

# Surfactant nebulization therapy during NIPPV ventilation in surfactant-deficient newborn piglets

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## Abstract

**Background:** In recent years, nasal intermittent positive pressure ventilation (NIPPV) has been growing in popularity as a form of noninvasive ventilation for respiratory support in the initial treatment of neonates with surfactant (SF) deficiency. The combination of this type of ventilation with noninvasive SF administration (by nebulization) is an attractive treatment option for respiratory distress syndrome (RDS)-associated pathophysiology of the neonatal lungs. In this study, we aimed to test the tolerability and efficacy of SF nebulization during NIPPV for the treatment of neonatal RDS.

**Methods:** Spontaneously-breathing newborn piglets ( $n = 6/\text{group}$ ) with bronchoalveolar lavage (BAL)-induced RDS were assigned to receive during NIPPV (180 min): poractant alfa (400 mg/kg) via an investigational customized vibrating-membrane nebulizer (eFlow-Neos) or poractant alfa (200 mg/kg) as a bolus using the Insure method or no surfactant (controls).

**Measurement and results:** We assessed pulmonary, hemodynamic and cerebral effects and performed histological analysis of lung and brain tissue. After repeated BAL, newborn piglets developed severe RDS ( $F_{\text{IO}_2} = 1$ ,  $\text{pH} < 7.2$ ,  $P_{\text{aCO}_2} > 70$  mmHg,  $P_{\text{aO}_2} < 70$  mmHg,  $C_{\text{dyn}} < 0.5$  ml/cmH<sub>2</sub>O/kg). In both SF-treated groups, we observed rapid improvement in pulmonary status and also similar hemodynamic, cerebral behavior, and lung and brain injury scores.

**Conclusion:** Our results in newborn piglets with severe BAL-induced RDS show the administration of nebulized poractant alfa using the eFlow-Neos nebulizer during NIPPV to be well tolerated and efficacious, suggesting that this noninvasive SF administration option should be explored further.

## KEYWORDS

brain, histology, nebulization, NIPPV, respiratory distress syndrome, surfactant

Carmen Rey-Santano and Victoria Mielgo contributed equally to this study.

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## 1 | INTRODUCTION

Over recent years, the approaches used for surfactant (SF) administration and ventilation in neonatal intensive care units (NICUs) have changed greatly seeking to minimize the use of invasive mechanical ventilation (MV) for the treatment of respiratory distress syndrome (RDS). New approaches that have emerged for SF administration include the intubation-SF-extubation (Insure) method, the less invasive surfactant administration (LISA) also known as minimally invasive SF therapy (MIST) methods, involving intratracheal SF instillation using a thin catheter (e.g., vascular catheter or nasogastric tube), and also various techniques not yet approved, such as nebulization, pharyngeal administration, and laryngeal mask airway-guided administration.<sup>1–3</sup> Of these options, the least invasive way to administer SF is nebulization, which avoids the risks related to laryngoscopy and bolus fluid therapy.<sup>2,4</sup> An investigational customized nebulizer, based on vibrating-membrane technology (eFlow-Neos Nebulizer; Pari Pharma GmbH) and miniaturized for use in neonates, has been shown to deliver therapeutically useful doses of SF to the lungs.<sup>5–8</sup> In particular, its clinical effects were shown to be long lasting when the SF was administered at a dose of 400 mg/kg,<sup>9,10</sup> and after receiving only 200 mg/kg, neonates with mild RDS were less likely to need MV.<sup>11</sup>

On the other hand, the use of noninvasive ventilation (NIV) as the primary mode of respiratory support in spontaneously breathing preterm infants with RDS is becoming more widely accepted. The two NIV techniques most commonly used in NICUs are nasal continuous positive airway pressure (NCPAP) and nasal intermittent positive pressure ventilation (NIPPV). In a small but randomized study in premature infants on either NCPAP or NIPPV, the latter was associated with less need for MV and SF treatment (administered using a LISA/MIST technique) in the first 72 h after birth.<sup>12</sup> High rates of lung deposition of aerosolized SF during NIPPV were observed in a study in newborn piglets<sup>6</sup>; however, though several studies have explored combining nebulized SF with NCPAP, this being the most commonly used ventilation strategy,<sup>13,14</sup> only one clinical study has been conducted so far with NIPPV.<sup>15</sup>

Our hypothesis was that the combination of NIPPV and SF nebulization, using the aforementioned eFlow-Neos nebulizer, would produce an improvement in physiological response similar to that achieved by administering SF using the Insure method. The aim of this study was to assess tolerability and efficacy of SF nebulization with this device applied while using NIPPV for NIV in the treatment of neonatal RDS. Specifically, in spontaneously-breathing newborn piglets with bronchoalveolar lavage (BAL)-induced RDS, we investigated the acute response to the combination of these two noninvasive treatments in terms of gas exchange and hemodynamics, as well as oxygen metabolism and brain and lung injury scores.

## 2 | MATERIALS AND METHODS

### 2.1 | Animal preparation

All experiments were conducted following a protocol that complies with Spanish and European regulations for research with animals (UE2010/63-RD53/2013) and was approved by the Ethics Committee for Animal Welfare of Biocruces Bizkaia Health Research Institute. The methods are similar to those in our previous study exploring SF dose-response relationships in the same animal model and are described in detail elsewhere.<sup>16</sup>

In brief, 2- to 4-day-old newborn piglets<sup>17–19</sup> were sedated with ketamine (15 mg/kg), diazepam (2 mg/kg) and atropine (0.05 mg/kg) i.m. and anesthetized with sevoflurane (2%–3%). They were ventilated with a positive pressure ventilator (VIP Bird; Bird Products Corp.) through a cuffed endotracheal tube (ET) and the initial settings were:  $F_{iO_2}$  = 0.21–0.28, respiratory frequency ( $f_R$ ) = 28 breaths/min, positive end-expiratory pressure (PEEP) = 3 cmH<sub>2</sub>O, and positive inspiratory pressure (PIP) = 9–11 cmH<sub>2</sub>O adjusted to achieve a tidal volume ( $V_T$ ) = 8–10 ml/kg.<sup>9,16,20</sup>

For monitoring mean arterial blood pressure (MABP) and heart rate (HR) and obtaining blood samples for gas analysis, an arterial catheter was inserted into the femoral artery. Further, for administering fluid therapy and obtaining venous blood samples, a 5Fr dual-lumen catheter was placed in the jugular vein. An ultrasonic flow probe (Transonic Systems Inc.) was used to measure blood flow in the right common carotid as a proxy for cerebral blood flow. Heat lamps were used to keep rectal temperature at 38–39°C.

