Positive Pressure Ventilation with the Open Lung Concept Optimizes Gas Exchange and Reduces Ventilator-Induced Lung Injury in Newborn Piglets

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ABSTRACT

Previous studies demonstrated that high-frequency oscillatory ventilation using the open lung concept (OLC) resulted in superior gas exchange and a reduction in ventilator-induced lung injury (VILI). We hypothesized that these beneficial effects could also be achieved by applying the OLC during positive pressure ventilation. After repeated whole-lung-lavage, newborn piglets were assigned to either OLC positive pressure ventilation (PPV_{OLC}), OLC high-frequency oscillatory ventilation ($HFOV_{OLC}$), or conventional positive pressure ventilation (PPV_{CON}) and ventilated for 5 h. In both OLC groups, collapsed alveoli were actively recruited and thereafter stabilized using the lowest possible airway pressures. In the PPV_{CON} group, ventilator settings were adjusted to prevent critical hypoxia. Airway pressure, blood gas analysis, pressure-volume curve, and alveolar protein infiltration was recorded. A lung injury score was used for histologic comparison. Mean airway pressures were comparable in the three ventilation groups over time (1.2–1.5 kPa). Arterial oxygenation increased to mean values above 60 kPa in both OLC groups compared with 10 kPa in the PPV_{CON} group ($p < 0.001$). Maximal lung compliance was superior in both OLC groups (PPV_{OLC}: 91 ± 23; $HFOV_{OLC}$: 90 ± 31 mL/kPa/kg, $p < 0.01$) compared with the PPV_{CON} group (39 ± 14 mL/kPa/kg). Alveolar protein infiltration was significantly reduced in the PPV_{OLC} group (0.33 ± 0.10 mg/mL, $p < 0.01$) and the $HFOV_{OLC}$ group (0.40 ± 0.13 mg/mL, $p < 0.01$) compared with the PPV_{CON} group (0.70 ± 0.15 mg/mL). Lung injury scores were significantly higher in the PPV_{CON} group (33.5 ± 9.5, $p < 0.01$) compared with both OLC groups (PPV_{OLC}: 10.5 ± 2.6; $HFOV_{OLC}$: 11 ± 2.2). There were no differences between the two OLC groups. We conclude that, in surfactant-depleted newborn piglets, application of the OLC during PPV is feasible and results in superior gas exchange and a reduction in VILI compared with conventional PPV. These beneficial effects are comparable to HFOV. (***Pediatr Res* 53: 245–253, 2003**)

Abbreviations
PPV, positive pressure ventilation
HFOV, high-frequency oscillatory ventilation
OLC, open lung concept
VILI, ventilator-induced lung injury
MawP, mean airway pressure
PIP, peak inspiratory pressure
PEEP, positive end-expiratory pressure
I/E, inspiration/expiration time
CDP, continuous distending pressure
Fio_2, fraction of inspired oxygen
Pao_2, arterial Po_2
$Paco_2$, arterial Pco_2
Crs_{max}, maximal lung compliance
TLC, total lung capacity
CVP, central venous pressure

Acute respiratory failure in newborn infants is often caused by surfactant deficiency and/or inactivation. These changes in surfactant result in alveolar collapse and subsequent hypoxia and hypercapnia. Treatment with conventional PPV is often indicated, but, in the attempt to optimize gas exchange, PPV often leads to secondary lung injury, also referred to as VILI (1). This may contribute to long-term respiratory morbidity (chronic lung disease), especially in newborn infants (2).

Animal studies have shown that alveolar overdistension (volutrauma) and cyclic reopening of alveoli collapsed during expiration (atelectrauma) are the major causes of VILI (3, 4).

During HFOV, alveolar overdistension is prevented by the use of small tidal volumes superimposed on a relatively high CDP to maintain adequate lung volume. However, animal studies have shown that this approach will only result in a reduction of VILI if alveoli are optimally recruited at the start of ventilation (5, 6).

The ventilation strategy based on these three important principles of lung protective ventilation (alveolar recruitment, prevention of alveolar overdistension and collapse) is also referred to as the high-volume strategy or the OLC (7–9).

In contrast to HFOV, the OLC during PPV has not been investigated in newborn animals or infants. Adult animal studies showed that PPV can effectively recruit alveoli and keep them open during the entire respiratory cycle (10). To accomplish this, PIP are increased for a short period of time to recruit collapsed alveoli and, subsequently, alveolar collapse and overdistension is prevented by sufficient amounts of PEEP and by using small pressure amplitudes (PIP − PEEP). Studies in adult patients treated for acute respiratory failure showed promising results (11).

Based on these findings, we decided to investigate the OLC during PPV (PPV_{OLC}) in a surfactant-deficient newborn animal model. PPV_{OLC} was compared with both conventional PPV (PPV_{CON}) and HFOV ($HFOV_{OLC}$), because these latter two strategies are mostly used in neonates (12–14). Primary outcome parameters in this comparison were gas exchange and VILI. Secondary outcome parameters were changes in ventilatory pressures and hemodynamics. We hypothesized that PPV_{OLC} would lead to superior gas exchange and less VILI compared with PPV_{CON}. Superior gas exchange was defined as improved oxygenation while preventing hypercapnia. A reduction in VILI was defined as a preservation of compliance, total lung capacity, and alveolar stability (lung mechanics); a decrease in alveolar protein influx; and a decrease in histologic evidence of lung injury expressed as a semiquantitative score. We further hypothesized that the magnitude of these beneficial effects on gas exchange and VILI would be comparable to $HFOV_{OLC}$.

METHODS

Animal Preparation

These experiments were performed at the Department of Anesthesiology, Erasmus University Rotterdam. The study was approved by the institutional animal investigation committee.

Anesthesia was induced in 45 mixed-breed newborn piglets, aged 23 ± 15 h (mean ± SD) and weighing 1.4 ± 0.3 kg, with ketamine hydrochloride [35 mg/kg intramuscularly (i.m.)] and midazolam (0.5 mg/kg i.m.). The animals were tracheotomized, connected to a Servo ventilator 300 (Siemens-Elema, Solna, Sweden) and ventilated in the pressure-controlled time-cycled mode, at a Fio_2 concentration of 1.0, a rate of 25–30 breaths/min, PIP of 0.9–1.2 kPa (9–12 cm H_2O), PEEP of 0.2 kPa (2 cm H_2O), and an I/E ratio of 1:2. A neuromuscular block was induced with pancuronium bromide (0.5 mg/kg i.v.), followed by a continuous infusion of fentanyl (20 μg/kg/h), midazolam (0.3 mg/kg/h), and pancuronium bromide (0.3 mg/kg/h).

