


Cerebral oxygenation associated with INSURE versus LISA procedures in surfactant-deficient newborn piglet RDS model

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Abstract

Background: Nasal continuous-positive airway pressure (nCPAP) with the INSURE (INTubation-SURfactant-Extubation) or LISA (Less-Invasive Surfactant Administration) procedures are increasingly being chosen as the initial treatment for neonates with surfactant deficiency. Our objective was to compare the effects on cerebral oxygenation of different methods for surfactant administration: INSURE and LISA, using a nasogastric tube (NT) or a LISAcath® catheter, in spontaneously breathing SF-deficient newborn piglets.

Methods: Eighteen newborn piglets with SF-deficient lung injury produced by repetitive bronchoalveolar lavages were randomly assigned to INSURE, LISA-NT, or LISAcath® groups. We assessed pulmonary (gas exchange, lung mechanics, lung histology) and hemodynamic (mean arterial blood pressure, heart rate) changes, cerebral oxygenation (cTOI) and cerebral fractional tissue extraction (cFTOE), with near-infrared spectroscopy, carotid blood flow and brain histology.

Results: SF-deficient piglets developed respiratory distress ($F_{iO_2} = 1$, $pH < 7.2$, $P_{aCO_2} > 70$ mmHg, $P_{aO_2} < 70$ mmHg, $C_{d_{yn}} < 0.5$ mL/cmH₂O/kg). Rapid improvements in pulmonary status were observed in all surfactant-treated groups without hemodynamic alterations. In the INSURE group, a transient decrease in cTOI occurred during and immediately after surfactant administration, while cTOI only decreased during surfactant administration in the LISA-NT group and did not change significantly in the LISAcath® group. Brain injury scores were low in all surfactant-treated groups.

Conclusion: In spontaneously breathing SF-deficient newborn piglets, short-lasting decreases in cerebral oxygenation are associated with surfactant administration by the INSURE method or LISA using an NT, while no cerebral oxygenation changes occurred with LISA using a LISAcath®. Notably, none of treatments studied seems to have a negative impact on the neonatal brain.

KEYWORDS

cerebral oxygenation, INSURE, LISAcath®, NIRS, respiratory distress syndrome, surfactant

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

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1 | INTRODUCTION

When surfactant (SF) treatment of neonatal respiratory distress syndrome (RDS) was introduced during the 1990s, it was exclusively administered as a bolus into intubated babies who required mechanical ventilation. In recent decades, there have been important developments in RDS treatment including the use of non-invasive ventilation such as nasal continuous positive airway pressure (nCPAP),¹ and the development of different strategies for SF replacement.^{2–5} Early nCPAP is associated with reduced pulmonary morbidity and lower rates of bronchopulmonary dysplasia (BPD).⁶ Moreover, early SF administration has been shown to be more efficient than late treatment and is associated with decreases neonatal mortality, incidence of air leak syndromes and BPD.⁷ Hence, combining early nCPAP with early SF administration during spontaneously breathing seemed logical.

The administration of SF during nCPAP has been investigated considering two main options. On the one hand, the INSURE (INTubation-SUrfactant-Extubation) procedure combined with nCPAP reduces the need for and duration of mechanical ventilation, and length of oxygen therapy, as well as the need for additional SF doses.^{8,9} Nevertheless, the INSURE procedure carries its own risks, as a brief period of endotracheal intubation and mechanical ventilation is still required,¹⁰ and it can also be difficult to extubate infants following the procedure.¹¹ On the other hand, some authors have suggested using a less invasive SF administration (LISA) method, which involves the insertion of a nasogastric tube (NT), vascular catheter or a new catheter purpose-built for LISA, the LISAcath®, into the trachea and its removal immediately after SF instillation, while the patient is spontaneously breathing on nCPAP. Use of the LISA method has been shown to have beneficial effects on mortality, BPD, need for mechanical ventilation and survival in preterm infants.^{12–14} Nonetheless, both the LISA and INSURE procedures have been associated with side effects such as transient hypoxemia, bradycardia, and desaturation.^{12–14} Such alterations may produce changes in cerebral hemodynamics, oxygenation and electrical brain activity^{15–19} which could negatively influence long-term neurodevelopment.²⁰ To date, a relatively small number of studies have assessed the impact on the brain of different methods of SF administration (INSURE vs LISA).^{15–19} Moreover, no one has evaluated the effects on the lung and brain of using the new purpose-built catheter, LISAcath®, for SF administration.

In this context of uncertainty about the impact of different SF administration strategies on the brain, the hypothesis of the study was that the LISA procedure using an NT or LISAcath® catheter may potentially be as efficient and safe as the INSURE procedure. Our main objective was to compare these methods of SF administration evaluating their impact on the brain in development. In addition, the effect of SF administration on gas exchange, hemodynamic parameters and lung injury scores were assessed using each of these SF administration methods in spontaneously breathing SF-deficient newborn piglets.

2 | MATERIAL AND METHODS

2.1 | Animal preparation

The experimental protocol meets European and Spanish regulations for the protection of experimental animals (UE2010/63-RD53/2013) and was approved by the Ethics Committee for Animal Welfare of Biocruces Bizkaia Health Research Institute.