### 2.2 | Study design including induction of lung injury

BAL (30 ml/kg; 37°C with  $F_{iO_2}$ : 1) was performed to induce SF-deficient lung injury.<sup>20,21</sup> At the end of BAL procedure, positive pressure ventilation settings were  $F_{iO_2}$  = 1.0<sup>21–23</sup> and PEEP = 5 cmH<sub>2</sub>O, and to avoid barotrauma,  $f_R$  and PIP were adjusted to a maximum of 42 breaths/min and 25 cmH<sub>2</sub>O, to maintain  $V_T$  = 8–10 ml/kg. Lavage procedures were repeated (every 5 min) until arterial blood partial pressure of oxygen ( $P_{aO_2}$ ) < 100 mmHg. After allowing the piglets to stabilize for 30 min on positive pressure ventilation, they received an i.v. bolus dose of 20 mg/kg of caffeine citrate (Peyona 20 mg/ml; Chiesi Farmaceutici, Parma) to stimulate spontaneous breathing and were fitted with short binasal prongs (made by cutting and joining two pieces of ET, with an internal diameter of 3–5 mm and length of 4 cm, matched to the size of our piglets' nasal orifice). Having established spontaneous breathing, piglets were randomly assigned, using a sealed envelope method, to one of three groups:

- NIPPV alone group ( $n = 6$ ): the ET was removed and animals were maintained on NIPPV for 180 min, without SF treatment.
- NIPPV-Insure (Insure) group ( $n = 6$ ): 200 mg/kg of poractant alfa (Curosurf; Chiesi Farmaceutici, Parma) was administered through the ET, which was then immediately removed, and animals were maintained on NIPPV and followed up for 180 min from SF administration.
- NIPPV-Neb-Surf (NS) group ( $n = 6$ ): the ET was removed, a volume of 400 mg/kg of poractant alfa was placed in the reservoir and SF was administered using the eFlow-Neos nebulizer (Pari Pharma) placed between the prongs and the NIPPV circuit, the nebulizer was removed (immediately after SF nebulization), and animals were maintained on NIPPV and followed up for 180 min from SF administration.

Initial NIPPV settings were:  $f_R$  of 40 breaths/min; PEEP 5 cmH<sub>2</sub>O and PIP 15–17 cmH<sub>2</sub>O at  $F_{IO_2} = 1$ . These were then adjusted based on individual animal's pulmonary status, seeking to keep  $P_{aO_2}$  and  $P_{aCO_2}$  within the ranges of 80–100 mmHg and 35–45 mmHg, respectively. To avoid oxygen induced lung injury,  $F_{IO_2}$  was reduced as soon as  $P_{aO_2}$  improved to maintain values within the range of 80–100 mmHg.

### 2.3 | Physiological measurements

In all randomized piglets, we measured directly or calculated the following measurements:

- Arterial pH,  $P_{aO_2}$ ,  $P_{aO_2}/F_{IO_2}$  ratio,  $P_{aCO_2}$  and base excess, lactic acid, and glucose (GemPremier4000; Instrumentation Laboratory);
- Hemodynamic parameters, namely, HR, MABP, and carotid blood flow;
- Oxygen delivery (OD), oxygen consumption ( $VO_2$ ) and intrapulmonary shunt ratio ( $Q_s/Q_t$ ) (IntelliVue Monitor; Philips Medical System), using the following equation:  $Q_s/Q_t$  (%) =  $100 \times (1.34 \times Hb + 0.0031 \times P_{AO_2} - C_{aO_2}) / (1.34 \times Hb + 0.0031 \times P_{AO_2} - C_{vO_2})$ , where Hb is hemoglobin (g/dl);  $P_{AO_2} = F_{IO_2} \times (P_{atmospheric} - 47) - P_{aCO_2}$ ;  $C_{aO_2}$  is arterial O<sub>2</sub> content and  $C_{vO_2}$  is mixed venous O<sub>2</sub> content.

All these measurements were obtained at the following time points: immediately after surgery; on intubation at baseline (basal values); immediately after inducing RDS (BAL values); after the stabilization (ST) period (to confirm respiratory failure); immediately after extubation; 15 and 30 min after the start of NIPPV, and then every 30 min during NIPPV until the end of the experiment, at 180 min. The measurements in NS group started from the beginning of nebulization for first 15–30 min measurement on NIPPV.

In addition, airway flow, mean airway pressure and  $V_T$  with a flow sensor (placed between the circuit and the ET), and dynamic compliance ( $C_{dyn}$ ),  $V_T$  and airway resistance with a computerized system (M1014A; Philips Medical System) were measured at baseline; immediately after inducing RDS; and after the ST period. In all

animals, it was not feasible to measure lung mechanics after extubation, when NIPPV was established. Hence, at the end of the experiment, they were re-intubated and connected to mechanical ventilation (using the same settings as at baseline), and after 5 min of ST, lung mechanics were measured.

### 2.4 | Lung tissue analysis

After animal sacrifice, the lungs were removed and perfused with saline. For biochemical analysis, the left lung was isolated, occluded, submerged in liquid nitrogen and stored at  $-80^\circ\text{C}$  until use, and for histological analysis, the right lung was fixed in 4% formalin at 15 cmH<sub>2</sub>O.