A 4-Fr double-lumen polyurethane catheter (Vygon, Ecouen, France) was introduced through the external jugular vein for measurement of CVP and infusion of fluids and medication. The carotid artery was cannulated for monitoring of blood pressure and blood sampling. In addition, a sensor for continuous blood gas monitoring (Paratrend/Trendcare, Philips Medical, Böblingen, Germany) was inserted through a femoral artery catheter.

A continuous infusion of 5% dextrose was started (100 mL/kg/d) and all animals received one dose of cefotaxime (100 mg/kg). Body temperature was kept between 38° and 39°C during the experiment.

Surfactant Depletion

After the instrumentation period, respiratory failure was induced by repeated saline lavage (50 mL/kg; 37°C) as described by Lachmann *et al.* (15). The first five lavages were performed in the prone position, after which the animals were placed in a supine position for the remainder of the lavage procedure. Lavages were repeated at 3-min interval until Pao_2 was below 10.7 kPa and $Paco_2$ above 5.3 kPa at the following ventilator settings: PIP/PEEP, 2.45/0.49 kPa (25/5 cm H_2O); rate, 40 breaths/min; I/E ratio, 1:2; and Fio_2, 1.0.

Experimental Protocol

Immediately after induction of anesthesia, five animals were killed and served as a healthy nonventilated control group. An additional group of four animals was killed immediately after the lavage procedure and served as histologic controls.

Within 10 min after the last lavage, the remaining animals were randomly allocated (T = 0 h) to one of the three treatment groups ($n = 12$ each) and ventilated for a period of 5 h. Fio_2 was kept at 1.0 during all experiments.

PPV_{OLC} group. In this group, the ventilatory rate was increased to 120 breaths/min with an I/E ratio of 1:1. These settings remained unchanged during the experiment. PEEP was increased to 1.47 kPa (15 cm H_2O) and PIP was stepwise increased [0.49 kPa (5 cm H_2O) each step] to open up the lung. Recruitment of previously collapsed alveoli during this procedure will decrease intrapulmonary shunt and thus increase oxygenation (Fig. 1). Based on Pao_2 levels in the healthy piglets, ventilated with an Fio_2 of 1.0, we defined optimal alveolar recruitment as a $Pao_2 \geq 60$ kPa. The level of PIP needed to recruit the lung was called the opening pressure (PIP_O).

After this recruitment procedure, PIP and PEEP were simultaneously decreased in equal steps every 2 to 3 min until Pao_2 dropped below 60 kPa, indicating increased intrapulmonary shunting due to alveolar collapse. The level of PEEP at this stage of alveolar collapse was called the closing pressure ($PEEP_C$). PEEP was then raised to a level of 0.2 kPa (2 cm H_2O) above $PEEP_C$ and PIP was momentarily (about 10 s) raised to PIP_O to fully recruit the lung. Then, the pressure amplitude was set to keep the $Paco_2$ within the target range (4–6 kPa). PEEP was only decreased if there were signs of alveolar overdistension defined as increasing $Paco_2$, decreasing
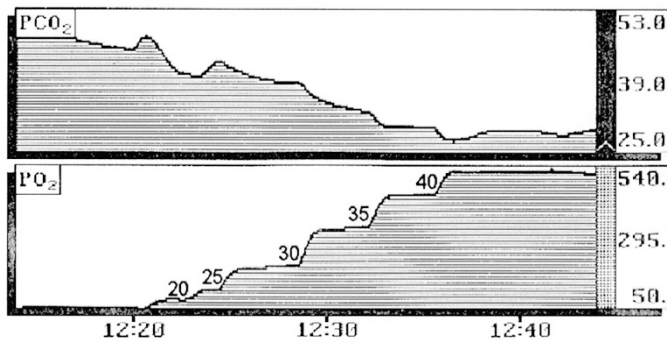
Figure 1. Registration from a single representative experiment showing continuous measurement of arterial Pao_2 (mm Hg) and $Paco_2$ (mm Hg) during the lung recruitment phase in the PPV_{OLC} group. Numbers indicate the actual peak inflation pressures (cm H_2O) used in stepwise recruitment of the lung. Each increase is followed by an increase in Pao_2 and a decrease in $Paco_2$ over time. To convert mm Hg to kPa, divide by 7.5.

Pao_2, or decreasing blood pressure. After 5 h of ventilation, PIP_O and $PEEP_C$ were once again determined (T = 5 h).

$HFOV_{OLC}$ group. Animals in this group were ventilated with HFOV (model 3100; SensorMedics Critical Care, Yorba Linda, CA, U.S.A.) using a frequency of 10 Hz and an I/E ratio of 1:2. These settings remained unchanged throughout the experiment. Opening and closing pressures (CDP_O and CDP_C, respectively) were determined as described in the PPV_{OLC} group, by adjusting the CDP. The delta pressure during oscillation was set at a level that kept the $Paco_2$ within the target range. CDP was only decreased if there were signs of alveolar overdistension as described earlier. After 5 h of ventilation, CDP_O and CDP_C were once again determined (T = 5 h).

PPV_{CON} group. In this third group, animals were ventilated in the pressure-controlled mode, applying a conventional strategy. As preliminary experiments showed that it was not possible to achieve a target $Pao_2 \geq 60$ kPa, the ventilator settings after the lavage procedure were adjusted to prevent critical hypoxia ($Pao_2 < 8$ kPa). First, the I/E ratio was set at 1:1 and, if necessary, PIP was increased with a limit of 3.43 kPa (35 cm H_2O). If the level of PIP exceeded 2.94 kPa (30 cm H_2O), PEEP was increased to 0.59 kPa (6 cm H_2O).

The rate was preferentially changed (between 25 and 60 breaths/min) to keep the $Paco_2$ within the target range. If adjusting the rate proved unsuccessful in controlling $Paco_2$, PIP could be altered as long as the changes did not compromise Pao_2.

During the experimental period, intravascular volume expansion (albumin 5%; 10 mL/kg/dose) was initiated if there were two or more signs of circulatory failure: heart rate > 200 beats/min, mean arterial blood pressure < 6.7 kPa (50 mm Hg), and deteriorating Pao_2 (> 10%).

