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Alveolar Recruitment Promotes Homogeneous Surfactant Distribution in a Piglet Model of Lung Injury

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ABSTRACT

Uneven distribution of exogenous surfactant contributes to a poor clinical response in animal models of respiratory distress syndrome. Alveolar recruitment at the time of surfactant administration may lead to more homogeneous distribution within the lungs and result in a superior clinical response. To investigate the effects of three different volume recruitment maneuvers on gas exchange, lung function, and homogeneity of surfactant distribution, we studied 35 newborn piglets made surfactant deficient by repeated airway lavage with warm saline. Volume recruitment was achieved by either a temporal increase in tidal volume or an increase in end-expiratory pressure during surfactant administration, yielding an increase in dynamic compliance of the respiratory system of 77% in the first group and an increase in functional residual capacity of 108% in the second group. A third group of piglets (all $n = 7$) received a combination of both volume recruitment maneuvers, with increases in dynamic compliance of the respiratory system of 100% and in functional residual capacity of 192%. Those animals subjected to increased tidal volume showed an improved surfactant response in terms of oxygenation, ventilation, lung volumes, lung mechanics, and homogeneity of surfactant distribution. Increased end-expiratory volume augmented the surfactant effect only to some extent. The combination of both volume recruitment maneuvers, however, needed lung volumes beyond total lung capacity (approximately 56 mL/kg), thus probably inducing early sequelae of ventilator-induced lung injury. We conclude that volume recruitment by means of increased tidal volumes at the time of surfactant administration leads to a superior surfactant effect owing to more homogeneous surfactant distribution within a collapsed lung. **(*Pediatr Res* 50: 34–43, 2001)**

Abbreviations

C_{RS}, dynamic compliance of the respiratory system
ETT, endotracheal tube
Fio_2, fraction of inspired oxygen
FRC, functional residual capacity
$Paco_2$, arterial P_{CO_2}
Pao_2, arterial P_{O_2}
P_{AO_2}, alveolar P_{O_2}
PEEP, positive end-expiratory pressure
RDS, respiratory distress syndrome
TLC, total lung capacity
V_A, alveolar volume
V_T, tidal volume
VILI, ventilator-induced lung injury
VRM, volume recruitment maneuver

Surfactant treatment has reduced mortality and morbidity in infants with RDS (1). However, detrimental factors affecting response to therapy are numerous, such as uneven distribution of exogenous surfactant, insufficient dosage, inability of exogenous surfactant to enter the metabolic pathways, inhibition of surface activity by plasma-derived proteins, or respiratory failure caused by factors other than surfactant deficiency (2). Two clinical studies (3, 4) have identified patent ductus arteriosus, gas accumulation outside the alveolar level, and pulmonary infection as the main reasons for nonresponse in neonates with RDS, leading to an increased mortality as high as 40% of affected infants. Poor response, however, is thought to be predominantly caused by uneven surfactant distribution. Animal studies have shown that the distribution of exogenous surfactant is often nonuniform, and that nonuniform distribution patterns are associated with poor clinical response (5–7).

The immediate increase in FRC after surfactant administration to infants with RDS (8–10) can be considered to be the result of two mechanisms: stabilization of previously collapsing alveoli at end-expiration, and stabilization of alveoli already being ventilated at higher end-expiratory volumes. When

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end-inspiratory and end-expiratory airway pressures are held constant during mechanical ventilation, stabilization without recruitment results in a decrease in lung compliance measured between the two pressures whereas recruitment of previously unventilated alveoli increases compliance (11). The lack of improvement in compliance immediately after surfactant treatment in infants with RDS (8–10, 12, 13) at a time when gas exchange and lung volume have already improved suggests the possibility that a substantial compartment of unventilated alveoli still exists. If so, mechanical recruitment of unventilated alveoli accompanying surfactant administration might lead to a superior clinical response of exogenous surfactant in terms of gas exchange and lung function owing to a more homogeneous pattern of distribution.

To test this hypothesis, three different VRMs were evaluated in newborn piglets made surfactant deficient by repeated airway lavage. The first maneuver used increased tidal volumes in an attempt to recruit previously unventilated alveoli, the second used increased PEEP to achieve higher end-expiratory volumes, and the third consisted of a combination of both maneuvers. We expected an improved lung compliance by the first maneuver and an increased FRC by the second maneuver promoting a more homogeneous surfactant distribution when compared with surfactant administration without alveolar recruitment. In a second step we tried to match improved gas exchange and lung function with a more homogeneous pattern of surfactant distribution at the alveolar level by the use of a new staining technique for exogenous surfactant.

MATERIALS AND METHODS

Animal preparation. The experimental protocol was approved by the local Review Board for the Care of Animal Subjects in accordance with the German law for animal protection and the European Community guidelines (86/609/EC).

Thirty-five piglets (mixed country breed) of either sex from five litters were studied between day 2 and 14 of age, weighing 2.4 ± 0.4 kg (range, 1.7–4.3 kg). Initially 0.05 mg/kg atropine, 10 mg/kg ketamine, and 1 mg/kg midazolam hydrochloride were administered intramuscularly to provide anesthesia. Oral intubation with an uncuffed 3.0-mm inner diameter ETT was performed after the administration of 2 mg/kg propofol i.v. *via* a catheter in an ear vein. Anesthesia and muscle paralysis were maintained by continuous i.v. infusion of 15 mg/kg propofol and 0.2 mg/kg pancuronium bromide per hour throughout the study period. To prevent leakage the ETT was tightly secured in place by a peritracheal ligature. A polyvinyl catheter was inserted into the left common carotid artery for monitoring of arterial blood pressure and arterial blood gases. The right ventricle was catheterized *via* a catheter inserted into the right external jugular vein to obtain mixed venous blood samples and monitoring of right ventricular blood pressure.

Mechanical ventilation and airway lavage. Mechanical ventilation was provided by two time-cycled pressure-limited infant ventilators (Babylog 1, Dräger, Germany). The following ventilator settings were used: Fio_2, 0.6; PEEP, 0.4 kPa; flow, 8 L/min; inspiratory time, 0.4 s; and ventilator rate, 35 breaths/min before lavage and 60 breaths/min during and immediately after lavage. PIP was adjusted to keep tidal volume (V_T) at 8 mL/kg.