Samples were taken from the frozen lungs to measure interleukin-8 (IL-8), IL-1 $\beta$ , and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels using specific enzyme-linked immunosorbent assay kits for porcine interleukins (Abnova), and protein levels using the Bradford method (Bio-Rad).<sup>24</sup> The formalin-fixed tissue was cut into 5- $\mu\text{m}$  sections, which were placed on slides and stained with hematoxylin-eosin. Lung injury was assessed with light microscopy by a pathologist blinded to group allocation who rated the extent of injury using a semi-quantitative scoring system. As described elsewhere, alveolar and interstitial hemorrhage, alveolar and interstitial inflammation, atelectasis, edema, and necrosis were each scored on a 0- to 4-point scale: 0 indicating no lung injury; 1, 2, and 3 injury to 25%, 50%, and 75% of the field, respectively; and 4 injury across the field.<sup>25,26</sup>

### 2.5 | Brain tissue analysis

For histological analysis, the brain was fixed (4% formalin) and divided into brain stem and cerebellum, cortex, and inner regions (striatum, thalamus, and hippocampus). As for lung injury, brain injury was assessed with light microscopy by a pathologist blinded to group allocation who rated the extent of injury using a semi-quantitative scoring system. As described elsewhere, edema, hemorrhage, inflammation, infarction and necrosis were each scored on a 0- to 3-point scale: 0 indicating no injury; and 1, 2, and 3 mild, moderate, and severe injury across the field. In total, 20 fields were analyzed and more than five necrotic cells/field was considered to indicate neuronal necrosis (score range: 0–20).<sup>16,26</sup>

### 2.6 | Statistical analysis

Data were expressed as mean  $\pm$  SEM. Levene's test was used to assess the homogeneity of variance between the different treatments and the Kolmogorov-Smirnov test to assess whether the data were normally distributed (JMP8; Statistical Discovery, SAS). One- and two-way analysis of variance were performed to analyze gas exchange, hemodynamics, oxygen metabolism, and lung mechanics by group and time of repeated measures. The Wilcoxon test was used to assess lung biochemical results and injury score and brain injury score. A  $p$  value of less than .05 was considered significant.

### 3 | RESULTS

The 18 newborn piglets used in the study were from different litters but similar in age ( $3 \pm 1$  days) and size ( $2.0 \pm 0.1$  kg). To induce appropriately severe lung injury, at least 13 BALs (range: 13–15) were required (resulting parameters:  $P_{aO_2} < 100$  mmHg; NIPPV:  $61 \pm 2$  mmHg; Insure:  $64 \pm 3$  mmHg; NS:  $63 \pm 3$  mmHg). Differences between groups in the numbers of BALs required and the volume of lavage fluid recovered did not reach significance. The mean SF nebulization time was  $48 \pm 1$  min.

#### 3.1 | Pulmonary outcomes

##### 3.1.1 | Gas exchange and lung mechanics

Values of pH,  $P_{aO_2}/F_{iO_2}$ ,  $P_{aCO_2}$ , and  $C_{dyn}$  were similar across the groups at baseline, after induction of SF-deficient lung injury and after 30 min of ST (Table 1 and Figure 1). In all groups, BAL was followed by significant decreases in  $P_{aO_2}/F_{iO_2}$  (Figure 1A),  $C_{dyn}$  (Figure 1B), and pH (Table 1) and a significant increase in  $P_{aCO_2}$  (Figure 1C), consistent with severe RDS.

Improvements were observed in  $P_{aO_2}/F_{iO_2}$  ratio,  $P_{aCO_2}$ , and pH in all groups. Comparing Insure and NIPPV alone, the parameters improved more rapidly in the Insure group (Figure 1; Table 1). Specifically, RDS had resolved in the Insure group by the end of the experiment ( $P_{aO_2}/F_{iO_2}$  ratio  $> 350$  mmHg), whereas animals given only NIPPV continued to have mild-to-moderate RDS ( $P_{aO_2}/F_{iO_2}$  ratio  $< 280$  mmHg). In line with the improvements observed in the Insure group, the values of these parameters also improved significantly after the administration of 400 mg/kg of nebulized SF. Further, in the NS group,  $P_{aCO_2}$  was  $\geq 50$  mmHg during the nebulization period (Figure 1C), and decreased rapidly after nebulizer removal, carbon dioxide returning to within the normal range by 60 min after starting nebulization. Notably,  $P_{aCO_2}$  remained higher in animals in the NIPPV alone group than those in the SF-treated groups throughout the experiment.

Regarding lung compliance,  $C_{dyn}$  returned to or close to baseline (80%–85%) in both SF-treated groups, the extent of recovery being significantly greater than in the NIPPV alone group ( $C_{dyn}$  returning to 60% of baseline). In contrast,  $V_T$  and resistance parameters did not differ between groups (data not shown).

For the first 30 min after SF administration, respiratory frequency was significantly higher in both SF-treated groups (Table 1). After that, no significant between-group differences were detected.

##### 3.1.2 | Lung inflammatory markers and lung injury

After 3 h on NIPPV, both SF-treated groups had lower cytokine levels than those seen in animals only given NIPPV. Specifically, compared to levels in the NIPPV alone group (IL-8:  $33 \pm 3$  pg/mgprot; TNF- $\alpha$ :  $38 \pm 3$  pg/mgprot; IL-1 $\beta$ :  $360 \pm 27$  pg/mgprot), Insure group

animals had significantly lower levels of all cytokines (IL-8:  $19 \pm 2$  pg/mgprot; TNF- $\alpha$ :  $27 \pm 2$  pg/mgprot; IL-1 $\beta$ :  $260 \pm 28$  pg/mgprot), while NS group animals had significantly lower levels of IL-8 and TNF- $\alpha$  (IL-8:  $24 \pm 3$  pg/mgprot; TNF- $\alpha$ :  $27 \pm 3$  pg/mgprot), but the difference in the case of IL-1 $\beta$  did not reach significance (IL-1 $\beta$ :  $280 \pm 25$  pg/mgprot,  $p = .053$  vs. NIPPV group). Although all values were within normal physiological ranges, significantly more edema and interstitial hemorrhage were observed in both SF-treated groups than in the NIPPV alone group (Table 2 and Figure 2).