Mean arterial blood pressure, heart rate, CVP, ventilator settings, and pressures were recorded at the end of the instrumentation period, at the end of the lavage procedure, and hourly after randomization. Although blood gas values were recorded continuously, reference samples were drawn at these same time points (ABL 505, Radiometer, Copenhagen, Denmark).

Pressure-Volume Curves

After the 5 h ventilation period, eight animals in each treatment group were killed by an overdose of pentobarbital and used to assess lung mechanics. Static pressure-volume (P/V) curves were recorded using the syringe technique as previously described (16). In short, after opening the thorax and diaphragm, the endotracheal tube was connected to a pressure transducer with a syringe attached to it (model DP 45–32, Validyne Engineering, Northridge, CA, U.S.A.), and pressures were recorded on a polygraph (model 7B, Grass Instruments, Quincy, MA, U.S.A.). Using a syringe, the lungs were first inflated using steps of 1 mL up to a volume of 10 mL and thereafter steps of 5 mL until an airway pressure of 3.43 kPa (35 cm H_2O) was reached. The lungs were deflated in the same manner starting with 1-mL steps, followed by 5-mL steps until the airway pressure reached 0 kPa.

Crs_{max} was calculated from the maximal slope on the deflation limb (17). Total lung capacity (TLC) was defined as lung volume at inflation with a distending pressure of 3.43 kPa (35 cm H_2O). The Gruenwald index, which characterizes the surfactant system *in situ*, was calculated from the P/V curve, defined as $(2V_5 + V_{10})/2V_{max}$, where V_5, V_{10}, and V_{max} are the lung volumes at transpulmonary pressures of 0.49, 0.98, and 3.43 kPa (5, 10, and 35 cm H_2O), respectively, on the deflation limb (18).

Bronchoalveolar Lavage Procedure

After recording P/V curves, bronchoalveolar lavage was performed five times (40 mL/kg) with saline-$CaCl_2$ 1.5 mM. The recovered fluids from these five lavages were pooled and analyzed as one sample. The percentage of lung lavage fluid recovered was calculated. Samples were centrifuged for 10 min at 1500 *g* to remove cell material, and protein concentration was measured using the Bradford method (protein assay, Bio-Rad, Munich, Germany) (19).

Lung mechanics and alveolar protein infiltration as described above were also assessed in the healthy nonventilated control group

Histologic Processing

At the end of the experiment, the lungs of the four remaining animals in each ventilation group and in the histologic control group were fixated as previously described (20). Briefly, after perfusion, the lung was fixated with a solution consisting of 3.6% formaldehyde and 0.25% glutaraldehyde. Before fixation, the airway pressure was momentarily increased to 2.94 kPa (30 cm H_2O) and thereafter maintained at 1.96 kPa (20 cm H_2O).

Blocks of tissue were taken from the center of the upper and middle lobe and from the ventral and dorsal part of the lower lobe. The specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

A semiquantitative morphometric analysis of lung injury was performed under blinded conditions, scoring atelectasis, hyaline membrane formation, and cellular infiltration as none, minimal, light, moderate, or severe (scores 0, 1, 2, 3, or 4, respectively). As there were no differences in lung injury

scores between the left and right lung, only the right lung was used for further analysis. The lung injury score for each lung was defined as the sum of all four segments taken (maximum score 48). The total lung injury score for the different treatment groups was obtained by averaging the lung injury scores from the animals included in each group.

Statistical Analysis

Statistical analysis was performed using SPSS version 10 (SPSS, Chicago, IL, U.S.A.). Intergroup differences were analyzed with ANOVA or the t test. If ANOVA resulted in $p < 0.05$, a Bonferroni posthoc test was performed. Intragroup comparisons were analyzed with the paired t test. Lung injury data were analyzed after logarithmic transformation. A p value < 0.05 was considered statistically significant.

RESULTS

All animals survived the study period. There were no intergroup differences in age, weight, or number of lavages (14 ± 4 SD) needed to induce lung injury. No air leaks were observed during the study period. Blood gas values before and immediately after lavage were comparable in the three treatment groups.

Mean Pao_2 values increased after the recruitment maneuver in the PPV_{OLC} and $HFOV_{OLC}$ groups and remained significantly higher than in the PPV_{CON} group at all time points (Fig. 2A).

$Paco_2$ decreased in all ventilation groups after randomization, but was significantly lower in the PPV_{CON} group compared with the PPV_{OLC} and $HFOV_{OLC}$ group (Fig. 2B). Because $Paco_2$ gradually increased over time in the PPV_{CON} group, this difference in $Paco_2$ was no longer significant *versus* the $HFOV_{OLC}$ group during the last 2 h of the study period.

The MawP in both PPV groups and the CDP in the HFOV group showed no significant differences between ventilation groups during the entire experiment (Table 1). A higher level of PEEP and a lower level of PIP resulted in a significantly smaller pressure amplitude during PPV_{OLC} compared with PPV_{CON}. To establish adequate CO_2 removal, the ventilatory rate was significantly higher during PPV_{OLC} compared with PPV_{CON}. It is important to mention that the delta pressure during HFOV as reported in Table 1 represents the values as displayed by the oscillator. Because we did not measure intratracheal pressures, the reported delta pressure cannot be compared with the pressure amplitude measured during PPV.

Comparison of the opening pressures (PIP_O and CDP_O; Fig. 3A) between both OLC groups revealed no significant differences either at the start (T = 0) or at the end (T = 5) of the ventilation period. This was also true for the closing pressures ($PEEP_C$ and CDP_C; Fig. 3B). However, analyzing opening and closing pressure within each OLC group separately showed a significant decrease of both parameters over the 5-h ventilation period (T = 0 *versus* T = 5 h; Fig. 3).