We used a modification of the original lavage protocol described by Lachmann *et al.* (14). Each lavage involved the instillation and removal of 35 mL/kg of warmed normal saline *via* the ETT carried out over a 30-s period. After the first lavage, PEEP was increased to 0.8 kPa and the ventilator rate to 60 breaths/min to ensure adequate oxygenation and ventilation and to hasten surfactant removal and alveolar collapse (15). The first six lavages were performed in changing side positions. Airway lavage was repeated every 3–6 min until both the Pao_2 decreased to 5.3–6.6 kPa, and a minimum PIP of >1.8 kPa was required to maintain V_T at 8 mL/kg. Twenty minutes later two more lavages were performed to remove surfactant released into the alveoli by the sympathetic stimulus from the lavage procedure (16, 17). Before baseline measurements the original PEEP level of 0.4 kPa was reestablished following a modified protocol by Dijk *et al.* (18).

Loss of lavage fluid within the airways was noted for comparability of study animals among groups. In addition, the lavage fluid was centrifuged at 2500 rpm for 10 min to determine the amount of alveolar wash when separated from saline and cellular debris.

Experimental protocol. Measurements of gas exchange (Pao_2 and $Paco_2$), lung function indices (FRC, V_A, V_T, and C_{RS}) and hemodynamic variables (heart rate, systemic and right ventricular blood pressures, and venous admixture) were made before airway lavage and after airway lavage at baseline and at 5, 30, 60, 90, and 120 min after intervention (*i.e.* surfactant administration with or without a VRM). Ventilator rate was maintained at 60 breaths/min until completion of the surfactant administration protocol and was then switched back to 35 breaths/min throughout the remainder of the study. In contrast, animals of the control group continued being ventilated at 60 breaths/min.

At baseline the animals were randomized to one of the following protocols (all $n = 7$): a control group (Control) receiving 5.5 mL/kg of air into a second lumen of the ETT; a group (Surf) receiving 5.5 mL/kg surfactant only (this group was serving as the true control group for statistical analysis); a group (V_T16) receiving 5.5 mL/kg surfactant concomitantly with an increased V_T of 16 mL/kg (intervention group 1); a group (P8) receiving 5.5 mL/kg surfactant concomitantly with an increased PEEP of 0.8 kPa (intervention group 2); and a group (V_T16+P8) receiving 5.5 mL/kg surfactant concomitantly with an increased V_T of 16 mL/kg and an increased PEEP of 0.8 kPa (intervention group 3).

The VRMs were applied for 5 min before surfactant administration, during surfactant administration (2 min), and for another 5 min after surfactant administration. After this period of 12 min, the ventilator rate was switched back to 35 breaths/min as was done in the Surf group immediately after surfactant administration. V_T was then allowed to vary along with increases or decreases in C_{RS} after surfactant administration.

Surfactant preparation and administration. The surfactant preparation used in this study was Curosurf (Serono, Unterschleißheim, Germany), an organic solvent of pig lung purified by chromatography containing phospholipids B and C, at a

concentration of 40 mg/mL after dilution with equal amounts of normal saline to promote its physiologic response (6). A diluted green histology dye (Green Tissue Marking Dye, WAK-Chemie Medical, Bad Soden, Germany) was mixed with the surfactant preparation, adding an additional 1/10 volume to the surfactant preparation, and hand-stirred for 5 min. The original dye preparation was diluted 1:10 with normal saline before adding to surfactant as previously described (19). All animals of the intervention groups received 200 mg/kg Curosurf equivalent to a volume of 5.5 mL/kg *via* a second lumen of the ETT within 2 min (20) regardless of the absolute amount of fluid administered and without interrupting mechanical ventilation.

Measurement of gas exchange, hemodynamics, and lung function indices. Pa_{O_2} and Pa_{CO_2} were measured from blood samples taken from the carotid arterial catheter.

Intrapulmonary right-to-left shunt (venous admixture) was calculated by Fick equation $[(Ca_{O_2} - Cc_{O_2})/(Cv_{O_2} - Cc_{O_2})]$. Pa_{O_2} and P_{CO_2} were measured from arterial and mixed venous blood samples taken from the carotid arterial catheter and the right ventricular catheter, respectively, and used to calculate arterial (Ca_{O_2}) and mixed venous (Cv_{O_2}) oxygen contents. Alveolar capillary O_2 content (Cc_{O_2}) was calculated by using P_{AO_2} from the alveolar gas equation with $P_{AO_2} = [(P_B - 6.25) \times Fi_{O_2} - Pa_{CO_2}]$, where P_B is barometric pressure. Blood gases were analyzed using an ABL 500 (Radiometer, Copenhagen, Denmark), and oxygen saturation was measured with a hemoximeter (OSM3, Radiometer, Copenhagen, Denmark), which adjusts oxygen saturation to the specific Hb of pigs.

To calculate FRC, V_A, V_T, and C_{RS}, we used the lung function technique described by Sjöqvist *et al.* (21, 22) and Edberg *et al.* (23). The airflow signal was derived from a differential pressure transducer (Dr. Fenyves und Gut, Hechingen, Germany) and a Fleisch No. 00 pneumotachometer (Fleisch, Lausanne, Switzerland) attached to the ETT connector.

FRC and V_A were measured using a multiple-breath nitrogen washout technique (22) with a model 721 Nitralyzer (KaeTech Instruments, Green Bay, WI, U.S.A.) to measure nitrogen concentration in respiratory gas mixtures. C_{RS} was estimated by the least squares method by fitting airflow and V_T signals to proximal airway pressure using the standard equation of motion (21–23).

Airflow, pressure, and nitrogen signals used for measuring lung function indices were sampled at a rate of 200 Hz, digitized, and stored in a personal computer for subsequent analysis. Data acquisition and analysis was done using a lung function analysis software by Ants R. Silberberg from Chalmers University of Technology, Gothenburg, Sweden, also using the software program Matlab 5.3 (The MathWorks Inc., Natick, MA, U.S.A.) for data analysis.

Clinical care. A heating pad was used to maintain a constant core temperature of the piglets between 38° and 39°C, as measured by a rectal probe. Each piglet received an infusion of 10% dextrose in water at 5.5 $mg \cdot kg^{-1} \cdot min^{-1}$ glucose and a fluid intake of 80 $mL \cdot kg^{-1} \cdot d^{-1}$. The pressure transducers were flushed with normal saline containing 2 IU heparin/mL.