As previously described,²¹ 2- to 4-day-old newborn piglets were sedated with an i.m. ketamine-diazepam-atropine injection and anesthetized with sevoflurane (2–3%). A cuffed-tracheal tube was inserted and connected to a positive pressure ventilator (Carescape R860, Datex-Ohmeda Inc, Madison, WI) with the following initial settings: F_{iO_2} = 0.21–0.28, respiratory rate (RR) = 28 breaths/min, positive end-expiratory pressure (PEEP) = 3 cmH₂O and positive inspiratory pressure (PIP) = 9–11 cmH₂O.

An arterial catheter was inserted into the femoral artery to monitor mean arterial blood pressure (MABP) and heart rate (HR) and obtain blood samples for gas analysis. Moreover, pulse oximetry oxygen saturation (SpO₂) was continuously monitored.

The right common carotid blood flow was measured, with an ultrasonic flow probe (Transonic Systems Inc., NY), as a proxy for cerebral blood flow. Rectal temperature was maintained between 38 and 39°C with heating lamps.

2.2 | Lung injury and study design

SF-deficient lung injury was achieved by repetitive saline lavage (30 mL/kg; 37°C with F_{iO_2} = 1).^{21,22} After the first bronchoalveolar lavage (BAL), the PEEP was increased to 5 cmH₂O, and PIP and RR were adjusted to a maximum of 25 cmH₂O and 45 bpm, respectively. Lavage procedures were repeated at 5 min intervals until P_{aO_2} < 100 mmHg was obtained. After 30 min of stabilization, all newborn piglets received a bolus dose of 20 mg/kg of caffeine citrate (Peyona® 20 mg/mL; Chiesi Farmaceutici, Parma, Italy) before extubation. Customized tightly-fitting short binasal prongs were fitted to all animals. Once spontaneous breathing had been established, piglets were randomly assigned to one of the following groups:

1. INSURE group ($n = 6$): newborn piglets with SF-deficient lung injury that received 200 mg/kg of SF (Poractant-alfa, Curosurf®, Chiesi Farmaceutici, Parma, Italy) by the INSURE method.¹⁹ With the animal in a supine position, the SF bolus was administered over 30–60 s. After SF administration, mechanical ventilation was delivered for 1 min set at 18/5 cmH₂O. Then the piglets were immediately extubated and placed on nCPAP for 180 min.
2. LISA-NT group ($n = 6$): newborn piglets with SF-deficient lung injury that received 200 mg/kg of SF by the LISA method.¹⁹ With the animal maintained on nCPAP, the LISA procedure was performed using 5Fr flexible NT that had been appropriately shortened. It was placed in the trachea with direct visualization of the vocal cords with a laryngoscope and using Magill forceps.
3. LISAcath® group ($n = 6$): newborn piglets with SF-deficient lung injury that received 200 mg/kg of SF by the LISA method. With

the animal maintained on nCPAP, the LISA procedure was performed using a LISAcath® catheter (Chiesi Farmaceutici, Parma, Italy). It was placed in the trachea with direct visualization of the vocal cords with a laryngoscope (without the use of Magill forceps).

After NT or LISAcath® placement, the laryngoscope was removed, and 200 mg/kg of SF was intratracheally administered over 30–60 s. The piglets' mouth was closed during the procedure, in order to avoid loss of pressure in the airways during SF administration. After instillation, the NT or LISAcath® was immediately removed. The nCPAP support was maintained for 180 min, at a level of 5 cmH₂O with a flow of 3 L/min.

2.3 | Lung measurements

Gas exchange and cardiovascular parameters

- Arterial pH, P_{aO₂} and P_{aCO₂} were measured.
- Alveolar-arterial (A-a) oxygen tension difference (A-aDO₂), arterial/alveolar (a/A) ratio and P_{aO₂}/F_{IO₂} parameters were calculated.
- Intrapulmonary intrapulmonary-shunt (Q_s/Q_t) and hemodynamic parameters, namely, HR and MABP (Intellivue MP70, Philips-Medical, Eindhoven, The Netherlands) were measured.

All these parameters were obtained at baseline, after the BAL procedure, during the 30 min of stabilization under conventional mechanical ventilation and, after extubation, during nCPAP every 30 min until the end of experiment, at 180 min.

2.3.1 | Lung mechanics

Lung mechanics were measured with a computerized system (M1014A, Philips Medical, Eindhoven, The Netherlands). The system reported values for dynamic compliance (C_{dyn}), tidal volume and airway resistance at baseline, after the BAL procedure, after 30 min of stabilization, and at the end of the experiment (animals being intubated and lung mechanics measured).

2.3.2 | Lung tissue analysis

Postmortem, the lungs were fixed in formalin 4% at 15 cmH₂O for histological analysis. Lung injury was scored by a pathologist blinded to treatment group using a semi-quantitative scoring system. Pathological signs of lung injury (atelectasis, alveolar and interstitial inflammation, alveolar and interstitial hemorrhage, edema, and necrosis) were each scored on a 0–4 point scale: 0 corresponding to no injury; 1, 2, and 3 to injury to 25%, 50%, and 75% of the field, respectively; and four to injury across the field.^{21,23} Moreover, all seven lung injury scores were summed in order to obtain a mean total lung injury score for each group, with a minimum value of 0 and maximum of 28, values higher than 12 corresponding to quite severe lung injury.²³

2.4 | Brain measurements

As for the aforementioned physiological parameters, brain measurements were taken at baseline, after the BAL procedure, during the 30 min of stabilization and, after extubation, every 30 min until the end of experiment, at 180 min. Moreover, in order to assess changes induced during the period of SF administration, the brain measurements were also taken: 2 min before SF administration (–2 min), during its administration (0 min), and 1 and 5 min after its administration.