With the exception of the CVP, circulatory parameters showed no significant differences between ventilation groups throughout the experiment (Table 2). CVP was significantly higher in the PPV_{OLC} group compared with the PPV_{CON} and $HFOV_{OLC}$ groups during the 5-h ventilation period. There were no significant differences in acid-base status and in the
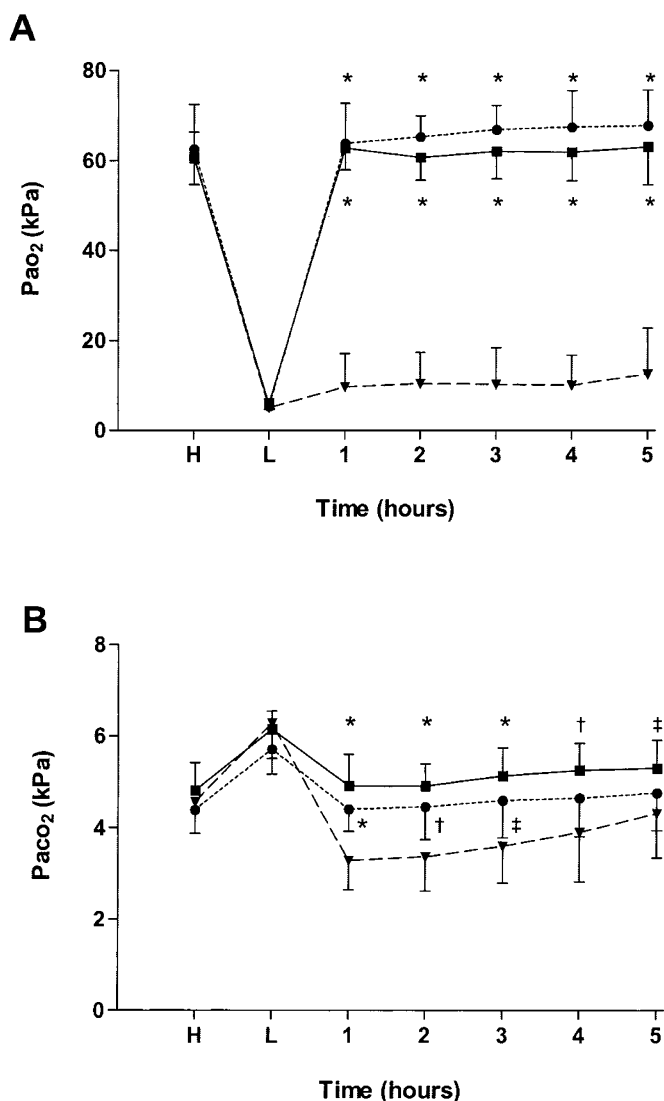
Figure 2. Changes (mean $\pm$ SD) in Pao_2 (*A*) and $Paco_2$ (*B*) before lung lavage (*H*), after lung lavage (*L*), and during the 5-h ventilation period in the three ventilation groups (PPV_{OLC}, *squares*; $HFOV_{OLC}$, *circles*; PPV_{CON}, *triangles*). *$p < 0.001$, †$p < 0.01$, ‡$p < 0.05$ *vs* PPV_{CON} group. To convert to mm Hg, multiply by 7.5.

amount of fluid support between the groups (29 ± 15 mL/kg in PPV_{OLC}, 23 ± 21 mL/kg in $HFOV_{OLC}$, and 18 ± 13 mL/kg in PPV_{CON}).

Figure 4 shows P/V curves of the three ventilation groups. There were no significant differences in lung mechanics between the PPV_{OLC} and the $HFOV_{OLC}$ groups, which were comparable to the nonventilated healthy controls. However, TLC, Crs_{max} and the Gruenwald index were significantly lower in the PPV_{CON} group compared with the PPV_{OLC} and the $HFOV_{OLC}$ group (Table 3).

The protein concentration in the bronchoalveolar lavage fluid of the PPV_{CON} group was significantly higher than in both OLC groups (Table 3). Protein concentration in the nonventilated healthy controls was comparable to that in the OLC groups. There were no significant differences between the groups in the amount of bronchoalveolar lavage fluid recovered.

Table 1. *Ventilatory parameters at different time points and mode of ventilation**

	MawP/CDP (kPa)	PIP (kPa)	PEEP (kPa)	PA/Delta P (kPa)	Rate (breaths/min)
Healthy					
PPV$_{OLC}$	0.45 ± 0.07	1.09 ± 0.22	0.25 ± 0.10	0.84 ± 0.25	31 ± 5
PPV$_{CON}$	0.41 ± 0.02	0.99 ± 0.08	0.21 ± 0.07	0.78 ± 0.10	29 ± 2
HFOV$_{OLC}$	0.42 ± 0.05	1.01 ± 0.16	0.23 ± 0.05	0.78 ± 0.15	29 ± 2
Lavaged‡					
PPV$_{OLC}$	1.00 ± 0.09	2.45 ± 0.00	0.49 ± 0.00	1.96 ± 0.00	40 ± 0
PPV$_{CON}$	0.98 ± 0.06	2.45 ± 0.00	0.49 ± 0.00	1.96 ± 0.00	40 ± 0
HFOV$_{OLC}$	1.03 ± 0.09	2.45 ± 0.00	0.49 ± 0.00	1.96 ± 0.00	40 ± 0
1 h					
PPV$_{OLC}$	1.47 ± 0.31	1.91 ± 0.47†	1.11 ± 0.25†	0.80 ± 0.25†	120 ± 0†
PPV$_{CON}$	1.40 ± 0.18	2.62 ± 0.17	0.52 ± 0.05	2.11 ± 0.14	31 ± 6
HFOV$_{OLC}$	1.35 ± 0.25	—	—	1.28 ± 0.40	600 ± 0
2 h					
PPV$_{OLC}$	1.45 ± 0.32	1.89 ± 0.47†	1.08 ± 0.27†	0.81 ± 0.24†	120 ± 0†
PPV$_{CON}$	1.38 ± 0.19	2.61 ± 0.17	0.52 ± 0.05	2.10 ± 0.14	30 ± 5
HFOV$_{OLC}$	1.33 ± 0.25	—	—	1.31 ± 0.45	600 ± 0
3 h					
PPV$_{OLC}$	1.43 ± 0.31	1.90 ± 0.42†	1.05 ± 0.26†	0.83 ± 0.21†	120 ± 0†
PPV$_{CON}$	1.38 ± 0.19	2.61 ± 0.17	0.52 ± 0.05	2.11 ± 0.13	30 ± 6
HFOV$_{OLC}$	1.31 ± 0.26	—	—	1.26 ± 0.37	600 ± 0
4 h					
PPV$_{OLC}$	1.43 ± 0.31	1.89 ± 0.42†	1.04 ± 0.27†	0.84 ± 0.20†	120 ± 0†
PPV$_{CON}$	1.39 ± 0.19	2.63 ± 0.18	0.52 ± 0.05	2.11 ± 0.14	30 ± 6
HFOV$_{OLC}$	1.28 ± 0.29	—	—	1.23 ± 0.34	600 ± 0
5 h					
PPV$_{OLC}$†	1.25 ± 0.47	1.84 ± 0.41†	1.02 ± 0.27†	0.83 ± 0.19†	120 ± 0†
PPV$_{CON}$	1.38 ± 0.19	2.63 ± 0.18	0.52 ± 0.05	2.11 ± 0.14	30 ± 6
HFOV$_{OLC}$	1.26 ± 0.29	—	—	1.23 ± 0.33	600 ± 0

* Data are mean ± SD. To convert kPa to cm H_2O, multiply by 10.2. PA, pressure amplitude; Delta P, delta pressures as displayed by the oscillator.

