg VT and *IncrVT and PressSI (all P < 0.05; Sidak posttest, repeated-measures ANOVA).
Ventilation strategies. Twenty-four lambs were randomized to receive one of the following ventilation strategies.

1. A PressSI at an inflating pressure of 40 cmH\textsubscript{2}O for 20 s, followed by volume-controlled, pressure-limited (40 cmH\textsubscript{2}O) intermittent mandatory ventilation (IMV) with a VT of 7 ml/kg, PEEP 5 cmH\textsubscript{2}O, and inflation time of 0.4 s in 0.3 fraction of inspired oxygen (F\textsubscript{IO}\textsubscript{2}).

2. Gradual increase in VT via IMV (IncrVT) using 3 ml/kg VT for the first 2 min of life, then 5 ml/kg for the next 3 min of life, and, finally, 7 ml/kg at 5 min of life.

3. A control group receiving IMV from birth with a constant 7 ml/kg VT.

The IncrVT and 7 ml/kg VT groups received IMV with the same inflation time, PEEP, and F\textsubscript{IO}\textsubscript{2} as the PressSI group. In all groups, IMV was continued for 15 min, and F\textsubscript{IO}\textsubscript{2} was adjusted from 5 min of life to maintain preductal Sp\textsubscript{O}\textsubscript{2} 90–95%.

At 15 min, the lungs were ventilated at 1.0 F\textsubscript{IO}\textsubscript{2} for 2 min, after which the tracheal tube was clamped for 3 min to facilitate lung collapse by oxygen reabsorption. Lambs were euthanized with intravenous sodium pentobarbitone (100 mg/kg), after which an in situ postmortem super-syringe static pressure-volume curve to a maximal pressure of 40 cmH\textsubscript{2}O was performed (16).

Data acquisition and analysis. Sp\textsubscript{O2}, heart rate, arterial and central venous pressure, pressure at the airway opening, and flow were recorded at 1 kHz and digitalized using PowerLab and LabChart V7.2.5 (AD Instruments, Castle Hill, NSW, Australia). Delivered VT and dynamic respiratory system compliance (C\textsubscript{dyn}) were calculated from the pressure and flow data.

Respiratory variables were determined from 10 consecutive stable inflations at 30 s, and 1, 2, 3, 5, 10, and 15 min after birth. Input lung impedance was obtained using the low-frequency forced oscillation technique (2, 17): impedance spectra were derived from measurements of pressure and volume (2) obtained during application of an optimized ventilator waveform (average tracheal VT 7 ml/kg, 0.5–13 Hz, 8-s duration) (21) to the airway at 5, 10, and 15 min. The constant phase tissue model (9) was fitted to the impedance spectra to determine a Newtonian airway resistance (R\textsubscript{aw}), constant-phase tissue damping (G, similar to tissue resistance), tissue elastance (H). Tissue hysteresivity (\eta) was calculated as G/H (7).

EIT was analyzed offline using AUSPEX V1.6 (CareFusion). To isolate EEV, the trough of each respiratory cycle was determined after low-pass filtering the impedance signal to the respiratory domain (3, 6, 10, 36, 37). The filtered EIT EEV data were divided into three regions of interest (ROI): the global (entire chest cross-sectional data) and gravity-dependent (ventral) and nondependent (dorsal) hemithoraces. Relative change in EEV (\Delta EEV) from the unaerated reference state within each ROI was expressed as a percentage of the vital capacity for that ROI. Vital capacity was defined as the difference in impedance at 0 and 40 cmH\textsubscript{2}O in a ROI during the postmortem super-syringe static pressure-volume curve (6, 36, 37). The global EEV signal was calibrated to units of milliliters per kilogram using the postmortem super-syringe absolute volume data (1, 19, 22) to provide a global \Delta EEV from birth.

Functional EIT images of VT at 1 min and 15 min of life were constructed using the method of Frerichs and coworkers (8) to determine the spatial distribution of VT within the thorax. The relative impedance changes due to VT within 32 nondependent to dependent equally sized ROI were calculated from the functional EIT images. The impedance changes were expressed as a fraction of total VT (%) for intersubject comparison. Subtraction histograms were created to determine the change in fraction of VT within each ROI between 1 and 15 min of life (37). The gravity-dependent geometric center of ventilation was calculated for each image (8).

Postmortem analyses. Bronchoalveolar lavage was obtained by triplicate washouts of the left lung (14), and total protein content determined by the Lowry method (20). Representative samples were taken from the right lower lobe for quantitative real-time polymerase chain reaction analysis of early markers of lung injury including...
connective tissue growth factor (CTGF), cysteine-rich 61 (CYR61), and early growth response protein 1 (EGR1) mRNA (39). Quantitative real-time polymerase chain reaction results were analyzed using the 2^{ΔΔCT} method.