2.4.1 | Carotid blood flow and near-infrared spectroscopy (NIRS) measurements

Carotid blood flow (CBF) was recorded to assess changes in cerebral blood flow. Changes in cerebral perfusion-oxygenation were assessed using a near-infrared-spectroscopy NIRS system (NIRO-200, Hamamatsu Photonics, Joco Cho, Japan). The sensor was placed on and fixed to the frontoparietal region of the piglet's head. The cerebral tissue oxygen index (cTOI) was continuously monitored. This index represents the cerebral oxygen saturation expressed as a percentage and was also used to calculate the cerebral fractional tissue oxygen extraction (cFTOE): cFTOE = (SpO₂–cTOI)/SpO₂.

2.4.2 | Brain tissue analysis

To perform histological analysis, the brain was fixed (formalin 4%) and divided into cortex, inner regions (striatum, thalamus and hippocampus), and cerebellum and brain stem. A total of 20 fields were analyzed with light microscopy. Pathological features of brain injury (necrosis, inflammation, hemorrhage, edema and infarction) were each scored on a 0- to 3-point scale: 0 corresponding to no injury; and 1, 2, and 3 to injury to mild, moderate, and severe injury across the field, respectively. The presence of more than five necrotic cells/field was considered to indicate neuronal necrosis (score range: 0–20).²¹

2.5 | Statistical analysis

Results are expressed as mean ± standard deviation (SD). Data were assessed using Levene's test to confirm the homogeneity of variance between the treatments and the Kolmogorov-Smirnoff test for normality (JMP8, Statistical Discovery, SAS, NC). Intragroup comparisons were performed with one-factor analysis of variance (ANOVA). Short-term cerebral effects, and lung and brain injury scores were analysed using the Wilcoxon nonparametric test. A *P* < 0.05 was considered significant.

3 | RESULTS

Animals in each group were similar in age (4 ± 1 days) and size (INSURE group: 2.1 ± 0.2 kg; LISA-NT group: 2.3 ± 0.3 kg; LISAcath group: 2.3 ± 0.3 kg). Multiple BALs (INSURE group: 12 ± 4; LISA-NT group: 12 ± 3; LISAcath® group: 11 ± 3) were needed to induce the lung injury, no significant differences being observed between groups in the number required. The procedure was well tolerated.

3.1 | Pulmonary outcomes

All animals had similar pH, P_{aO_2}/F_{iO_2} , P_{aCO_2} , A-aDO₂, and C_{dyn} parameters at baseline, after induction of SF-deficient lung injury and after 30 min of stabilization (Table 1 and Figure 1). BAL produced an abrupt decrease in P_{aO_2}/F_{iO_2} (Figure 1A), C_{dyn} (Figure 1C), and pH (Table 1), with a significant increase in A-aDO₂ and P_{aCO_2} Levels (Table 1 and Figure 1B), without significant differences between groups.

The time course of response to SF administration was similar in INSURE, LISA-NT, and LISAcath® groups, with rapid improvement in the P_{aO_2}/F_{iO_2} ratio, P_{aCO_2} , pH and A-aDO₂ values in all SF-treated groups (Figure 1 and Table 1), while the F_{iO_2} ratio rapidly reduced in all groups (Table 1). These improvements were maintained over time, and were similar in all SF-treated groups.

Regarding C_{dyn} , it recovered close to baseline levels with all methods of SF administration (Figure 1C). Moreover, no significant differences were observed between groups in tidal volume or resistance parameters (data not shown).

The LISAcath® group obtained significantly higher scores for atelectasis, alveolar inflammation and total lung injury than INSURE and LISA-NT groups (Table 2). Nevertheless, the scores for all parameters studied were low, none indicating relevant lung injury (Figure 2).

3.2 | Intrapulmonary shunt and hemodynamic assessment

BAL produced an abrupt increase in Q_s/Q_t (Figure 3A). Subsequently, rapid improvements in Q_s/Q_t were observed with all SF treatment

strategies, levels reaching baseline 2 h after SF administration (Figure 3A).

At baseline, there were no differences between groups in the hemodynamic parameters studied. No significant changes in MABP were observed after BAL (Figure 3B), while the HR rose (Figure 3C). MABP and HR did not change significantly during the experimental period and did not differ significantly between the groups at any point.

3.3 | Cerebral assessment

3.3.1 | Overall cerebral effect

Carotid blood flow increased in all groups after the BAL procedure, while cTOI, cFTOE, and systemic oxygenation (SpO₂) decreased (Figure 4). SF administration was followed by a continuous decrease in carotid blood flow, this reaching baseline levels after 60 min in all SF-treated groups (Figure 4A). On the other hand, cTOI, cFTOE, and SpO₂ increased after SF treatment, reaching baseline levels after 15-30 min of treatment, with no significant differences between groups (Figures 4B-4D).