† $p < 0.001$ *vs* PPV$_{CON}$ group.

‡ Ten minutes after lavage.

Figure 5 shows the lung injury scores of the different treatment groups. Both PPV$_{OLC}$ and HFOV$_{OLC}$ showed significantly less lung injury than the PPV$_{CON}$ group.

Histologic changes seen in the PPV$_{CON}$ group consisted of large atelectatic areas containing hyaline membranes and a high degree of interstitial cellular infiltration (Fig. 6*A*). Histologic changes seen in both OLC groups were mainly increased interstitial cellular infiltration (Fig. 6, *B* and *C*), resulting in a higher lung injury score compared with the animals that were lavaged only (Fig. 6*D*). More detailed quantitative comparisons between the two OLC groups were not made.

DISCUSSION

The present study demonstrates that applying the OLC during PPV in surfactant-depleted newborn piglets optimizes gas exchange and reduces VILI compared with conventional PPV.

Previously, most newborn and adult animal studies investigated the beneficial effects of the OLC during HFOV. Comparison of this ventilation modality with conventional PPV in animals showed the superiority of HFOV with regard to gas exchange and several parameters of VILI (5, 6, 21–23). A recent meta-analysis confirmed these animal findings in human newborn infants with respiratory distress syndrome (24). However, some have stated that it is not the ventilation mode (HFOV or PPV) but the ventilation strategy (OLC) that is responsible for these beneficial effects (7, 25).

Until now, only two studies explored this theory. These latter studies showed that application of the OLC during PPV and HFOV resulted in comparable gas exchange and VILI (16, 26). However, neither of these studies included a conventional PPV group, making direct comparison with this (still widely used) mode of ventilation impossible. Secondly, both studies used adult animal models, which may not be ideal for testing new ventilation modalities in newborns. The newborn lung is both structurally (*e.g.* gas exchange unit) and biochemically (*e.g.* surfactant metabolism) different from the adult lung (27, 28). Some believe this makes the newborn lung much more susceptible for VILI than the adult lung (29).

We therefore decided to compare the OLC during PPV and HFOV with conventional PPV in newborn piglets. We chose the newborn piglet because it has a lung morphology similar to that of humans (30, 31).

Acute respiratory failure was induced by whole-lung lavage, which has been postulated to reflect a primary surfactant deficiency, as seen in neonatal respiratory distress syndrome (15).

Our study clearly shows that both PPV$_{OLC}$ and HFOV$_{OLC}$ are able to recruit and maintain adequate lung volume, leading to superior oxygenation compared with the PPV$_{CON}$ group (Fig. 2*A*). We used arterial oxygenation to determine optimal recruitment as it has been demonstrated that this parameter increases with increasing lung volume (5, 32). We did not measure absolute lung volume because application of this
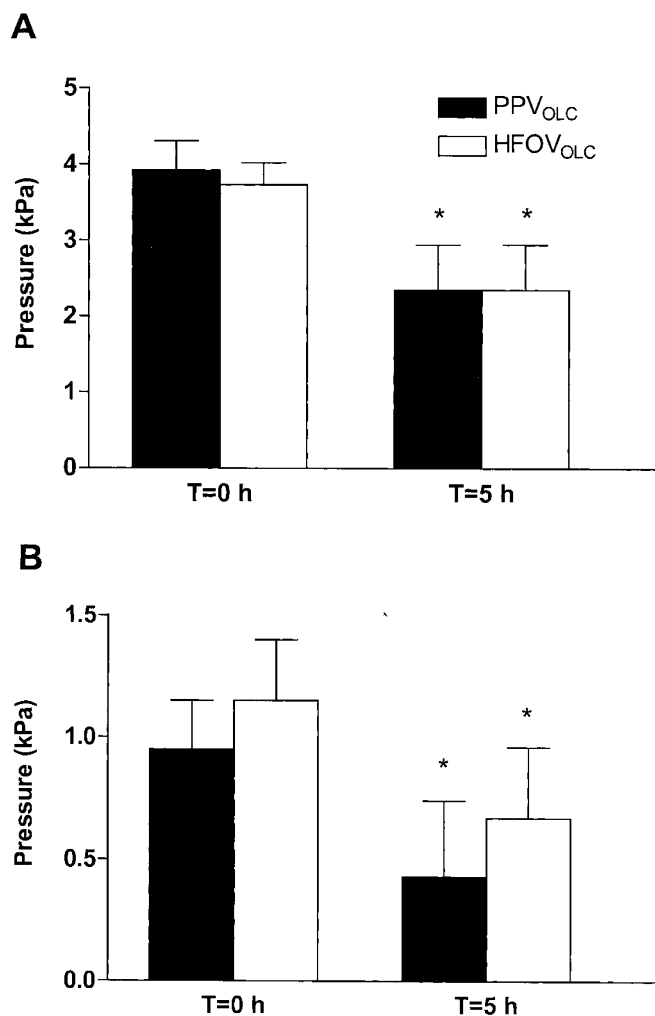
A

B

Figure 3. Changes (mean $\pm$ SD) in opening (*A*) and closing (*B*) pressures immediately after randomization (T = 0 h) and at the end of the 5-h ventilation period (T = 5 h). There is a significant decrease in opening and closing pressures within each ventilation group over time. *$p < 0.001$ *vs* T = 0 h. To convert to cm H_2O, multiply by 10.2.