#### 3.2 | Intrapulmonary shunt and oxygen transport

BAL was followed by a significant increase in  $Q_s/Q_t$  (Figure 3A), but none of the systemic oxygen metabolism parameters changed significantly (Table 1). In the NIPPV alone group, there were no significant changes in OD or  $V_{O_2}$  after 3 h on ventilation (Table 1), and though  $Q_s/Q_t$  gradually improved, it did not reach baseline (Figure 3A). In contrast, in both SF-treated groups,  $Q_s/Q_t$  recovered to baseline by 2 h after treatment (Figure 3A). Further,  $V_{O_2}$  values were significantly higher in the groups receiving SF treatment as well as NIPPV than that on NIPPV alone (Table 1), without significant differences in OD.

#### 3.3 | Hemodynamic assessment

Hemodynamic parameters did not differ significantly between the groups at baseline. Further, following BAL, there were no significant changes in MABP (Figure 3B), but the HR increased significantly (Figure 3C). Over the study period, MABP values remained similar in all groups studied, while HR was significantly higher in both Insure and NS groups at 2 h after the start of treatment than in those given NIPPV alone.

#### 3.4 | Cerebral evaluation

In all groups, carotid blood flow increased significantly following BAL (Figure 3D). Subsequently, during NIPPV with or without SF treatment (i.e., in all groups), carotid blood flow decreased steadily reaching baseline values by 1 h after the start of treatment. Further, brain injury scores were low in all three groups, with similar scores for necrosis, edema, hemorrhage, inflammation, and infarction for all regions studied (Table 3 and Figure 2).

## 4 | DISCUSSION

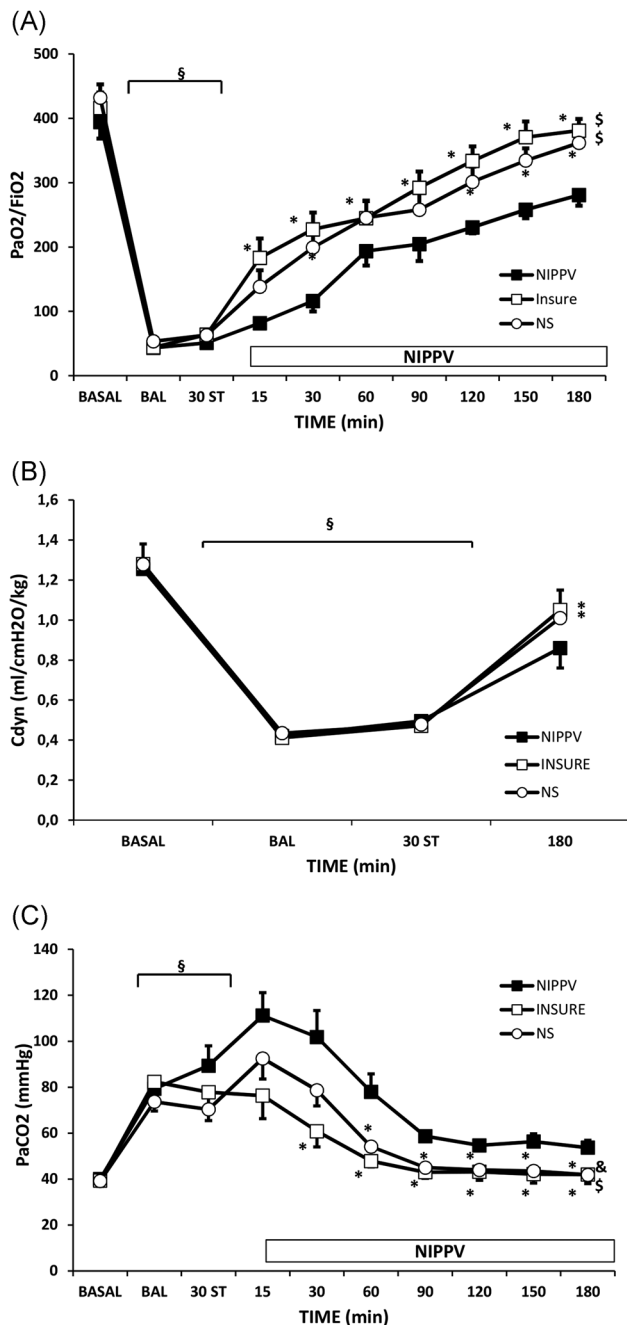
In our spontaneously-breathing newborn piglet model of SF-deficient lung injury, we have shown that the investigational customized eFlow-Neos nebulizer is well-tolerated and efficacious, in that SF nebulization administered using this device during NIPPV is

**TABLE 1** pH, respiratory rate, and oxygen metabolism in BAL-induced RDS newborn piglets treated with noninvasive ventilation (NIPPV) with surfactant treatment, using the Insure method or nebulized surfactant (NS)