3.3.2 | Cerebral effects around the time of SF administration

Assessing the impact of SF during and immediately after its administration, we observed transient decreases in SpO₂ and cTOI in INSURE and LISA-NT groups (Figures 4Bb and 4Dd). The effect of the INSURE procedure on SpO₂ was observed 1 min after SF administration, while cTOI showed a continuous decrease immediately on administration of SF and during the first minute following its

TABLE 1 Gas exchange and ventilatory parameters in surfactant deficiency newborn piglets treated with SF administered using INSURE or LISA technique (by nasogastric tube (NT) or by LISAcath®)

Groups	Basal	BAL	30 ST	15 min	30 min	60 min	90 min	120 min	150 min	180 min
pH										
INSURE	7.38 ± 0.04	7.12 ± 0.09&	7.10 ± 0.12&	7.29 ± 0.05	7.36 ± 0.06	7.39 ± 0.07	7.42 ± 0.08	7.46 ± 0.08	7.46 ± 0.07	7.44 ± 0.08
LISA-NT	7.38 ± 0.03	7.09 ± 0.08&	7.07 ± 0.13&	7.31 ± 0.02	7.33 ± 0.08	7.42 ± 0.05	7.43 ± 0.05	7.43 ± 0.04	7.46 ± 0.04	7.46 ± 0.03
LISAcath	7.37 ± 0.05	7.06 ± 0.05&	7.10 ± 0.10&	7.27 ± 0.06	7.30 ± 0.06	7.34 ± 0.06*	7.42 ± 0.02	7.42 ± 0.05	7.40 ± 0.05	7.42 ± 0.04
A-aDO ₂ (mmHg)										
INSURE	77 ± 11	744 ± 17&	728 ± 27&	507 ± 102	338 ± 89	247 ± 98	193 ± 91	141 ± 61	111 ± 36	91 ± 23
LISA-NT	79 ± 7	731 ± 23&	716 ± 38&	554 ± 125	389 ± 69	267 ± 87	206 ± 67	163 ± 47	117 ± 13	103 ± 10
LISAcath	79 ± 11	740 ± 18&	730 ± 22&	547 ± 119	406 ± 115	292 ± 60	226 ± 66	160 ± 36	119 ± 20	110 ± 18
FiO ₂										
INSURE	0.28 ± 0.01	1.00 ± 0.00&	1.00 ± 0.00&	0.83 ± 0.15	0.62 ± 0.12	0.47 ± 0.11	0.41 ± 0.12	0.34 ± 0.07	0.31 ± 0.05	0.29 ± 0.01
LISA-NT	0.28 ± 0.01	1.00 ± 0.00&	1.00 ± 0.00&	0.89 ± 0.09	0.65 ± 0.21	0.50 ± 0.11	0.41 ± 0.08	0.37 ± 0.07	0.31 ± 0.02	0.29 ± 0.01
LISAcath	0.28 ± 0.01	1.00 ± 0.00&	1.00 ± 0.00&	0.85 ± 0.13	0.66 ± 0.14	0.51 ± 0.08	0.44 ± 0.07	0.36 ± 0.08	0.31 ± 0.03	0.30 ± 0.02
RR (breath/min)										
INSURE	27 ± 1	42 ± 1&	42 ± 1&	50 ± 26	50 ± 12	43 ± 10	42 ± 14	44 ± 14	42 ± 12	40 ± 13
LISA-NT	27 ± 1	42 ± 1&	42 ± 1&	64 ± 27	57 ± 20	63 ± 18*	55 ± 13	46 ± 11	46 ± 10	45 ± 17
LISAcath	28 ± 1	42 ± 1&	42 ± 1&	68 ± 22	65 ± 16*	59 ± 22	51 ± 12	46 ± 9	41 ± 4	36 ± 6

Statistical differences (&) $P < 0.05$ versus basal point; (*) $P < 0.05$ versus INSURE group. Values are expressed as mean ± SD. Alveolar-arterial oxygen tension difference (A-aDO₂); inspiratory oxygen fraction (FiO₂); respiratory rate (RR)