Volume-pressure curves. At the end of the clinical study period, at 120 min after surfactant administration, the lungs of the animals were degassed by applying an Fi_{O_2} of 1.0 for 3 min before clamping the ETT and discontinuing mechanical ventilation; the animals were in deep narcosis with propofol. After 3 min, the animals were killed with an overdose of 1 M KCl. Quasi-static volume-pressure curves were obtained by manual inflation with calibrated syringes in pressure steps of 0.66 kPa up to a maximum of 3.3 kPa and by withdrawing volume in discrete steps of 0.66 kPa back to atmospheric airway pressure as described by Venegas *et al.* (24). Comparisons of the three groups were made at opening pressure of 1.33 kPa as the lungs were inflated, at maximal lung volume (3.3 kPa), and at a point that reflects deflation stability at 0.66 kPa as lungs were deflated, according to Jobe (25).

Histologic processing. After tying off the trachea to prevent efflux of stained surfactant from the airways into the fixation fluid, the lungs were removed from the thorax and put into 4% formaldehyde for several days. Ten cuts of lung at a width of 3 μm were taken from central and peripheral locations of each lobe and mounted on slides for fixation. All slides were stained with hematoxylin and eosin using standard methods. A score was established [0–5 points for each index (we also used steps of 0.5 points): 0, not at all; 1, very little; 2, little; 3, moderate; 4, moderate to severe; 5, severe] describing emphysema and atelectasis; edema; infiltration with polymorphonuclear leukocytes and lymphocytes; invasion with macrophages, giant macrophages, and erythroblasts; bleeding; and hyaline membranes. Distribution of the stained surfactant within the lung was assessed by another score (0–5 points: from 0 indicating very inhomogeneous to 5 indicating very homogeneous) differentiating between right upper lobe, right middle lobe, right lower lobe, left upper lobe, and left lower lobe. Photographs were taken to demonstrate morphologic changes and the pattern of surfactant distribution at the alveolar level.

Statistical methods. To establish comparability of the five experimental groups before surfactant administration, we used a Kruskal-Wallis test to assess differences in the number of lavages, loss of lavage fluid, and alveolar wash (Table 1). The indices of gas exchange (Pa_{O_2}, Pa_{CO_2}), venous admixture, and the four indices of lung function (FRC, V_A, V_T, and C_{RS}) after lavage (at baseline) were compared using a one-way ANOVA. Efficacy of volume recruitment (Table 2) was assessed using a paired *t* test. Repeated measures ANOVA with Student-Neuman-Keuls *post hoc* testing was used to assess differences

Table 1. *Comparability of experimental groups: number of lavages, loss of lavage fluid, and amount of alveolar wash*

Variable	Control	Surf	V_T16	P8	V_T16 + P8	*p*
Number of lavages	10.4 ± 2.7	18.4 ± 6.3	15.4 ± 5.1	17.0 ± 9.3	15.7 ± 4.6	0.12
Loss of lavage fluid (%)	10.1 ± 2.0	6.8 ± 2.6	7.5 ± 2.5	8.2 ± 3.8	6.5 ± 1.7	0.11
Alveolar wash (mg)	373 ± 205	912 ± 517	841 ± 317	712 ± 380	822 ± 311	0.07

$n = 7$ for all groups.

Table 2. *Impact of VRM (by increased PEEP or PIP) on FRC, V_A and C_{RS}*

Variable	VT16	P8	VT16 + P8
PEEP (kPa)	0.4	0.8	0.8
PIP (kPa)			
At baseline	2.20 ± 0.15	2.15 ± 0.19	2.25 ± 0.23
Volume recruitment	2.94 ± 0.34***	2.38 ± 0.13**	3.37 ± 0.22***
Delta	0.74 ± 0.25	0.22 ± 0.12	1.11 ± 0.25
FRC (mL/kg)			
At baseline†	13.2 ± 3.4	13.4 ± 4.2	13.7 ± 3.4
Volume recruitment	23.9 ± 7.8***	28.0 ± 10.4***	40.1 ± 8.4****
Delta	10.6 ± 5.6	14.5 ± 6.5	26.4 ± 6.9
V_A (mL/kg)			
At baseline†	1.7 ± 0.4	1.6 ± 0.7	1.8 ± 0.5
Volume recruitment	4.2 ± 1.5**	1.7 ± 0.3	5.9 ± 1.4****
Delta	2.5 ± 1.5	0.1 ± 0.4	4.2 ± 1.3
C_{RS} ($mL \cdot kPa^{-1} \cdot kg^{-1}$)			
At baseline†	8.0 ± 1.3	7.1 ± 1.3	6.8 ± 0.8
Volume recruitment	14.2 ± 1.6****	9.0 ± 1.5***	13.6 ± 1.5****
Delta	6.2 ± 1.7	1.8 ± 0.6	6.7 ± 1.4

** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ (paired t test).

† There were no significantly different results for FRC, V_A, and C_{RS} at baseline among groups.

among groups in change over time for the seven indices (Figs. 1 and 2). For missing values a one-way ANOVA was also used to assess differences in pressure-volume curves (Fig. 3) among groups at 1.33 kPa (opening pressure), 3.3 kPa (maximal lung volume), and 0.66 kPa (deflation stability). A Friedman test was used to evaluate differences in histology score and surfactant distribution (Figs. 4 and 5), followed by Dunn's multiple comparison test in case of significant differences. Comparison of surfactant distribution among groups summarizing the scores of all five lung lobes (Fig. 5, "All lobes") were assessed using a Wilcoxon rank sum test.

Mean values ± SD are expressed unless specified otherwise. Histology scores are displayed as box and whisker plots. Significant differences among groups were assumed to be present at values of $p < 0.05$.

RESULTS

Comparability of study groups. Thirty-five piglets from five litters at an age of 5.9 ± 3.3 d (range, 2–14 d) and weighing 2.4 ± 0.4 kg (range, 1.7–4.3 kg) were consecutively studied after randomization at the completion of airway lavage. [Five more piglets were studied that died before randomization because of hypoxia and acidosis ($n = 2$), right atrial catheter perforation ($n = 2$), and congenital heart disease with severe pulmonary hypertension ($n = 1$)]. Table 1 displays some differences, although statistically insignificant, in the number of lavages used and the amount of alveolar wash gathered from airway lavage. The control group animals (stemming from two litters only in accord with the randomization protocol; differences among litters probably because of study animals not from inbred species) needed fewer lavages to achieve a Pa_{O_2} between 5.3 and 6.6 kPa, also yielding less alveolar wash than piglets of the other groups.