	Groups	Basal	BAL	30 ST	15 min	30 min	60 min	90 min	120 min	150 min	180 min
pH	NIPPV	7.41 ± 0.03	7.16 ± 0.03\$	7.14 ± 0.04\$	7.06 ± 0.05	7.13 ± 0.05	7.24 ± 0.05	7.36 ± 0.02	7.38 ± 0.02	7.38 ± 0.02	7.39 ± 0.02
	Insure	7.38 ± 0.02	7.13 ± 0.02\$	7.14 ± 0.03\$	7.26 ± 0.03*	7.35 ± 0.02*	7.41 ± 0.02*	7.45 ± 0.01*	7.45 ± 0.02*	7.46 ± 0.02*	7.47 ± 0.02*\$
	NS	7.41 ± 0.02	7.17 ± 0.02\$	7.18 ± 0.02\$	7.14 ± 0.04	7.20 ± 0.03#	7.33 ± 0.02#	7.40 ± 0.01*	7.43 ± 0.01*	7.44 ± 0.01*	7.43 ± 0.01* <sup>‡</sup>
Respiratory rate (bpm)	NIPPV	28 ± 1	42 ± 0\$	42 ± 0\$	44 ± 3	49 ± 3	48 ± 3	48 ± 3	44 ± 4	44 ± 3	41 ± 3
	Insure	28 ± 1	42 ± 0\$	42 ± 0\$	54 ± 2*	57 ± 3*	50 ± 3	48 ± 3	43 ± 2	42 ± 1	39 ± 1
	NS	28 ± 1	42 ± 0\$	42 ± 0\$	54 ± 3*	57 ± 2*	49 ± 3	43 ± 3	44 ± 4	43 ± 3	43 ± 3
OD (ml/min)	NIPPV	62 ± 6	50 ± 5	59 ± 7	87 ± 8	104 ± 10	100 ± 7	92 ± 6	87 ± 6	91 ± 7	86 ± 8
	Insure	61 ± 4	49 ± 5	59 ± 7	84 ± 6	98 ± 7	99 ± 4	90 ± 5	86 ± 4	92 ± 4	83 ± 2
	NS	60 ± 4	56 ± 7	57 ± 3	84 ± 10	91 ± 10	94 ± 6	90 ± 4	80 ± 7	85 ± 6	83 ± 7
VO <sub>2</sub> (ml/min)	NIPPV	12 ± 2	12 ± 2	12 ± 2	10 ± 1	12 ± 2	13 ± 2	13 ± 3	13 ± 2	13 ± 2	15 ± 2
	Insure	12 ± 2	12 ± 2	17 ± 3	16 ± 3	21 ± 3*	21 ± 3*	19 ± 3	24 ± 5*	25 ± 3*	23 ± 4*
	NS	14 ± 1	14 ± 1	12 ± 1	12 ± 3	18 ± 1*	21 ± 1*	23 ± 2*	18 ± 1*	20 ± 2	21 ± 1*

Note: Values are expressed as mean ± SEM. Statistical differences <sup>§</sup>*p* < .05 versus basal point; \**p* < .05 versus NIPPV group and #*p* < .05 versus Insure (one-way ANOVA); <sup>‡</sup>*p* < .05 versus NIPPV group and <sup>‡</sup>*p* < .05 versus Insure group (two-way ANOVA).

Abbreviations: ANOVA, analysis of variance; BAL, bronchoalveolar lavage; NIPPV, nasal intermittent positive pressure ventilation; OD, oxygen delivery; RDS, respiratory distress syndrome; VO<sub>2</sub>, oxygen consumption.



**FIGURE 1** PaO<sub>2</sub>/F<sub>iO<sub>2</sub></sub> ratio, dynamic compliance (C<sub>dyn</sub>) and P<sub>aCO<sub>2</sub></sub> in newborn piglets with bronchoalveolar lavage-induced respiratory distress syndrome treated with noninvasive ventilation (nasal intermittent positive pressure ventilation [NIPPV]) with or without surfactant therapy (administered using the Insure method or nebulization [nebulized surfactant {NS}]). Values of (A) PaO<sub>2</sub>/F<sub>iO<sub>2</sub></sub>, (B) C<sub>dyn</sub>, and (C) P<sub>aCO<sub>2</sub></sub> in the NIPPV alone (black square), insure (white square), and NS (white circle) groups. C<sub>dyn</sub> value at 180 min was measured after the reintubation of the animals at the end of the study. <sup>§</sup>p < .05 versus baseline; \*p < .05 versus NIPPV alone group and #p < .05 versus Insure group (one-way analysis of variance); <sup>§</sup>p < .05 versus NIPPV alone group and <sup>§</sup>p < .05 versus Insure group (two-way analysis of variance). Values are expressed as mean ± SEM. 30 ST, 30-min period of stabilization

associated with a clinically-relevant improvement in acute physiological parameters, in particular, in oxygenation and lung function, and similar pulmonary, hemodynamic and cerebral and lung behavior to that observed with SF administration by the Insure method followed by NIPPV.

Natural SF administration is the most effective treatment of neonatal RDS, reducing mortality and morbidity in premature neonates. For many years, the traditional administration of SF via endotracheal intubation, bolus SF administration and prolonged MV has been the only approved method for administering SF in premature neonates with RDS. In recent years, however, the use of less invasive SF administration techniques (such as Insure, LISA/MIST, and even nebulization) and NIV strategies as the primary mode of respiratory support (NCPAP, NIPPV, etc.)<sup>2,4,12,27,28</sup> have been gaining acceptance in NICUs, seeking to avoid the side effects and risks<sup>29</sup> associated with more invasive approaches to SF administration and MV.

The use of less invasive SF administration techniques during NCPAP ventilation has been evaluated in animal and clinical studies, with positive results.<sup>1,2,4,9,10,16</sup> Further, there is some evidence that the positive effects observed may be enhanced if NIPPV were to be used instead of NCPAP.<sup>12,20,30</sup> Specifically, NCPAP only provides continuous distending pressure, to open the lungs and thereby prevent collapse of the alveoli during expiration, while NIPPV offers the same support plus ventilator breaths delivered at a set peak pressure, providing the benefits of NCPAP with less work of breathing.<sup>31</sup> Advantages of using NIPPV compared with NCPAP have been shown previously in infants given SF therapy with the Insure method.<sup>30,32</sup> Consistent with these findings, in a previous study with our animal model,<sup>20</sup> as in the current study, we observed that improvements in pulmonary outcomes (namely, gas exchange, lung mechanics, and lung inflammatory markers) were more rapid and significantly greater with NIPPV plus SF replacement therapy administered using the Insure method than with NIPPV alone.<sup>20</sup> Further, benefits of NIPPV over NCPAP were observed in a small randomized study in preterm infants, with a reduction in the need for MV and also for SF treatment (administered with a LISA/MIST technique) in the first 72 h after birth.<sup>12</sup> Nonetheless, only one previous study has investigated a minimally invasive method for SF administration (e.g., using a nebulizer) during NIPPV to treat neonatal RDS.<sup>15</sup> Although just 10% of patients were treated with SF delivered via a nebulizer plus NIPPV, the authors concluded that SF nebulization using non-invasive respiratory support reduced rate of intubation and SF instillation by nearly one-half.<sup>15</sup>