Table 2. *Circulatory parameters at different time points and mode of ventilation**

	MAP (kPa)	CVP (kPa)	HR (beats/min)
Healthy			
PPV_{OLC}	9.2 ± 1.6	0.44 ± 0.19	148 ± 18
PPV_{CON}	8.7 ± 1.6	0.33 ± 0.20	136 ± 15
$HFOV_{OLC}$	8.7 ± 2.5	0.28 ± 0.12	151 ± 17
Lavaged§			
PPV_{OLC}	10.1 ± 2.3	0.67 ± 0.13	171 ± 25
PPV_{CON}	9.7 ± 2.3	0.52 ± 0.19	158 ± 25
$HFOV_{OLC}$	8.8 ± 2.7	0.48 ± 0.19	185 ± 29
1 h			
PPV_{OLC}	11.1 ± 2.1	1.15 ± 0.23	187 ± 31
PPV_{CON}	10.7 ± 2.4	$0.71 \pm 0.36†$	189 ± 29
$HFOV_{OLC}$	9.3 ± 2.1	$0.72 \pm 0.27†$	174 ± 31
2 h			
PPV_{OLC}	9.3 ± 1.6	1.04 ± 0.20	186 ± 30
PPV_{CON}	9.3 ± 2.1	$0.67 \pm 0.29‡$	190 ± 32
$HFOV_{OLC}$	8.9 ± 2.3	$0.63 \pm 0.33†$	189 ± 42
3 h			
PPV_{OLC}	8.9 ± 1.5	0.95 ± 0.16	189 ± 34
PPV_{CON}	8.4 ± 1.6	0.71 ± 0.21	190 ± 34
$HFOV_{OLC}$	8.5 ± 2.3	$0.60 \pm 0.33†$	202 ± 37
4 h			
PPV_{OLC}	8.7 ± 1.5	0.93 ± 0.15	185 ± 40
PPV_{CON}	7.9 ± 1.6	$0.60 \pm 0.17†$	199 ± 34
$HFOV_{OLC}$	7.9 ± 1.6	$0.55 \pm 0.33†$	206 ± 41
5 h			
PPV_{OLC}	8.5 ± 1.9	0.91 ± 0.13	197 ± 37
PPV_{CON}	8.0 ± 1.9	$0.53 \pm 0.25†$	206 ± 30
$HFOV_{OLC}$	7.6 ± 1.9	$0.53 \pm 0.36†$	199 ± 36

* Data are mean $\pm$ SD. To convert kPa to mm Hg, multiply by 7.5. MAP, mean arterial pressure; HR, heart rate.

† $p < 0.01$ *vs* PPV_{OLC} group.

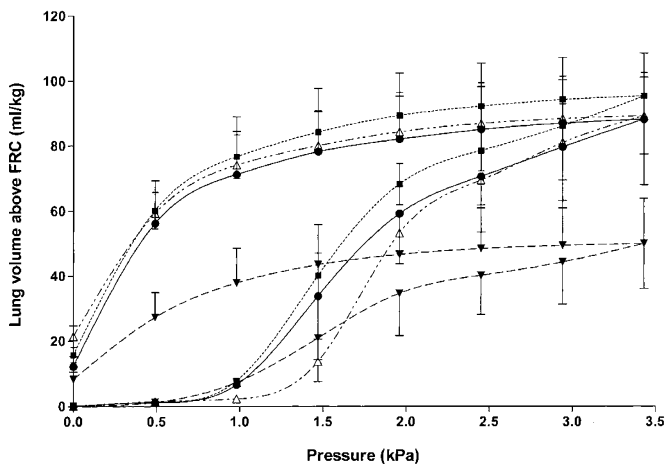‡ $p < 0.05$ *vs* PPV_{OLC} group.

§ Ten minutes after lavage.

Figure 4. Static pressure-volume curves (mean $\pm$ SD) in healthy controls (*open triangles*) and the three ventilation groups (PPV_{OLC}, *squares*; $HFOV_{OLC}$, *circles*; PPV_{CON}, *solid triangles*). Significant differences are given in Table 3. To convert to cm H_2O, multiply by 10.2.

measurement bedside is still unpractical and often not available. Furthermore, a measured increase in lung volume can result from either an increased expansion of already opened alveoli (distension) or opening of previously collapsed alveoli (recruitment). Only arterial oxygenation can differentiate between these two possibilities because only recruitment will reduce intrapulmonary shunting and thus improve Pao_2.

As the MawP in the PPV_{CON} group was not different from the PPV_{OLC} group, the relative hypoxia in the PPV_{CON} group was probably caused by inadequate levels of PEEP (unable to prevent end-expiratory alveolar collapse) and the absence of an active recruitment maneuver. This, however, is in accordance with daily practice in the neonatal intensive care unit, where PEEP during PPV_{CON} in newborns seldom exceeds 0.6 kPa (6 cm H_2O) and recruitment maneuvers are not performed (7, 33).

Increasing PIP in the PPV_{CON} group resulted in an only modest increase in Pao_2. To keep the PIP within a clinically acceptable range and MawP comparable with the OLC groups, a target Pao_2 of 8 kPa was used, as described elsewhere (22).

The higher pressure amplitude during PPV_{CON} resulted in a significantly lower mean level of $PaCO_2$ compared with both OLC groups (Fig. 2*B*). In some animals, it proved difficult to keep $Paco_2$ within the target range because of progressive hypoxia after lowering PIP. During the 5 h ventilation period, mean $Paco_2$ levels in the PPV_{CON} group slowly increased,

Table 3. *Data on lung mechanics and total protein concentration in lavage fluids**

	Healthy controls	PPV_{OLC}	$HFOV_{OLC}$	PPV_{CON}
TLC (mL/kg)	88 ± 12	95 ± 20†	88 ± 20‡	50 ± 14
Crs_{max} (mL/kPa/kg)	78 ± 17	91 ± 23‡	90 ± 31‡	39 ± 14
Gruenwald index	1.00 ± 0.07	1.03 ± 0.10§	1.04 ± 0.04‡	0.92 ± 0.05
Protein (mg/mL)	0.30 ± 0.21	0.33 ± 0.20‡	0.40 ± 0.23‡	0.70 ± 0.15
Recovery BAL (%)	85 ± 5	90 ± 4	87 ± 7	87 ± 4

* Data are mean ± SD. BAL, bronchoalveolar lavage.
† $p < 0.001$ *vs* PPV_{CON} group.
‡ $p < 0.01$ *vs* PPV_{CON} group.
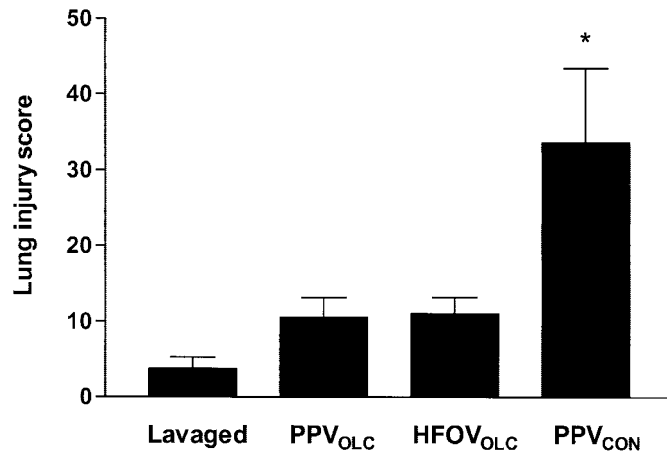§ $p < 0.05$ *vs* PPV_{CON} group.