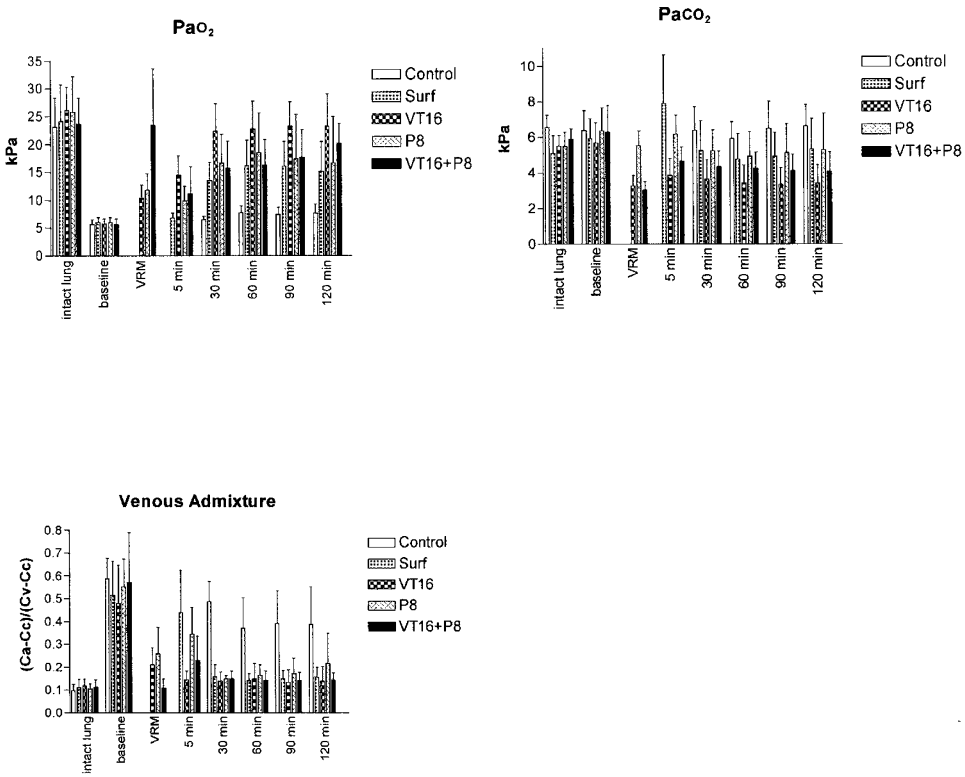

Figure 1. Pa_{O_2}, Pa_{CO_2}, and venous admixture before and after lavage, during a VRM, and over time for a study period of 120 minutes. Control group not included for statistical assessment (repeated measures ANOVA). Pa_{O_2} and Pa_{CO_2} showed highly significant differences among groups (both $p < 0.0001$); venous admixture was not different ($p = 0.18$). Student-Newman-Keuls *post hoc* testing revealed significant differences among the three groups receiving a VRM in favor of the VT16 group, yielding superior results when compared with P8 and VT16+P8 for Pa_{O_2} and Pa_{CO_2}.

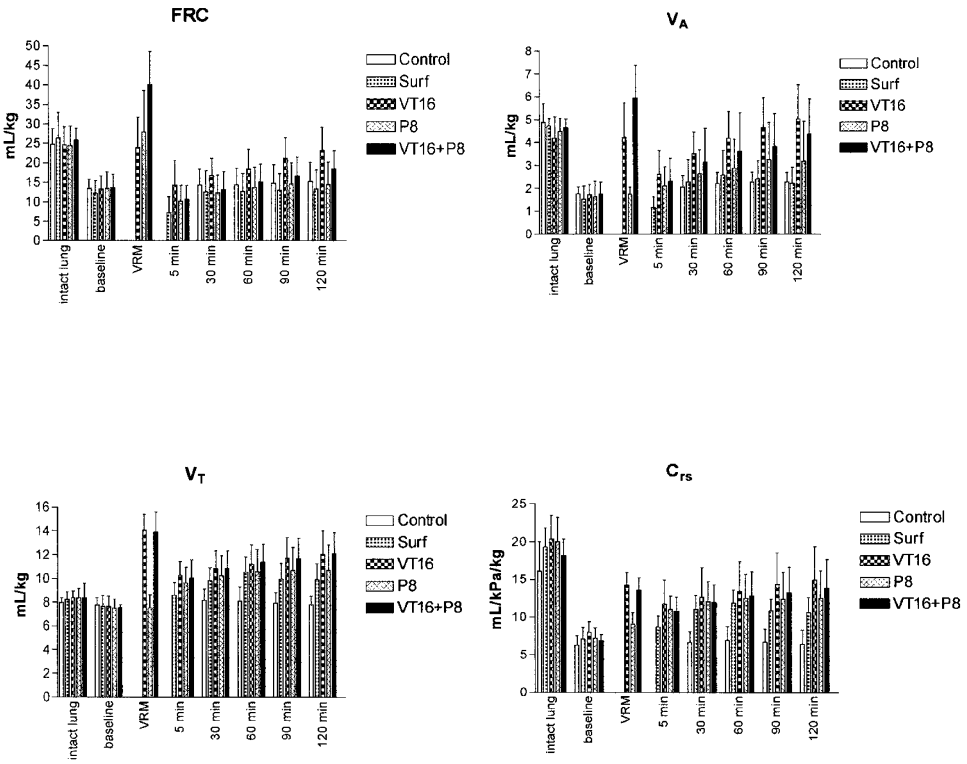

Figure 2. Four indices of lung function (FRC, V_A, V_T, and C_{RS}) before and after lavage, during a VRM, and over time for a study period of 120 minutes. Control group not included for statistical assessment (repeated measures ANOVA). FRC, V_A, V_T, and C_{RS} showed highly significant differences among groups (all $p < 0.0001$). Student-Newman-Keuls *post hoc* testing revealed significant differences among the three groups receiving a VRM in favor of the V_T16 group, yielding superior results when compared with P8 and V_T16+P8 for FRC, V_A, V_T (only V_T16+P8 *vs* P8), and C_{RS}.

At baseline (completion of airway lavage) there were no statistically significant differences among the five groups for Pa_{O_2}, Pa_{CO_2}, venous admixture, FRC, V_A, V_T, and C_{RS}.