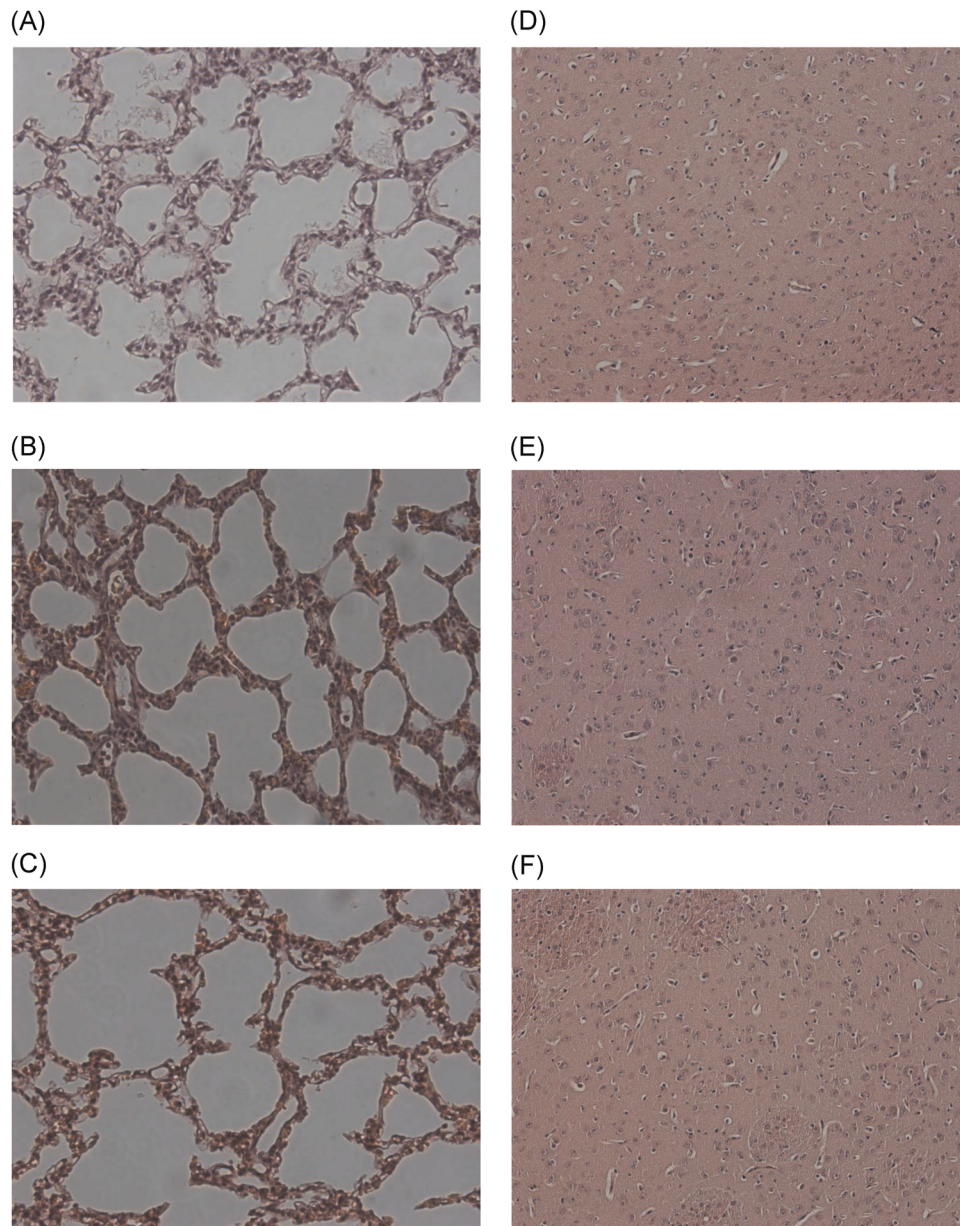
Nebulization of SF during NCPAP treatment using the eFlow Neos nebulizer has been investigated in randomized control trial in premature infants,<sup>11</sup> as well as in animals,<sup>5,6,9,10,16,16,33</sup> the results suggesting that this approach is both safe and feasible. This nebulizer has been customized considering the distinctive characteristics of SF (in particular, its lipid-protein composition and high viscosity) and has been shown to enhance the delivery of SF to the neonatal respiratory system during NCPAP, studies having documented appropriate particle sizes (2.5–3.5 μm), high distal airway delivery efficacies

**TABLE 2** Total lung injury scores in BAL-induced RDS newborn piglets treated with nasal continuous positive airway pressure (NIPPV) without or with surfactant treatment, using the Insure method, or NS