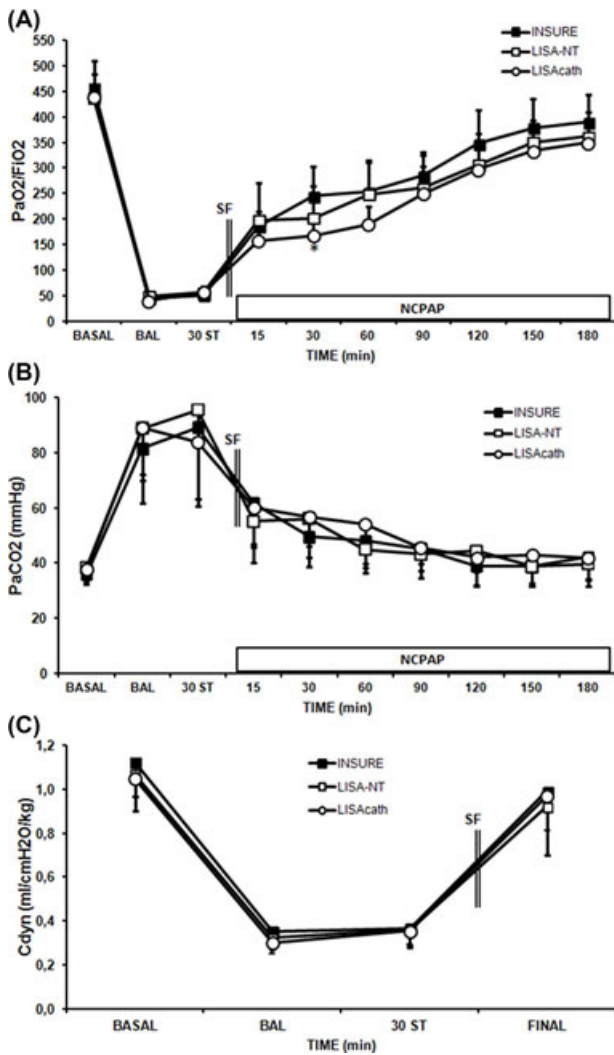


FIGURE 1 Changes in P_{aO_2}/F_{iO_2} ratio, P_{aCO_2} and dynamic compliance (C_{dyn}) in surfactant (SF)-deficient newborn piglets treated with SF administered by the INSURE method or LISA using a nasogastric tube (NT) or LISAcath®. P_{aO_2}/F_{iO_2} (A), P_{aCO_2} (B), and C_{dyn} (C) values in the INSURE (black square), LISA-NT (white square) and LISAcath® (white circle) groups. (* $P < 0.05$ versus INSURE group (one-way ANOVA). Values are mean \pm SD. BAL, bronchoalveolar lavage; ST, stabilization

administration and in the LISA-NT group, a significant decrease in both these variables was observed immediately on administration of SF (Figures 4Bb and 4Dd). In contrast, SpO_2 and $cTOI$ values did not significantly decrease during SF administration in the LISAcath® group. By 5 min after administering SF, SpO_2 and $cTOI$ had recovered to some extent in the INSURE group and completely (to baseline levels) in the LISA-NT group (Figures 4Bb and 4Dd).

Cerebral FTOE values increased in the LISA-NT group immediately on administration of SF and decreased thereafter, while no changes were observed in the INSURE or LISAcath® groups (Figure 4Cc). Progressive increases in $cFTOE$ were observed in both LISA-NT and LISAcath® groups, reaching baseline levels by 5 min after SF administration (Figure 4Cc).

TABLE 2 Total lung injury scores in surfactant deficiency newborn piglets treated with SF administered using INSURE or LISA technique (by nasogastric tube [NT] or by LISAcath®)

Lung region	Groups	Atelectasis	Necrosis	Edema	Alveolar inflammation	Interstitial inflammation	Alveolar hemorrhage	Interstitial hemorrhage	Total
UPPER	INSURE	0.17 \pm 0.41	0	0	0.17 \pm 0.41	0.67 \pm 0.52	0	0.17 \pm 0.41	1.17 \pm 1.17
	LISA-NT	0.17 \pm 0.41	0	0	0.50 \pm 0.54	1.16 \pm 1.16	0	0	1.83 \pm 1.17
	LISAcath	1.00 \pm 1.10	0	0	0.83 \pm 0.54*	1.33 \pm 0.81	0	0	3.16 \pm 2.04
MIDDLE	INSURE	0.33 \pm 0.51	0	0	0.33 \pm 0.51	1.00 \pm 0.89	0	0	1.67 \pm 1.51
	LISA-NT	0.83 \pm 0.75	0	0.17 \pm 0.40	0.33 \pm 0.51	1.33 \pm 1.03	0	0	2.67 \pm 1.75
	LISAcath	0.83 \pm 1.69	0	0	0.83 \pm 0.40	1.33 \pm 1.03	0	0	3.00 \pm 2.19
LOWER	INSURE	0.33 \pm 0.52	0	0	0.33 \pm 0.52	1.00 \pm 0.89	0.17 \pm 0.41	0	1.83 \pm 1.47
	LISA-NT	0.66 \pm 0.81	0	0	0.33 \pm 0.52	0.83 \pm 0.75	0	0	1.83 \pm 1.72
	LISAcath	1.33 \pm 1.02	0	0.17 \pm 0.40	1.33 \pm 0.52*\$	1.50 \pm 0.83	0	0.50 \pm 0.54	3.70 \pm 2.50*\$
TOTAL	INSURE	0.28 \pm 0.46	0	0	0.28 \pm 0.46	0.89 \pm 0.76	0.06 \pm 0.24	0.06 \pm 0.24	1.56 \pm 1.34
	LISA-NT	0.56 \pm 0.70	0	0.05 \pm 0.23	0.38 \pm 0.50	1.11 \pm 0.96	0	0	2.10 \pm 1.53
	LISAcath	1.00 \pm 1.00*	0	0.05 \pm 0.23	1.00 \pm 0.48*\$	1.38 \pm 0.85	0	0.16 \pm 0.38	3.6 \pm 2.25*\$

Statistical differences were assessed using Wilcoxon non-parametric test; (* $P < 0.05$ versus Insure group, (\$) $P < 0.05$ versus LISA-NT group. Values are expressed as mean \pm SD.