VRMs. There were no significantly different results for FRC, V_A, and C_{RS} at baseline for the three intervention groups subjected to VRMs (Table 2). The increase in FRC was most pronounced in the V_T16+P8 group, yielding exactly the sum of both increases from the V_T16 and P8 intervention groups. Significant increases in V_A were noted in the V_T16 and V_T16+P8 groups also, demonstrating effective alveolar recruitment by the increase in V_T.

Change in gas exchange, hemodynamics, and lung function indices over time. Figures 1 and 2 display seven variables of gas exchange, hemodynamics, and lung function during a study period of 120 min. With the exception of venous admixture, all variables were significantly improved by means of VRMs. V_T16 emerged as being superior to the P8 or V_T16+P8 VRMs, showing significant differences in all variables except for venous admixture.

Quasi-static volume-pressure curve. In contrast to the results from C_{RS}, postmortem distensibility of the respiratory system (Fig. 3) seemed to be better in the Surf than in the P8 group. However, the results were based only on three of seven animals in the Control group, four of seven animals in the Surf group, and five of seven animals in the P8 group. Only in the V_T16 and the V_T16+P8 groups could data be obtained from all seven animals. Differences among groups would probably have been more pronounced considering that the lungs of the sickest animals in each group experienced pneumothoraces leading to exclusion from statistical analysis.

Histology score and surfactant distribution. Figure 4 displays the impact of surfactant and VRM on six histology indices that were evaluated by light microscopy of central and peripheral cuts from each of the five lobes of the lungs. Among those groups receiving surfactant, a trend toward increased leakage of proteinaceous fluid into the airways as indicated by edema and hyaline membranes was noted in the P8 and V_T16+P8 groups (Fig. 4). The V_T16+P8 group regularly showed desquamated and dissolved epithelia within the small bronchi and alveoli as well as small bleeding areas (Fig. 6). Moreover, peripheral emphysema could most often be found especially in the right lower lobe and the left upper lobe. Most striking was the amount of macrophages in the P8 group also regularly containing giant macrophages in the alveoli. This group was also characterized by extensive diffuse and subpleural emphysema (Fig. 7).

On macroscopic examination (Fig. 8) surfactant distribution at the subpleural level was most inhomogeneous in the Surf group. Homogeneous surfactant distribution (Fig. 9) could most often be found in the V_T16 group, occasionally even extending to the subpleural spaces. Differences in surfactant deposits among lobuli were least in this group. In contrast surfactant was generally found as little clusters in the small bronchi of the Surf group, thus preventing surfactant from entering into the alveoli (Fig. 10). Failure to recruit atelectatic areas resulted in considerably less surfactant deposition to the

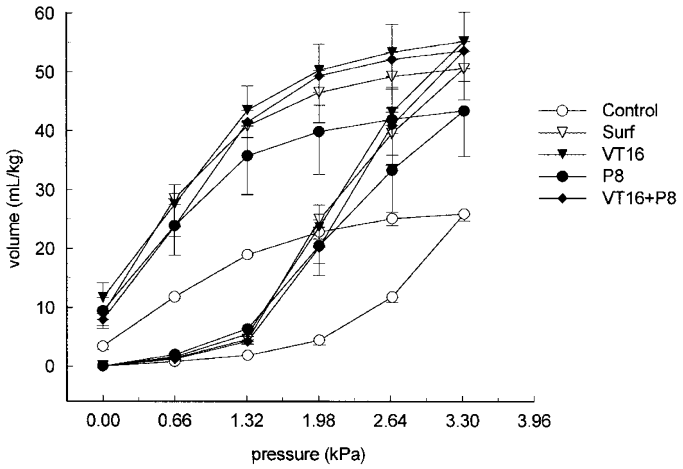

Figure 3. Quasi-static pressure-volume curves were assessed immediately postmortem with the lungs remaining *in situ*. Means ±SEM. There were no statistically differences (one-way ANOVA) among the four groups receiving surfactant at 1.32 kPa up (opening pressure), 3.3 kPa (maximal lung volume), and 0.66 kPa down (deflation stability).

collapsed lobuli. Lung specimens from the control group also showed erythroblasts in addition to macrophages and abundant polymorphonuclear granulocytes.

Stained surfactant deposits did not promote additional attraction of cells within the alveoli when compared with the control group (see also Fig. 4, "infiltration and invasion").

Figure 5 displays an overview of the homogeneity of stained surfactant distribution in all five lung lobes. Summarizing the scores of the five lobes in "All lobes" one can see a significant difference between the Surf group and those groups receiving a VRM while given surfactant (except Surf *versus* P8, $p = 0.05$).

DISCUSSION

This study shows that recruitment of previously unventilated alveoli at the time of surfactant administration leads to a superior response of exogenous surfactant in the saline-lavaged lungs of newborn piglets, in terms of gas exchange, lung function, and surfactant distribution at the alveolar level. Volume recruitment was proven by improved C_{RS} and increased FRC in all three intervention groups (Table 2). In contrast augmentation of V_A was only seen along with increased V_T (V_T16 and V_T16+P8 groups). If increased V_T had only resulted in further inspiratory distention of terminal airways already being ventilated, C_{RS} would have remained the same or decreased. The increase in C_{RS}, however, reflected the existence of a considerable compartment of alveoli not being ventilated after airway lavage. Recruitment of unventilated alveoli by the use of increased V_T promoted the exogenous surfactant effect more than higher end-expiratory volumes by means of increased PEEP. The combination of both recruitment maneuvers, however, showed no synergistic effects in terms of improvement in gas exchange and lung function. Increased alveolar ventilation at the end of the experiment (Fig. 2, observation at 120 min) was linked with a more homogeneous surfactant distribution within the lungs as proven by light microscopy (Fig. 5).