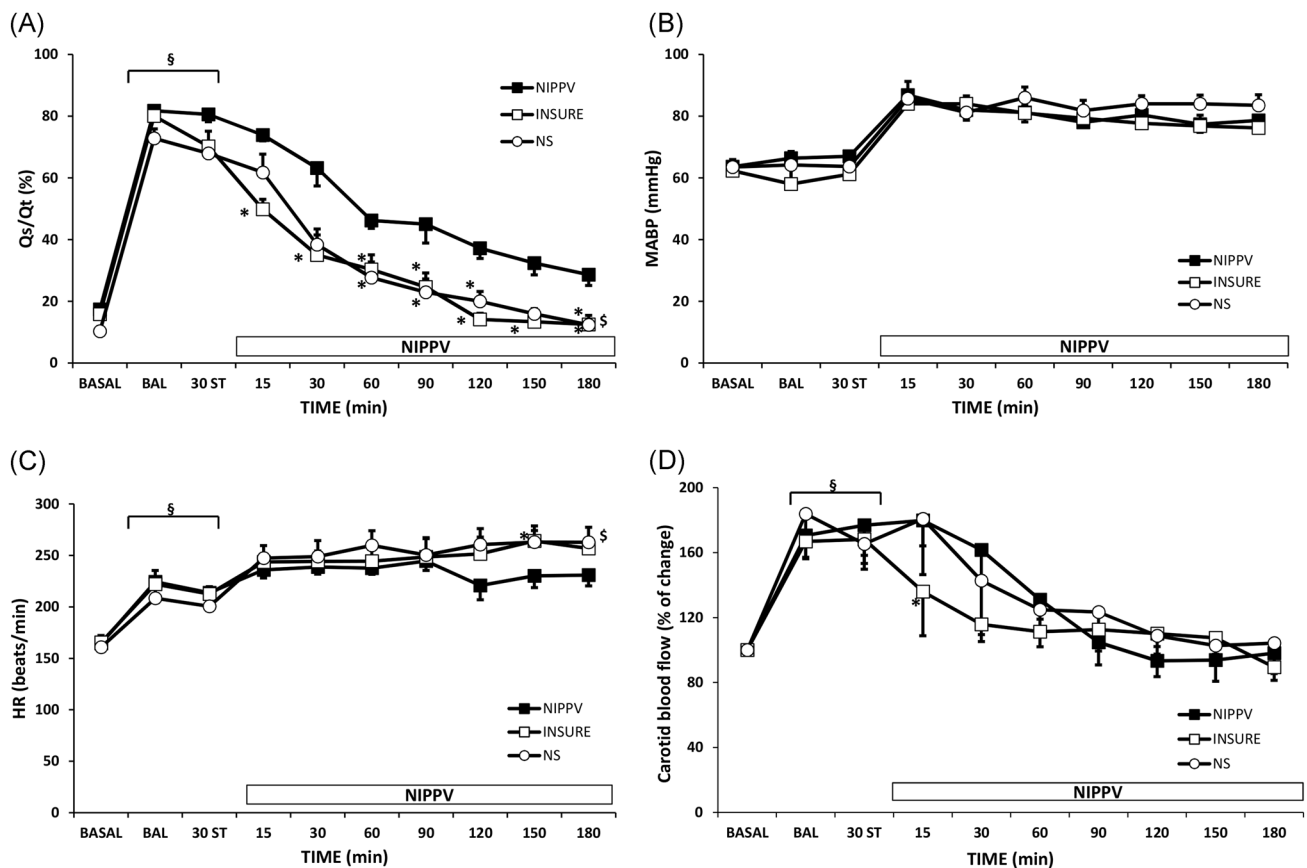
Groups	Atelectasis	Necrosis	Edema	Alveolar inflammation	Interstitial inflammation	Alveolar hemorrhage	Interstitial hemorrhage	Total
NIPPV	0.72 ± 0.19	0	0	0.66 ± 0.18	1.28 ± 0.21	0	0	2.67 ± 0.46
Insure	1.06 ± 0.18	0	0.20 ± 0.10*	1.00 ± 0.25	1.47 ± 0.21	0.06 ± 0.06	0.20 ± 0.11*	4.00 ± 0.56
NS	0.56 ± 0.18	0	0.33 ± 0.11*	0.94 ± 0.24	1.39 ± 0.26	0.06 ± 0.06	0.28 ± 0.11*	3.39 ± 0.62

Note: Values are expressed as mean ± SEM. Statistical differences \* $p < .05$  versus NIPPV group were assessed using analysis of variance.

Abbreviations: BAL, bronchoalveolar lavage; NIPPV, nasal intermittent positive pressure ventilation; NS, nebulized surfactant; RDS, respiratory distress syndrome.



**FIGURE 2** Photomicrographs ( $\times 200$  magnification) of representative sections of the (A–C) lung and (D–F) brain from animals in the NIPPV alone, Insure, and NS groups, respectively. Lung sections were cut from the middle lobe of the lung and brain sections from the striatum. NIPPV, nasal intermittent positive pressure ventilation; NS, nebulized surfactant [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 3** Intrapulmonary shunt ratio ( $Q_s/Q_t$ ) and carotid blood flow (D) in newborn piglets with bronchoalveolar lavage-induced respiratory distress syndrome treated with noninvasive ventilation (NIPPV) with or without surfactant therapy (administered using the Insure method or nebulisation [NS]). (A) Mean  $Q_s/Q_t$  and (B) mean carotid blood flow values in the NIPPV alone (black square), Insure (white square), and NS (white circle) groups.  $^{\S}p < .05$  versus baseline;  $^*p < .05$  versus NIPPV alone group and  $^{\#}p < .05$  versus Insure group (one-way analysis of variance);  $^{\$}p < .05$  versus NIPPV alone group and  $^{\S}p < .05$  versus Insure group (two-way analysis of variance). Values are expressed as mean  $\pm$  SEM. 30 ST, 30-min period of stabilization; NIPPV, nasal intermittent positive pressure ventilation; NS, nebulized surfactant

Groups	Necrosis	Edema	Inflammation	Hemorrhage	Infarct
NIPPV	8 (0–16)	0.4 (0–1)	0.3 (0–1)	0 (0–0)	0 (0–0)
Insure	8 (2–16)	0.4 (0–1)	0.3 (0–1)	0 (0–0)	0 (0–0)
NS	9 (0–17)	0.3 (0–1)	0.2 (0–1)	0 (0–0)	0 (0–0)

Note: No statistical differences were observed. Values are expressed as mean  $\pm$  range.

Abbreviations: BAL, bronchoalveolar lavage; NIPPV, nasal intermittent positive pressure ventilation; NS, nebulized surfactant; RDS, respiratory distress syndrome.