**Statistical analysis.** Data were tested for normality and analyzed with paired t-tests, Wilcoxon matched-pairs test, one-way ANOVA with Tukey post hoc tests, or repeated-measures ANOVA with Sidak post hoc tests (with ventilation strategy and time as variables) as appropriate. PRISM V6.0e for Mac (GraphPad Software, San Diego, CA) was used for analysis, and P < 0.05 considered significant.

**RESULTS**

**Baseline characteristics.** Table 1 summarizes the subject characteristics at birth. There were no differences in delivery order, birth weight, sex, or umbilical cord arterial partial pressure of O₂, arterial partial pressure of CO₂, and hematocrit at delivery. Cord arterial pH was lower in the PressSI group than the 7 ml/kg VT group (P = 0.029, Tukey posttest). All lambs completed the study without complications, including gross air leaks.

**Gas exchange.** Alveolar-arterial difference in oxygen was significantly higher in the IncrVT group at 10 min and 15 min of life compared with both of the other groups (P < 0.01; Fig. 1A). SpO₂ was lower in the IncrVT group at 2 min compared with both of the other strategies (P < 0.001; Fig. 1B) and remained lower than PressSI at 3 min (P < 0.001). Target SpO₂ range was obtained by 5 min in the PressSI and 7 ml/kg VT groups and by 15 min in the IncrVT group. Arterial partial pressure of CO₂ was not different between the three groups at any time point (Fig. 1C).

**Lung mechanics and ventilation parameters.** Delivered Vt was lower at 1 and 2 min in the IncrVT group (Fig. 2A), in accordance with the protocol (P < 0.05). There was no difference in the required change in pressure (Fig. 2B) during the first 2 min, despite the intentionally lower set Vt. Consequently, Cdyn at 1 and 2 min in the IncrVT group was lower than in the other groups (P < 0.05; Fig. 2C). There was no difference in Cdyn at any other time point.

The IncrVT strategy trended toward lower static volumes during the super-syringe pressure-volume curve (Fig. 2D); these differences were not statistically significant (P = 0.249).

**Partitioned forced oscillatory mechanics.** Figure 3 shows the Raw, G, H, and η data. IncrVT resulted in higher tissue G at 5 min compared with 7 ml/kg VT (P = 0.023; Sidak posttest; Fig. 3B). η was also higher at 5 min in the IncrVT compared with 7 ml/kg VT (P = 0.017) and PressSI (P = 0.05). There was no difference in groups at any other time points or for the Raw and H data.

**Hemodynamic measurements.** Carotid arterial blood pressure (P = 0.513; repeated-measures ANOVA), central venous pressure (P = 0.377), and heart rate (P = 0.688) were not different between groups at any stage of the ventilation strategy (data not shown).

**Regional aeration.** Ventilation strategy at birth influenced initial global ΔEEV (P = 0.004; Fig. 4): at 30 s of life, mean (95% confidence interval) ΔEEV was 17 (6, 29) ml/kg higher in the PressSI group compared with IncrVT group. Thereafter, there was no difference in global ΔEEV between groups.

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**Fig. 3.** Newtonian airway resistance (Raw; A), constant-phase tissue damping (G; B), tissue elastance (H; C), and tissue hysteresivity (η; D) for PressSI (○), 7 ml/kg VT (●), and IncrVT (○) groups. All data are means (SD). Significant difference between †IncrVT and both PressSI and 7 ml/kg VT and *IncrVT and 7 ml/kg VT (P < 0.05; Sidak posttest, repeated-measures ANOVA).
There was no statistical difference between the gravity dependent and nondependent hemithoraces in the PressSI group at any time point ($P = 0.054$), despite a pattern suggesting time-based derecruitment in the gravity-dependent hemithorax (Fig. 5). The gravity-dependent EEV was significantly lower than the nondependent hemithorax in the 7 ml/kg VT group overall ($P = 0.006$) and at 1 min ($P < 0.001$) on posttest analysis. Both time- ($P < 0.001$) and gravity-dependent ROI ($P < 0.001$) influenced the distribution of $\Delta$EEV in the IncrVT group, with significantly higher $\Delta$EEV in the nondependent hemithorax at 1, 5, 10, and 15 min of life (all $P < 0.05$).