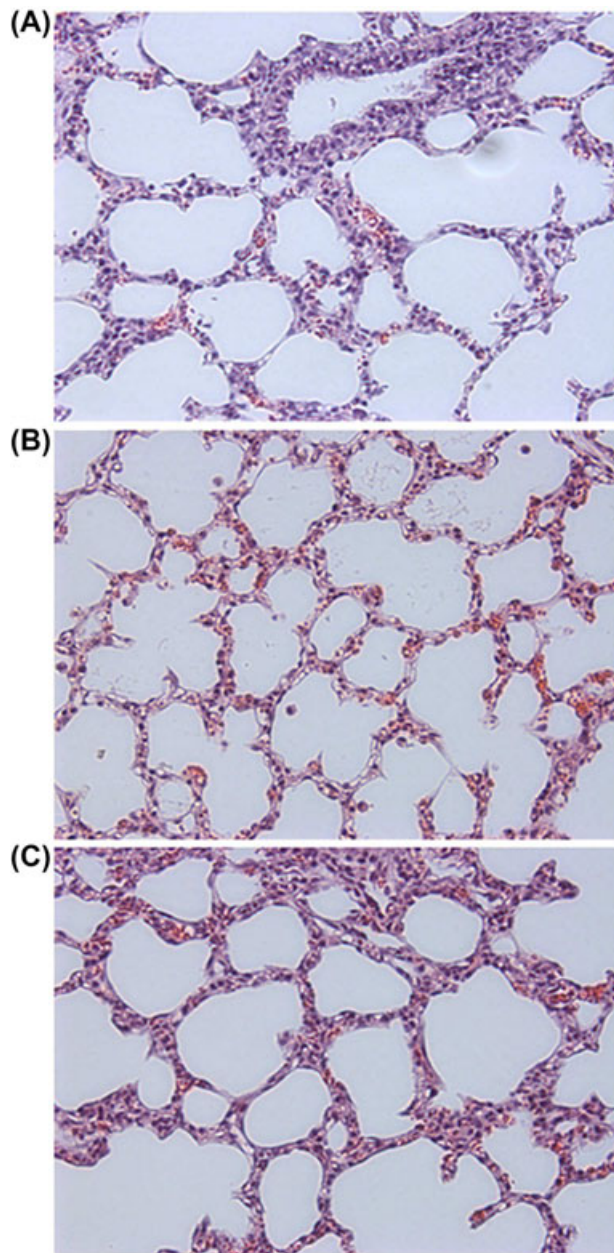


FIGURE 2 Photomicrographs (200 \times magnification) of representative lung sections from INSURE (A), LISA-NT (B) and LISAcath® (C) groups. Panels obtained from the middle region of the lung

3.3.3 | Brain injury

All groups studied obtained low brain injury scores, with similar necrosis, edema, hemorrhage, inflammation, and infarction scores in all regions studied (Table 3 and Figure 5).

4 | DISCUSSION

We have been able to demonstrate in our spontaneously breathing newborn piglet model of SF-deficient lung injury that two different methods of SF administration (INSURE and LISA) are both associated with

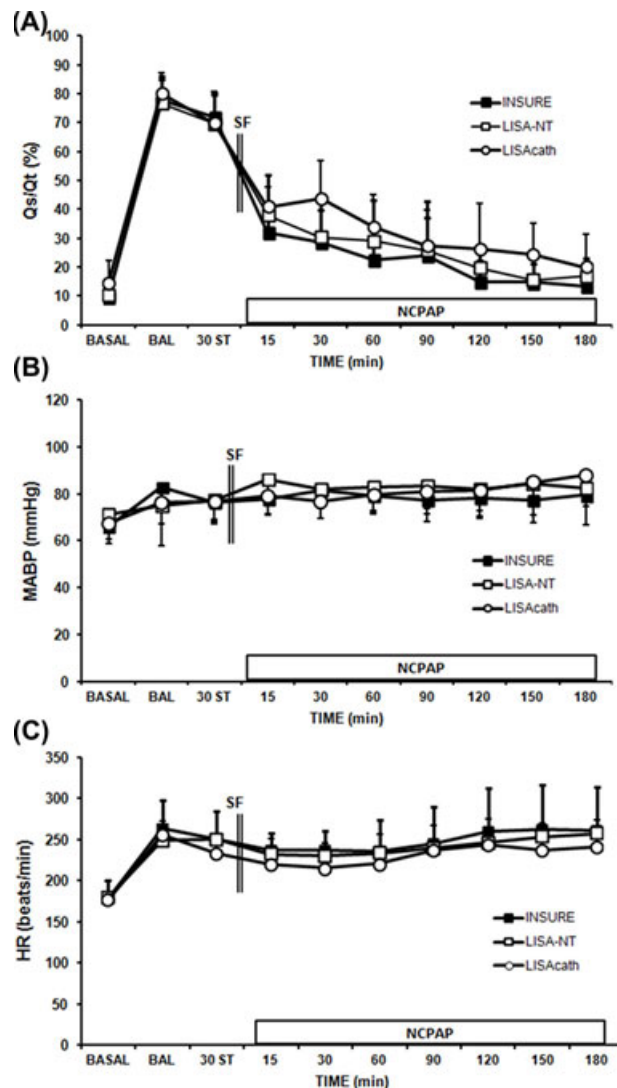


FIGURE 3 Changes in intrapulmonary shunt (Q_s/Q_t), arterial blood pressure (MABP), and heart rate (HR) in surfactant (SF)-deficient newborn piglets treated with SF administered by the INSURE method or LISA using a nasogastric tube (NT) or LISAcath®. Mean Q_s/Q_t (A), MABP (B), and HR (C) values in the INSURE (black square), LISA-NT (white square) and LISAcath® (white circle) groups. (* $P < 0.05$ versus INSURE group (one-way ANOVA). Values are mean \pm SD. BAL, bronchoalveolar lavage; ST, stabilization