Figure 5. Semiquantitative lung injury scores for lavaged histologic controls and the ventilation groups. *$p < 0.01$ *vs* both OLC groups.

indicating deteriorating pulmonary mechanics. These changes in Pa_{CO_2} levels during PPV_{CON} have also been reported in other animal models (22).

Although the pressure amplitude in the PPV_{OLC} group was much lower than in the PPV_{CON} group, mean Pa_{CO_2} levels in the PPV_{OLC} group remained stable and within the target range during the ventilation period. Hypercapnia during ventilation with small pressure amplitudes in the PPV_{OLC} group is prevented by more efficient ventilation (less pulmonary shunting) after alveolar recruitment and stabilization, and by increasing ventilatory rate. Although it seems inappropriate to compare two PPV groups with different ventilation settings and adjustments, we have to bear in mind that the present study did not aim to compare different settings but different ventilation strategies. The reported differences in ventilation settings are secondary to the ventilation strategy used and, in our opinion, therefore make the comparison valid.

Recruitment of collapsed alveoli requires inspiratory airway pressures that overcome the critical opening pressures of these alveoli, which implies application of high inspiratory airway pressures for a brief period (10). In the present study, initial mean opening pressures up to 3.92 kPa (40 cm H_2O) were needed to recruit collapsed alveoli in both OLC groups. These levels are somewhat higher than previously reported in newborn and adult animals, but, during these latter experiments, lower Pa_{O_2}/Fi_{O_2} ratios were used to define a fully recruited lung

(5, 22). Once again, similar to those previous reports, our study shows that the use of such high pressures are essential to fully recruit the lung and, more important, that these high-pressure levels themselves do not lead to additional VILI if applied for a short period of time.

Closing pressures in both OLC groups were much lower than the opening pressures. This phenomena can be explained by the law of LaPlace, which states that because of the increase of its radius, a recruited alveolus will require a lower airway pressure level to prevent alveolar collapse (8, 9).

Determination of opening and closing pressures at the end of the ventilation period showed a significant decrease in both OLC groups, indicating increased alveolar stability. This finding is in accordance with the superior lung mechanics (TLC, Crs_{max}, and Gruenwald index) found in the PPV_{OLC} and the $HFOV_{OLC}$ group at the end of the ventilation period (Table 3). Comparing both OLC groups to nonventilated healthy controls showed no significant differences in pulmonary mechanics after 5 h of ventilation.

These findings suggest that, during this short ventilation period, the lung of the newborn piglet is capable of regaining alveolar stability comparable to the healthy nonlavaged lung. One possible explanation could be an increased secretion of endogenous surfactant. Others have shown that stretching but not overdistending the alveoli can stimulate surfactant secretion, thus increasing alveolar stability (34). However, as we did not measure surfactant activity in the present study, we cannot substantiate this possibility.

The fact that, in contrast to both OLC groups, lung function did deteriorate in the PPV_{CON} group could be explained by the increased alveolar influx of protein. Besides being an important indicator for VILI, protein infiltrating the alveolar space is considered one of the important inhibitors of surfactant (35, 36). We found protein levels in broncho-alveolar lavage of both the OLC groups to be comparable to healthy nonventilated controls (Table 3). In contrast, alveolar protein levels in the PPV_{CON} group were significantly increased after 5 h of ventilation.

The histologic changes found in the PPV_{CON} group are consistent with this increase in alveolar protein infiltration and deterioration of lung mechanics (Fig. 5). The higher degree of atelectasis, hyaline membrane formation, and interstitial cellular infiltration in the PPV_{CON} group could be considered the start of alveolar and interstitial inflammation, currently considered one of the most important pathways for developing chronic lung disease of the newborn (29, 37).

The fact that the lung injury scores in both the OLC groups were higher than lavaged histologic controls once again shows that, independent of the strategy used, mechanical ventilation will always result in some degree of VILI in the surfactant-depleted lung. However, by applying the OLC during mechanical ventilation, the amount of VILI can be markedly reduced.