**Gravity-dependent distribution of VT.** Figure 6 shows the relative gravity-dependent spatial distribution of VT within the thorax at 1 and 15 min of life. There were no statistical differences in the geometric center of ventilation between groups at 1 min, although the PressSI group exhibited the most heterogeneous ventilation pattern, with a median (range) geometric center of ventilation located toward the nondependent thorax at 46.8 (42.4, 50.8)\% of the nondependent to dependent thoracic distance. By 15 min of life, there was a temporal shift in the distribution of VT toward the dependent lung, with a geometric center of ventilation at 48.2 (range 46.0, 53.1)\% ($P = 0.031$; Wilcoxon matched-pairs test). IncrVT and 7 ml/kg VT groups had relatively uniform VT distribution at 1 and 15 min, with no change in the geometric center of ventilation over time.

**DISCUSSION**

In preterm lambs, we investigated diametrically different approaches to respiratory support from birth: a fixed inflating pressure SI and an incremental increase in VT, with a control group receiving 7 ml/kg VT. Overall, there was no difference in early markers of lung injury, but the incremental VT strategy resulted in worse oxygenation and gravity-dependent heterogeneity of aeration that was persistent at 15 min of life. There were no substantial differences in the immediate outcomes of IMV with PEEP and 7 ml/kg VT, with or without a SI at birth, highlighting the importance of adequate tidal ventilation and PEEP beyond the first inflation.

Lung recruitment is achieved during inflation and maintained with PEEP (6, 29). This recruitment process was evident in our study: all groups received the same PEEP, but attainment of global EEV during the first 5 min of life was slowest in the IncrVT group. Thereafter, the IncrVT strategy achieved a steady-state EEV that was similar to the other two strategies. In contrast, oxygenation was worse and displayed greatest inter-subject variability in the IncrVT group at 10 and 15 min of life. Impaired oxygenation in the IncrVT group is explained by less uniform aeration. The pressure applied to the respiratory system at birth drives the transition from a fluid-filled to aerated state: the major airways are aerated first with progressively aeration of the respiratory tree until, finally, the distal and more dependent lung units, being the last, are aerated (13). Our data suggest that the initial lower inflating pressures in the IncrVT group were inadequate to facilitate aeration, especially in the dependent units. These initial events likely created the volume history that persisted throughout the study and emphasize the need to develop clinical strategies at birth that consider lung recruitment in a regional context.

PEEP is essential for optimal lung volume, maintaining EEV, and improving tidal mechanics (8). The role of PEEP as a lung-protective mechanism at birth is well accepted (23, 27, 28, 31, 36), but the optimal PEEP and how to achieve it remain unclear. Clinical trials of PEEP at birth are lacking. We chose
a PEEP of 5 cmH₂O, based on previous experience with our mode (12, 24) and clinical translation (38). Although there was no significant difference in regional EEV at 15 min of life, there was a pattern indicative of time-based gravity-dependent derecruitment in the PressSI group, suggesting that 5 cmH₂O might have been inadequate in the surfactant-deficient preterm lamb. In earlier preterm lamb studies, we showed that transient exposure to high PEEP during a stepwise PEEP open-lung approach at birth, with associated lower VT, resulted in sustained benefits in oxygenation and lung mechanics (36). The differences seen in the IncrVT group may be mitigated through the application of higher PEEP than used in this study. A higher PEEP may be particularly important for recruitment of the gravity-dependent lung regions that have higher opening pressures (11).

The 30-s SI quickly resulted in uniform attainment of EEV, with the least regional differences. Nonetheless, similar to other preterm animal model studies (26, 34), the initial benefits in lung volume were not sustained compared with IMV alone, using a lung protective strategy with PEEP. Thus augmenting initial lung aeration is simply the first step in any respiratory support strategy for the preterm lung. Ongoing attention to lung recruitment and/or retention of lung volume remains essential. Clinical guidelines provid-

Table 2. Markers of lung injury

<table>
<thead>
<tr>
<th></th>
<th>PressSI</th>
<th>7 ml/kg V̇T</th>
<th>IncrVT</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Total protein in</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL, µg/ml</td>
<td>1708 (989)</td>
<td>966 (540)</td>
<td>1755 (998)</td>
<td>0.154</td>
</tr>
<tr>
<td>EGR1</td>
<td>1.4 (0.7, 2.2)</td>
<td>1.0 (0.4, 1.3)</td>
<td>1.0 (0.5,1.3)</td>
<td>0.415</td>
</tr>
<tr>
<td>CTR61</td>
<td>1.1 (0.7, 2.1)</td>
<td>1.0 (0.6, 1.7)</td>
<td>0.9 (0.6,1.2)</td>
<td>0.811</td>
</tr>
<tr>
<td>CTGF</td>
<td>0.7 (0.4, 1.6)</td>
<td>1.0 (0.5, 1.4)</td>
<td>0.9 (0.4, 1.1)</td>
<td>0.854</td>
</tr>
</tbody>
</table>

Protein data values are means (SD), and mRNA values are median (with 25th to 75th percentile in parentheses). BAL, bronchoalveolar lavage fluid; EGR1, early growth response protein 1; CTR61, cysteine-rich 61; CTGF, connective tissue growth factor relative mRNA expression. mRNA expression is shown as means (SD) expressed relative to the PressSI group. Analysis is by one-way ANOVA on ranks.
ing advice on lung recruitment after initial transition to ex utero life are not established.