rapid and significant improvements in pulmonary status (gas exchange, respiratory parameters, and lung mechanics) together with low lung injury scores. Moreover, this is the first study that investigates the effect of two different LISA procedures (using an NT or a LISAcath® catheter) on cTOI and cFTOE and compares them to the INSURE procedure. The INSURE procedure and LISA using an NT were associated with brief transient decreases in SpO₂ and cTOI immediately after and during SF treatment, respectively, while LISA using a LISAcath® catheter was not associated with any changes in SpO₂ or cerebral oxygenation as measured by NIRS. The brain injury scores were similar across groups, implying that none of these SF administration procedures has any significant clinical effects on the brain in development.

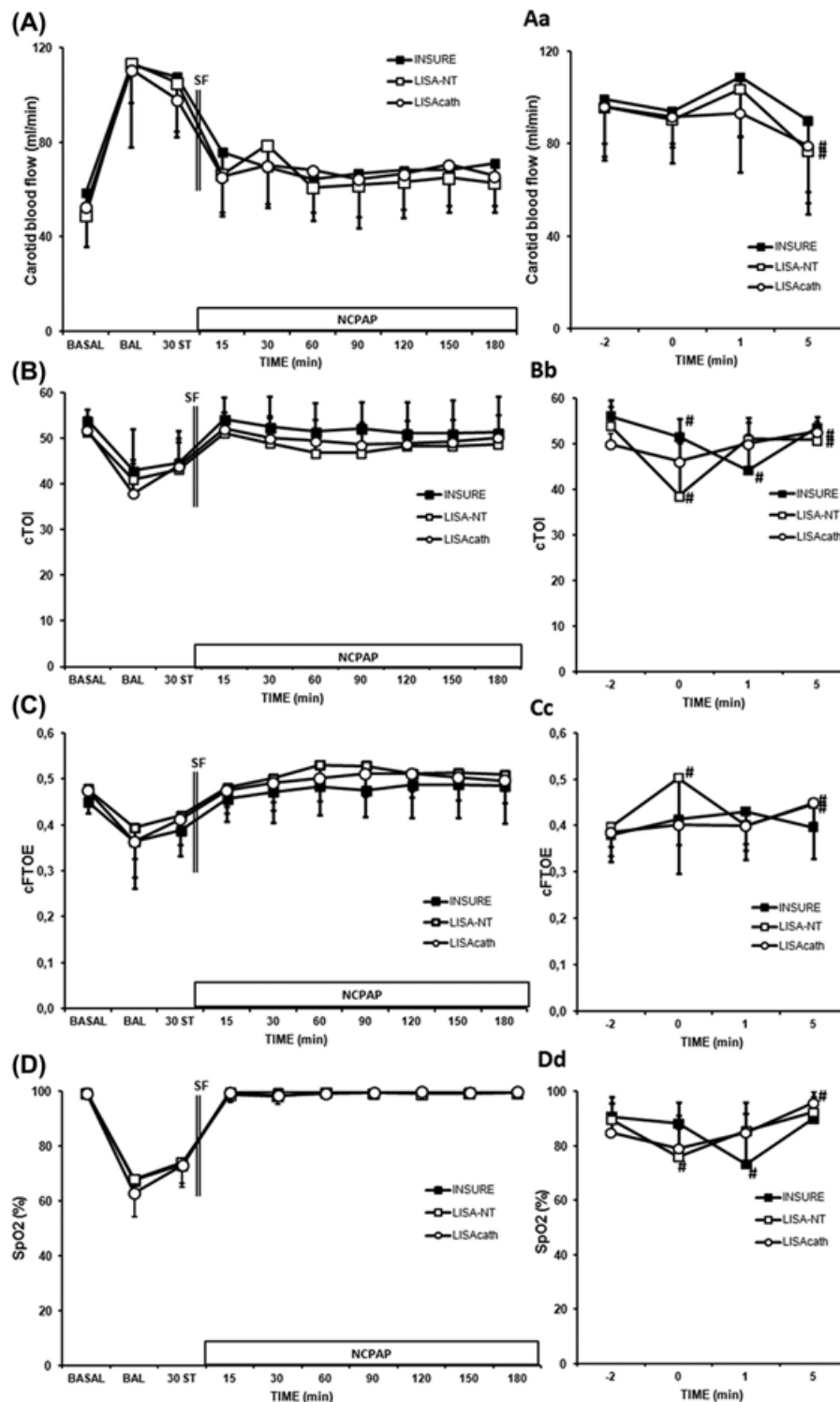


FIGURE 4 Changes in carotid blood flow, cerebral oxygenation (cTOI), cerebral fractional tissue oxygen fraction (cFTOE) and pulse-oximetry saturation (SpO₂) in SF-deficient newborn piglets treated with SF administered by the INSURE method or LISA using a nasogastric tube (NT) or LISAcath®. Cerebral effect on carotid blood flow (A), cTOI (B), cFTOE (C) and SpO₂ (D) values; SF administration interval effect on carotid blood flow (Aa), cTOI (Bb), cFTOE (Cc) and SpO₂ (Dd) values in the INSURE (black square), LISA-NT (white square) and LISAcath® (white circle) groups. (*)*P* < 0.05 versus INSURE group (one-way ANOVA); (#)*P* < 0.05 versus -2 min time point (Wilcoxon non-parametric test). Values are mean ± SD. BAL, bronchoalveolar lavage; ST, stabilization