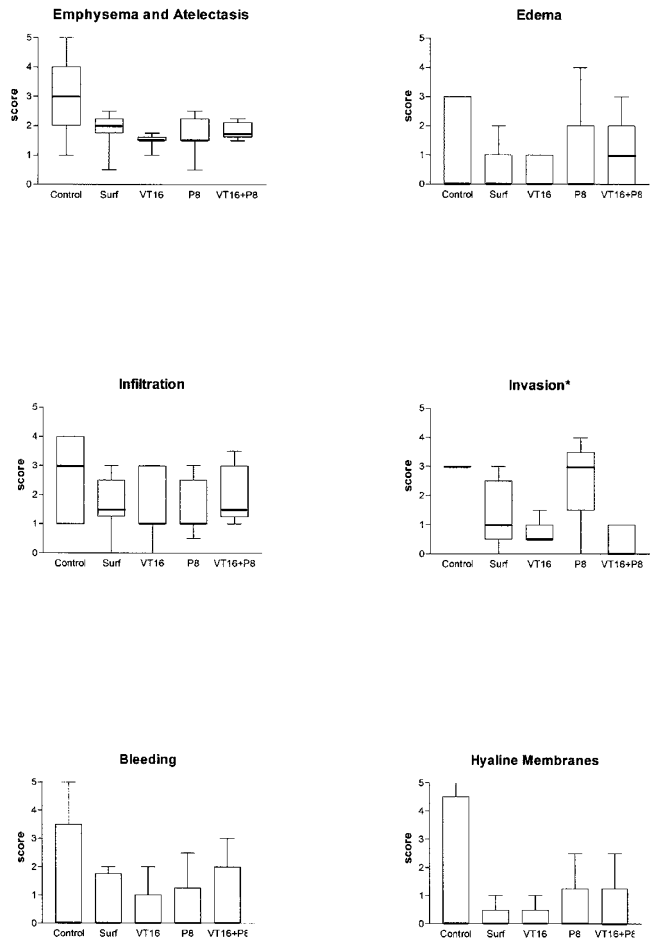

Figure 4. Box and whisker plots (boxes extending from the 25th to the 75th percentiles; median, fat line; whiskers showing the highest and lowest values) describing the following histology scores: 0, not at all; 1, very little; 2, little; 3, moderate; 4, moderate to severe; 5, severe (we also used steps by 0.5 points). Edema was essentially present only within the alveolar septa. Infiltration refers to the amount of polymorphonuclear leukocytes and lymphocytes in the interstitium, invasion to the presence of macrophages, giant macrophages, and erythroblasts in the lung interstitium or alveoli. To assess differences among intervention groups, the control group was not included for statistical analysis (Friedman test). *$p < 0.05$; Dunn's multiple comparison test also showing a difference between P8 and V_T16+P8 ($p < 0.05$).

Alveolar instability refers to the tendency for an alveolus to switch abruptly between the inflated state and the collapsed state. When unstable alveoli have been stabilized by surfactant repletion they deflate progressively without collapse so that they retain gas volume at end-expiratory pressures below their critical closing pressure (26). The lung model on which our hypothesis is based (11) assumes the presence of three compartments that differ in ventilation and stability: *1*) a compartment of alveoli that are not ventilated, *2*) a compartment of alveoli that are ventilated, but are unstable and collapse during expiration, and *3*) a compartment of alveoli that are ventilated and do not collapse during expiration. Inhomogeneity of ventilation has been demonstrated by indicator gas washout analysis in premature lambs (27) and in premature infants with RDS (28) and is normalized by surfactant treatment. Studies with premature lambs (5, 29) have shown that surfactant distribution is correlated with the distribution of ventilation.

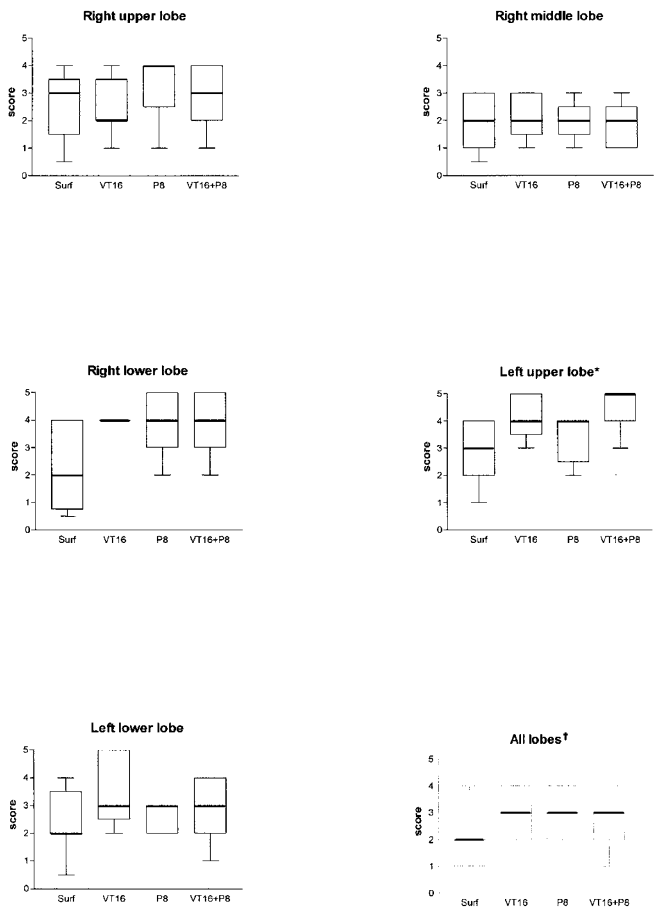

Figure 5. Box and whisker plots (boxes extending from the 25th to the 75th percentiles; median, fat line; whiskers showing the highest and lowest values) describing the following score in reference to the distribution of stained exogenous surfactant to the five lobes: 0–5 points from 0 indicating very inhomogeneous to 5 indicating very homogeneous (we also used steps by 0.5 points; Friedman test). *$p < 0.05$; Dunn's multiple comparison test also showing a difference between P8 and VT16+P8 ($p < 0.05$). "All lobes" summarizes the scores of all five lobes. †Comparisons among the Surf group and those groups receiving VRM along with surfactant administration yielded the following results: Surf *vs* VT16, $p < 0.01$; Surf *vs* P8, $p = 0.05$; Surf *vs* VT16+P8, $p < 0.05$ (Wilcoxon rank sum test).