Despite the improved oxygenation at 15 min in the PressSI and 7 ml/kg Vt groups, there was no difference in the measured markers of lung injury at that time. These data are interpreted with caution, as we restricted analysis to mRNA expression of known early markers of lung injury due to the short study duration. EGR1, CYR61, and CTGF are upregulated by at least 30 min in the injured lamb lung (39). Regional injury analysis may have provided additional information, given the observed regional differences in ventilation.

Our study describes the relative regional, or spatial, behavior of tidal ventilation during transition to ex utero life. Interestingly, the PressSI group demonstrated the least homogeneous pattern of tidal ventilation at 1 min, despite relatively uniform regional EEV pattern. The spatial pattern of tidal ventilation in the PressSI group distributed more uniformly with time. In contrast, there was minimal change in the spatial distribution of Vt over time in the 7 ml/kg Vt and IncrVt groups. As EIT measures relative volume patterns (8, 18), we cannot conclude that a safer volume state existed in the lung using these strategies: rather, all lung regions were behaving similarly, irrespective of volume state. Based on these data, and the EEV, oscillatory mechanics, and oxygenation data, the absence of altered spatial pattern of Vt over time is most likely due to persistent atelectasis in the IncrVt group. In contrast, uniform distribution of tidal ventilation in the 7 ml/kg Vt strategy likely results from optimal tidal ventilation throughout the lung, without the risk of transient initial overdistension.

This is the first study to consider a gentle, incremental approach to tidal ventilation at birth. The intention is to minimize excessive volume exposure to the lungs during aeration. It is arguable that the IncrVt is closest to current, albeit unintentional, clinical practice. Applied Vt is often very low and highly variable during resuscitation of preterm infants (30). The IncrVt strategy resulted in persistent differences in regional EEV, lower oxygenation, and likely greater atelectasis. Nonetheless, all other measures, particularly the early markers of lung injury, were similar to the other groups by 15 min. Our findings suggest that these inadvertent transient periods of low applied Vt at birth, while not optimal and ideally avoided, may not be significantly harmful.

Our study has a number of limitations. The preterm lamb is an established model of surfactant-deficient respiratory distress syndrome. However, unlike routine clinical practice, study lambs were intubated with cuffed tracheal tubes before birth and had spontaneous breathing suppressed to facilitate accurate delivery of the different resuscitation protocols and limit confounding between the groups. Our results are also primarily relevant to the preterm infant born without antenatal glucocorticoid exposure, as ewes did not receive antenatal steroids to avoid damping of the inflammatory responses. Like other animal studies of aeration at birth (33, 34), our study duration was short. This was to isolate the early inflammatory responses to initial resuscitation from the confounding injury associated with ongoing ventilation. This limits the use of other methods of injury analysis, such as histology, which may require longer than 15 min for meaningful inflammatory markers to be present. Consequently, we are unable to comment on the implications of each strategy for long-term lung, airway, and other organ injury, although increasing evidence of long-term injury from inappropriate resuscitation at birth exists (5, 12, 37, 39). Finally, measuring lung volumes, both globally and regionally, during IMV is difficult, especially at birth: the limitations of EIT in this setting are well described (18). Although the calibration of EIT signals to known volume measurements was validated (1, 22), we only calibrated the global signal (19) and limited our interpretation of regional volumetric behavior to relative rather than absolute changes.

Conclusions. In preterm lambs, the use of a gradual increase in Vt from birth resulted in less recruitment of the gravity-dependent lung and resultant poorer oxygenation at 15 min of life, but no substantial differences in lung volumes, mechanics, injury, and spatial distribution compared with similar IMV at a constant Vt of 7 ml/kg, with or without an initial SI. The use of a SI before IMV with a tidal ventilation of 7 ml/kg did not confer any benefit to regional ventilation distribution and suggests that the cornerstone of optimal respiratory support of the preterm lung at birth remains adequate tidal ventilation and PEEP.

REFERENCES


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