This optimization of gas exchange and the attenuation of VILI found in our study are consistent with earlier reports exploring the OLC during both HFOV and PPV, adding more evidence to the superiority of OLC ventilation and to the fact that the ventilation strategy is probably more important than the type of ventilator used (5, 6, 16, 22, 23, 26).
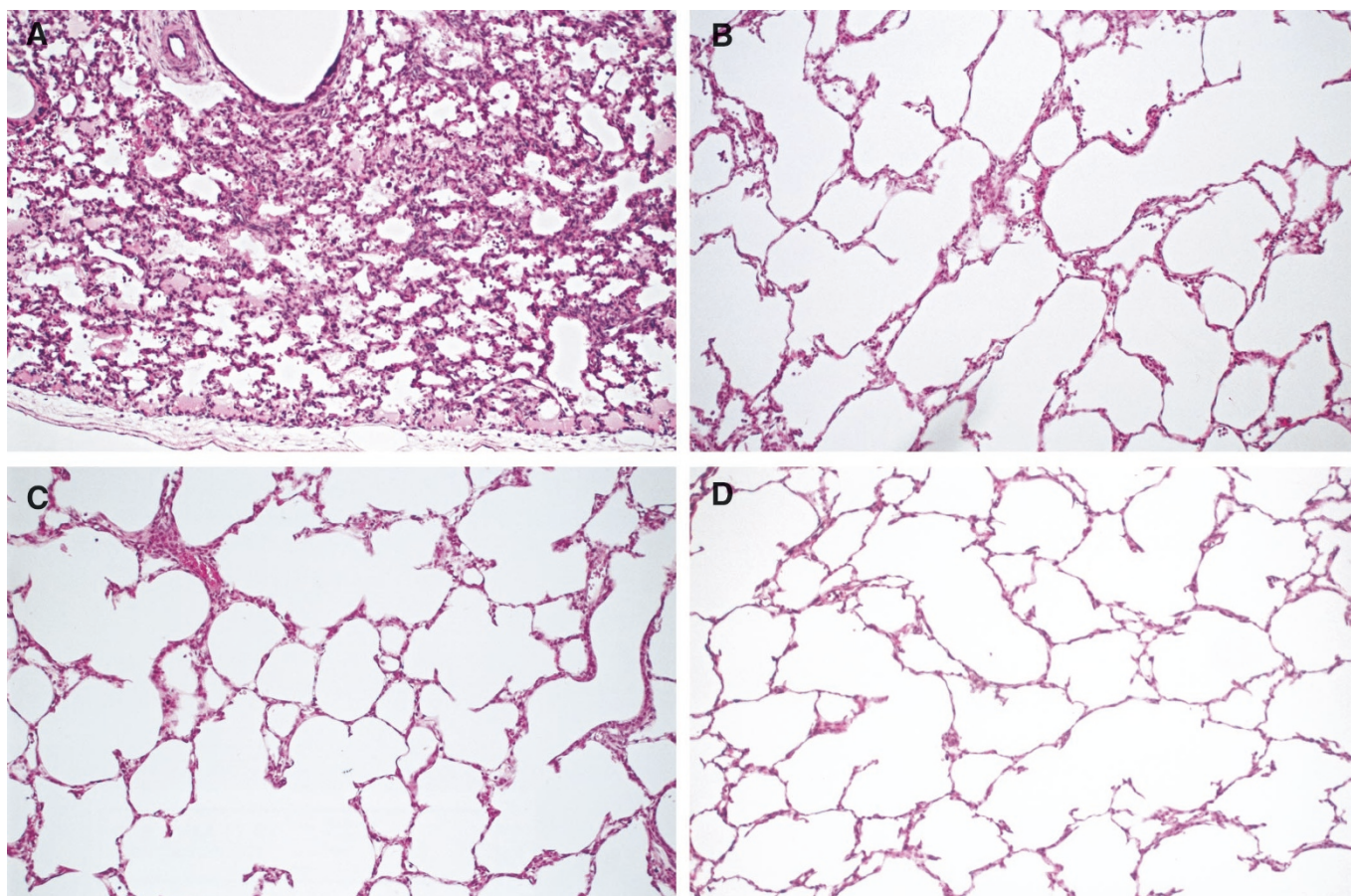
Figure 6. Sections of lungs stained with hematoxylin-eosin, magnification $50\times$. (*A*) Diffuse atelectasis, hyaline membrane formation, and cellular infiltration in the lung of an animal treated with PPV_{CON}. (*B*) Increased cellular infiltration in the lung of an animal treated with PPV_{OLC}. (*C*) Increased cellular infiltration in the lung of an animal treated with $HFOV_{OLC}$. (*D*) Minimal signs of structural damage to the lung of an animal undergoing lavage only.

Which aspect of the OLC contributes most to the differences between both OLC groups and the PPV_{CON} group cannot be answered by the present study. The aim of our study was to compare the OLC during PPV with conventional PPV as used in daily practice in the treatment of acute respiratory failure in neonates. However, based on previous studies it is reasonable to assume that all mentioned aspects of the OLC contribute to some extent to optimization of gas exchange and the reduction of VILI. Rimensberger *et al.* (38) clearly showed that, during PPV using optimal PEEP and small tidal volumes, application of a sustained inflation is essential in preventing deterioration of lung function. This beneficial effect of a sustained inflation on lung function during low tidal volume PPV was also reported by Bond *et al.* (39). On the other hand, McCulloch *et al.* (5) reported the importance of maintaining adequate lung volume after a recruitment maneuver during HFOV. Finally, Froese *et al.* (40) showed that establishing an adequate end-expiratory lung volume by using high tidal volumes and sub-optimal levels of PEEP during PPV will only result in a temporary improvement in gas exchange followed by deteriorating oxygenation and lung condition.

Recent studies have shown that hypercapnia can attenuate lung injury whereas hypocapnia may increase lung injury (41–43). Although these experiments were performed in isolated perfused lungs with Pa_{CO_2} levels in the extreme limits, we cannot rule out that the differences in Pa_{CO_2} reported in the present study influenced the differences in VILI. Future *in vivo* experiments should further clarify whether Pa_{CO_2} also influences lung injury if levels are closer to the normal range and differences are small, as in the present study.

Several studies reported no hemodynamic differences between HFOV and conventional PPV (22, 44); our study confirms these findings. However, in our PPV_{OLC} group, CVP was significantly higher than in the other two groups (Table 2). We can only speculate on the reasons for this difference. Because no differences were found in blood pressure, heart rate, and acid-base status, cardiac failure is unlikely to have caused this rise in CVP. Furthermore, the amount of volume expansion did not differ between the three groups.

Although not statistically significant, there was a clear trend to higher CVP values in the PPV_{OLC} group before randomization (healthy and lavaged), which could in part explain the differences found during the ventilation period. The difference could also be explained by the higher PEEP level in the PPV_{OLC} group compared with the PPV_{CON} group. However, the fact that the high CDP during $HFOV_{OLC}$ did not result in an increased CVP seems to weaken this explanation. Nevertheless, the question whether CVP is also influenced by the ventilation mode remains to be answered in future studies using more invasive hemodynamic monitoring.

In conclusion, the present study shows for the first time in a surfactant-depleted newborn animal model that applying the OLC during PPV is feasible, and results in superior gas exchange and a reduction in VILI compared with conventional PPV. Furthermore, the magnitude of these beneficial effects is comparable to HFOV. These results are important in those clinical situations where HFOV is not an option or is limited due to the lack of availability of proper equipment.

As the present study only explored the short-term effects of PPV$_{OLC}$, future animal studies should investigate long-term effects on gas exchange and VILI.

Furthermore, application of PPV$_{OLC}$ in different animal models of neonatal lung injury, may identify which infants will benefit most from this ventilation strategy. Depending on these results, implementation by randomized controlled trials in human newborn infants should be the next step and OLC PPV as described here should not be applied clinically outside controlled studies.

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