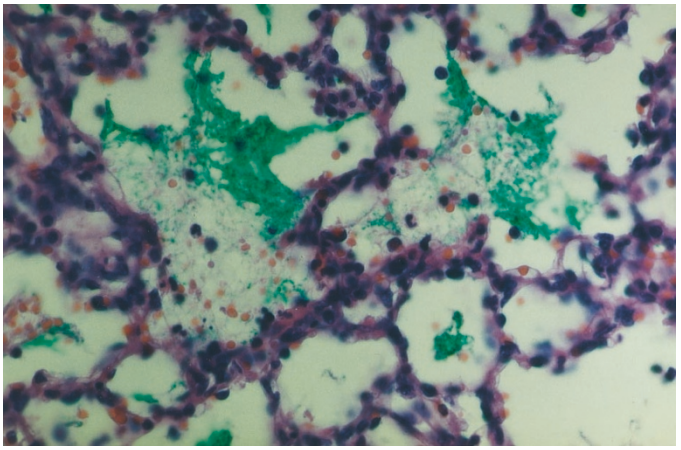

Figure 6. Proteinaceous alveolar edema stained with exogenous surfactant and some hyaline membranes in alveoli with minor intraalveolar (and interstitial) bleeding, desquamation of some pneumocytes (VT16+P8 group). Hematoxylin and eosin stain, oil ×1200.

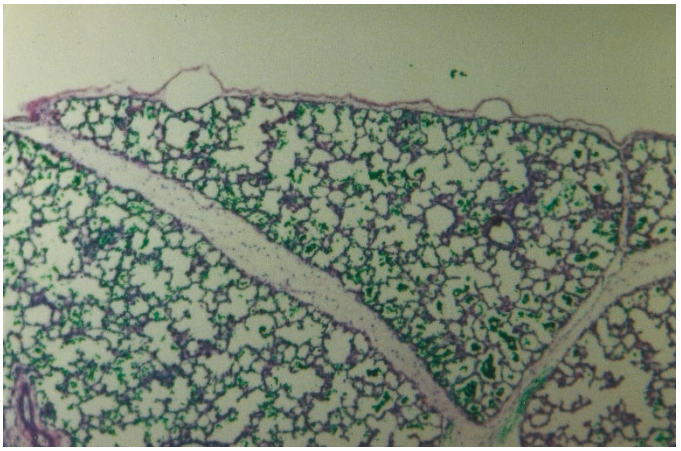

Figure 7. Subpleural emphysema and areas of alveolar overdistention in a lung demonstrating homogeneous surfactant distribution at the alveolar level (P8 group). Hematoxylin and eosin stain, ×300.

From these observations, it is reasonable to expect that recruitment of previously unventilated alveoli at the time of surfactant administration would facilitate the distribution of surfactant to this compartment.

The use of VRMs after surfactant administration apparently belongs to empirically based clinical practice (*e.g.* Survanta package insert) in premature infants with RDS to overcome alveolar hypoventilation and has been previously proven effective (increased VT) in an adult rabbit model (30). In contrast, the use of increased PEEP to augment end-expiratory lung volume at the time of surfactant administration does not belong to clinical routine.

All three VRMs positively influenced surfactant distribution when compared with the Surf group. The right upper and middle lobes were least influenced by VRM even though VRM in general promoted surfactant advancement from terminal bronchioli to the alveoli. Exogenous surfactant deposits to the subpleural areas was least in the small upper lobes of the piglet's lungs (Fig. 8), a finding that was also observed in a preterm lamb model of RDS (31). Differences in surfactant deposition between right and left upper lobes may be explained by the anatomic situation in the piglet with the right upper bronchus branching off directly from the trachea.

Application of large VT may be followed by VILI. VILI is likely to happen when the sum of FRC and VT exceeds maximal lung volume, being approximately 50 mL/kg in infants with nondiseased lungs and approximately 20 mL/kg in infants with RDS (32). Not only the degree but also the duration of overinflation is an important factor for VILI (33) and may happen after no more than a few minutes after mechanical ventilation with VT of 10 mL/kg in premature rabbits (34) or 46 mL/kg in adult rabbits (35). Six manual inflations of 35–40 mL/kg (bagging) for resuscitation before the start of regular mechanical ventilation led to severe compromise in gas exchange, lung mechanics, and lung histology in a preterm lamb model of RDS (36). There are several reasons for the occurrence of VILI when using high VT in animal models of surfactant deficiency: *1*) the conversion rate

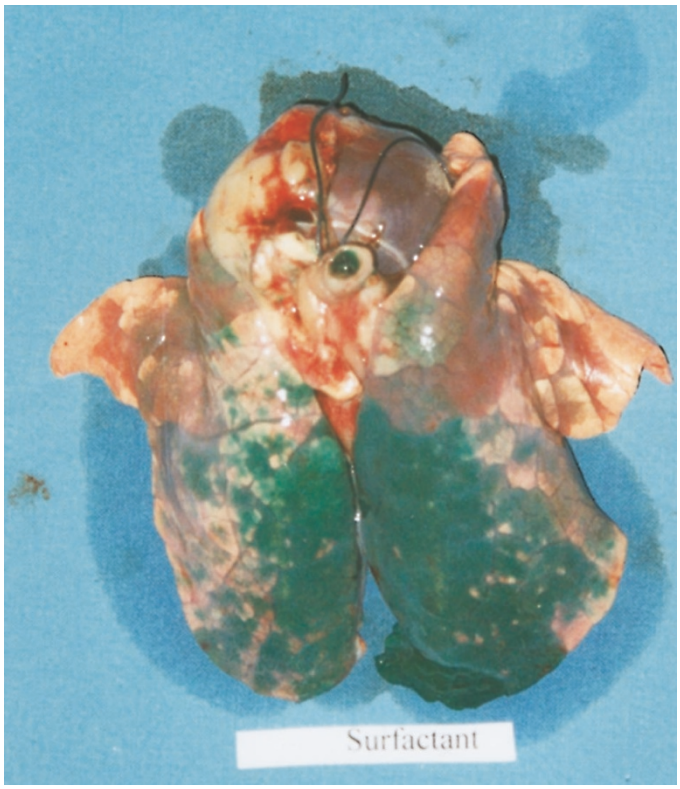

Figure 8. Dorsal aspect of excised lungs with the trachea tied off and cut off (Surf group). Grossly inhomogeneous distribution of stained surfactant at the subpleural level adjacent to areas of little or complete atelectasis. On top dorsal aspect of the heart beneath the right upper lobe.