**TABLE 3** Total brain injury scores in BAL-induced RDS newborn piglets treated with NIPPV without or with surfactant treatment, using the Insure method NS

(of >14%) and maintenance of SF activity after nebulization.<sup>5,34,35</sup> Testing the lung deposition of nebulized SF in healthy newborn piglets during NIPPV, high lung deposition of SF was also observed (>20%), similar to that reported during NCPAP.<sup>6</sup>

This study in spontaneously breathing newborn piglets with SF-deficient lung injury was designed to investigate the tolerability and efficacy of administering nebulized poractant alfa (at a dose of 400 mg/kg selected based on the results of a previous study)<sup>16</sup> during NIPPV for the treatment of neonatal RDS. We observed better oxygenation, intrapulmonary shunt and lung mechanics in

animals treated with SF by either of the methods of administration studied, namely, the Insure method and nebulization, than in untreated controls. A small delay in the improvement in oxygenation (not statistically significant) was observed in NS group compared to the Insure group, attributed to the time required for nebulized SF to reach the lung. Although one clinical trial has administered SF nebulization during NIV (including NIPPV) with positive results,<sup>15</sup> most of the findings on nebulized SF during NIV have been obtained when this treatment was administered during NCPAP. As in our study, similar pulmonary improvements have been previously observed



during NCPAP plus SF nebulization in short-<sup>10,16</sup> and long-term<sup>9,11</sup> follow-up studies. Moreover, a benefit of nebulization of natural SF (with known anti-inflammatory properties)<sup>36</sup> was also observed when assessing lung inflammatory mediators, with lung IL-8 and TNF- $\alpha$  levels being similar to those with the Insure method, but significantly lower than those observed with NIPPV alone.

In our study, the animals on NIPPV alone developed hypercarbia and we attribute this to a lower capacity to achieve lung recruitment after lung injury; then, as more of the lung was recruited, carbon dioxide levels fell, though they remained somewhat higher than in SF-treated groups. Further, a transient increase in  $P_{aCO_2}$  was observed following SF nebulization (likely related to external dead space), but this parameter returned to normal on nebulizer removal, as occurred when SF nebulization was applied during NCPAP.<sup>16</sup>

Based on our pathological injury scores, it seems that SF administration using Insure or nebulization procedures results in higher values of edema and interstitial hemorrhage than NIPPV alone. These higher values could be related to the effect produced by SF in the neonatal lung including a rapid improvement in lung compliance, rapid fall in pulmonary vascular resistance, increase in pulmonary blood flow, and so on.<sup>37</sup> Nonetheless, as when interpreting physiological outcomes, we should note that lung damage may be visible (and rated with a low score) without being clinically relevant. Specifically, data published by Zimmermann et al.<sup>25</sup> and our research group using the same animal model<sup>16</sup> suggest that all the values obtained in lung injury score in all the groups in this study should not be considered to reflect poor outcomes in terms of histological lung injury. Nonetheless, longer studies are needed to confirm this.

As in previous studies,<sup>10,16</sup> nebulization was not associated with any other significant changes in MABP, HR or systemic oxygen metabolism. Subsequently, although MABP did not change significantly in any groups, HR and  $VO_2$  were higher in SF-treated groups than the NIPPV alone group, though in this group they also remained within the physiological range as in our previous research.<sup>20</sup> Moreover, as previously observed with SF nebulization during NCPAP,<sup>9,16</sup> neither method for SF administration seemed to have a clinically significant effect on brain injury score, confirming the safety of SF nebulization during NIPPV.

We should recognize that this study has various limitations. In particular, we studied newborn piglets (2–4 days old) rather than premature piglets. Although in the context of neonatal RDS, premature animal models provide clinically relevant models of preterm neonatal physiology, SF washout by repeated lavage has been successfully used to develop models of acute pulmonary failure in the context of RDS in both adult and juvenile animals.<sup>21,22,38</sup> Moreover, one of the major limitations of the preterm pig model is associated with the large size of the sow (280–350 kg) and the requirement for equipment suitable for handling such weights. Further, the resources required, in terms of expert staff and NICU equipment to deal with the resuscitation and initiation of noninvasive ventilation for a large number of piglets at preterm Caesarean section, means that this animal model is not feasible for many researchers. Another limitation is that, evidently, the nasal and

pharyngeal anatomy of piglets differs from that of human infants; on the other hand, an advantage of the newborn piglet model is that brain maturation, lung volume, and birth weights have been shown to resemble those of newborn infants. Nonetheless, while animal models serve to bridge the gap between clinical and laboratory research, extrapolation of our results to humans requires caution. Further, animals were only followed up to 3 h.

## 5 | CONCLUSION

Delivery of a pulmonary SF (poractant alfa) with an investigational customized nebulizer (the eFlow-Neos) was both well tolerated and efficacious in acutely relieving SF deficiency in spontaneously breathing newborn piglets on NIPPV. Nonetheless, longer studies and clinical trials are required to assess outcomes in the long term, such as the need for SF re-dosing or intubation, as well as ventilation time and physiological stability, compared to outcomes obtained with NIPPV alone.

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## CONFLICT OF INTERESTS

Drs. Rey-Santano, Mielgo, Gomez-Solaetxe, Salomone, Bianco and Gastiasoro disclose off-label use of the vibrating-membrane nebulizer (eFlow-Neos). Drs. Bianco and Salomone disclose that they are Chiesi employees.

## AUTHOR CONTRIBUTIONS

Carmen Rey-Santano contributed to funding acquisition (lead), methodology (equal), project administration (lead), supervision (equal), writing original draft (lead). Elena Gastiasoro contributed to investigation (equal), validation (equal), visualization (equal). Federico Bianco contributed to conceptualization (equal), investigation (equal), resources (equal), writing review, and editing (equal). Fabrizio Salomone contributed to conceptualization (equal), methodology (equal), resources (equal), supervision (equal), writing review, and editing (equal). Miguel Gomez-Solaetxe contributed to formal analysis (equal), funding acquisition (equal), methodology (equal), project administration (equal), supervision (equal), writing review, and editing (equal).

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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