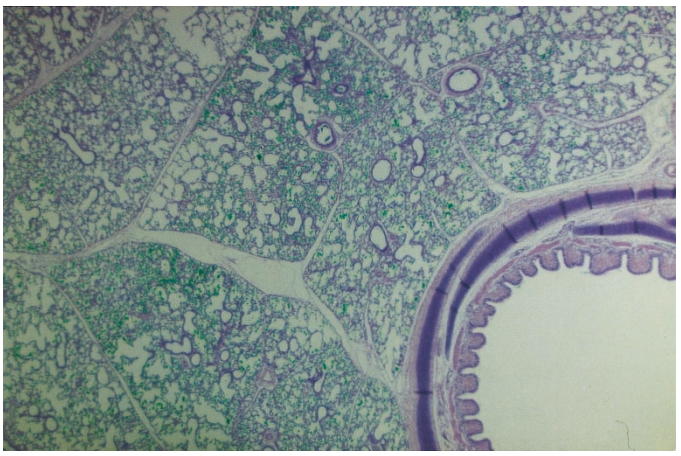

Figure 9. Homogeneous surfactant distribution showing only little variation in the amount of surfactant deposits among lobuli (V_T16 group). Hematoxylin and eosin stain, ×150.

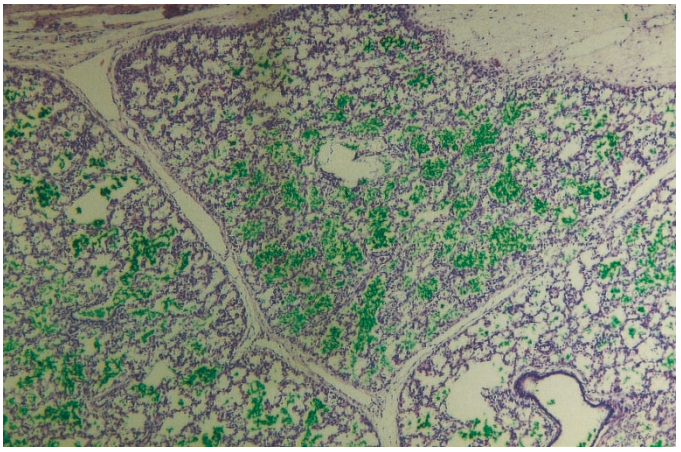

Figure 10. Clusters of stained surfactant obstructing terminal bronchi, thus preventing surfactant from entering into the alveoli or advancing to peripheral sites (Surf group). Hematoxylin and eosin stain, ×300.

of large aggregate to small (inactive) aggregate surfactant forms is enhanced (37, 38) and *2*) disruption of alveolar membranes leads to increased fluid influx into the lung parenchyma thus increasing the wet-to-dry weight ratio of the lungs (39).

It has been shown in different animal models (36, 40, 41) that VILI can be prevented when surfactant is given concomitantly with the initiation of ventilation. Moreover, VILI could not be found in a preterm sheep model of RDS using moderately high V_T of 15 mL/kg (as in our present study for volume recruitment) in comparison with 8 mL/kg (42). No differences in surfactant aggregate conversion or alveolar lavage protein content could be detected.

The sum of V_T and FRC in our V_T16+P8 group was approximately 56 mL/kg (Fig. 2), thus probably exceeding TLC. Gas exchange and lung function indices were inferior to those of the V_T16 group, suggesting some degree of VILI in this group as demonstrated by desquamation of bronchial epithelia and higher occurrence rates for edema and hyaline membranes (Figs. 4 and 6). In contrast we assume that a short period of large V_T ventilation, although less than TLC (V_T16 group), concomitantly with surfactant repletion of the lungs, will not result in VILI and that stretching of airways might rather lead to additional endogenous surfactant release (43). Not only VILI, but also peripheral emphysema at subpleural regions may have contributed to the inferior surfactant response in the V_T16+P8 group when compared with V_T16 alone.

In mammals, during unloaded quiet breathing, V_T appears to be cross-species constant when standardized to body weight, being approximately 6–8 mL/kg (44). We choose baseline PEEP levels of 0.4 kPa [accounting for approximately 50% of TLC in nondiseased adult rabbit lungs (45)] and a V_T of 8 mL/kg (until baseline) in an attempt to initially avoid lung volumes beyond TLC and to avoid circulatory compromise by lung overdistention when using $V_T \geq 20$ mL/kg and a PEEP $\geq$ 10 cm H_2O (46). Changes in V_T after surfactant administration were allowed to assess improvement in C_{RS}.

Evaluation of 10 histologic specimens for each experimental subject should be considered a conservative approach that tends to underestimate differences among groups. Despite limitations of the histologic method that we used to assess the absolute degree of injury, it is likely that differences in histology among groups reflect true differences in severity of injury (47). Occurrence rates of pneumothoraces while performing pressure-volume curves are another hint of differences in inhomogeneity of lung injury or, *vice versa*, for the homogeneity of surfactant distribution.

The surfactant staining technique used in this study (19) allows to describe surfactant distribution at the alveolar level, also giving some information about the timely responses of, for example, surfactant incorporation into macrophages or the build-up of the exogenous surfactant layer onto hyaline membranes.

The airway lavage model in term newborn piglets may not exactly represent the physiologic and histologic situation of the structurally more immature lung of premature human infants with RDS. Airway lavage in the adult rabbit (48) leads to widespread atelectasis alternating with areas of well-expanded or even hyperexpanded alveoli, necrosis and desquamation of airway epithelium, accumulation of edema fluid, hyaline membranes, nonspecific pneumonia, intraalveolar accumulation of macrophages, and areas of interstitial and intraalveolar hemorrhage, as also seen in our piglet model of airway lavage.

Further differences between the piglet lavage model and true animal models of RDS (premature lambs or monkeys) might influence the efficacy of VRM because impaired gas exchange occurs also because of the existence of proteinaceous intraalveolar fluid next to alveolar atelectasis as shown by Jackson *et al.* (49, 50) in a premature monkey model. Moreover Neumann *et al.* (51) demonstrated in an adult porcine model of lung injury that atelectatic areas induced by repeated airway lavage are more easily recruitable than when caused by lung injury induced by oleic acid injection. One difference between these two models of lung injury is the degree of intraalveolar fluid accumulation.

In summary, this study shows that volume recruitment by means of moderately increased tidal volumes at the time of surfactant administration leads to augmentation of alveolar ventilation, thus improving gas exchange and lung function owing to more homogeneous surfactant distribution within the lungs. These data obtained from a neonatal piglet model of airway lavage encourage further experimental trials in a true RDS model with preterm lambs for evaluation of a preclinical situation.

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