TABLE 3 Total brain injury scores in surfactant deficiency newborn piglets treated with SF administered using INSURE or LISA technique (by nasogastric tube (NT) or by LISAcath®)

	Necrosis	Edema	Inflammation	Hemorrhage	Infarct
CORTEX					
INSURE	8 (2-15)	0	0.7 (0-1)	0	0
LISA-NT	9 (4-14)	0.2 (0-1)	0.6 (0-1)	0	0
LISAcath	8 (4-14)\$	0.4 (0-1)*	0.6 (0-1)	0	0
INNER					
INSURE	7 (2-14)	0	0.4 (0-1)	0	0
LISA-NT	7 (2-14)	0.1 (0-1)	0.4 (0-1)	0	0
LISAcath	8 (0-12)	0.2 (0-1)	0.4 (0-1)	0	0
CB+B					
INSURE	9 (3-16)	0	0	0	0
LISA-NT	7 (3-16)	0	0	0	0
LISAcath	8 (4-16)	0	0	0	0

Statistical differences were observed between groups using Wilcoxon non-parametric test; (*) $P < 0.05$ versus INSURE group; (\$) $P < 0.05$ versus LISA-NT group. Values are expressed as mean \pm range. CB + B: cerebellum + brainstem.

Endotracheal intubation, SF bolus instillation and prolonged mechanical ventilation have been the standard treatment for neonatal RDS, but this approach carries a risk of acute lung injury in preterm infants.²⁴ Therefore, noninvasive ventilation such as nCPAP has been introduced as a primary treatment for preterm infants with spontaneous breathing after birth seeking to reduce acute lung injury. Moreover, the INSURE procedure was the first method used to combine nCPAP with SF administration,⁸ this approach being the most efficient to treat RDS. Nevertheless, it still requires a brief period of tracheal intubation and sedation, before the SF administration and rapid extubation, and the latter is not always possible. On the other hand, many researchers are investigating minimally invasive methods for SF administration,²⁻⁵ to avoid tracheal intubation and sedation altogether. With LISA, less invasive SF administration, SF is administered into the trachea by direct laryngoscopy, via a thin tube (NT, vascular catheter, or LISAcath®), possibly with the aid of Magill forceps, while the infant is supported with nCPAP.^{13,25,26} After SF instillation, the thin tube is immediately removed, avoiding the need for sedation and tracheal intubation.

It has previously been demonstrated that the process of SF administration itself may induce rapid changes in systemic and cerebral hemodynamic (blood pressure, cerebral blood flow velocities, cerebral blood volume), P_{aCO_2} and oxygenation values.²⁷⁻²⁹ Nevertheless, it is not well known how the new ways of SF administration affect the brain in development, this issue remaining relatively unexplored. This is of great importance, however, given that preterm infants, in whom RDS is relevant and common, have a significantly higher risk of acute and chronic brain injury than term infants.³⁰ Cerebral NIRS enables continuous non-invasive estimation of cerebral oxygenation, providing clinicians with important information concerning how and how long different SF administration procedures may affect the brain in development.

In our study, as expected, SF administration by the INSURE method rapidly improved pulmonary outcomes without significant

changes in MABP, HR or carotid blood flow. On the other hand, short-lasting hypoxemia (as demonstrated by pulse oximetry) and a brief transient decrease in cTOI (not associated with an increase in cFTOE), without changes in carotid blood flow were observed during and after SF administration, though levels recovered thereafter, as described previously.^{18,19,31} In contrast, van der Berg et al¹⁶ did not observe a perturbation of cerebral oxygen delivery or extraction, whereas electrical brain activity decreased for a prolonged period of time. Moreover, Li et al¹⁸ suggest that cerebral autoregulation may be affected transiently by SF administration using the INSURE technique, the impact lasting for 5-10 min. The effect of the INSURE procedure on cerebral oxygenation and perfusion observed in our study may be explained by two main factors. Firstly, in our study, 200 mg/kg of SF was used, while van der Berg et al and Li et al used low doses (100 mg/kg and 120 mg/kg, respectively) and thus low volumes of SF, and it is known that the amount of fluid instilled endotracheally plays an important role in determining changes in cerebral oxygenation and cerebral blood flow due to SF treatment.²⁷ Secondly, mechanical ventilation was maintained during and 1 min after SF administration, which may in some way help SF to spread and increase lung recruitment, as reflected in the gradual decrease in cTOI observed in our study.

SF administration by LISA using an NT or a LISAcath®, like the INSURE procedure, rapidly improved pulmonary outcomes without significant changes in hemodynamic parameters. Currently, there is not a clear consensus on the best catheter to use for LISA or the need for Magill forceps, among other options. Notably, based on our data, the NT and LISAcath® catheter seem to be associated with slightly different effects on cerebral oxygenation. On the one hand, when SF was administered through an NT, we observed transient decreases in SpO₂ and decrease in cTOI with a significant increase in cFTOE during SF administration, these alterations lasting for less than 1 min. The short increase in cFTOE may represent a compensatory mechanism for

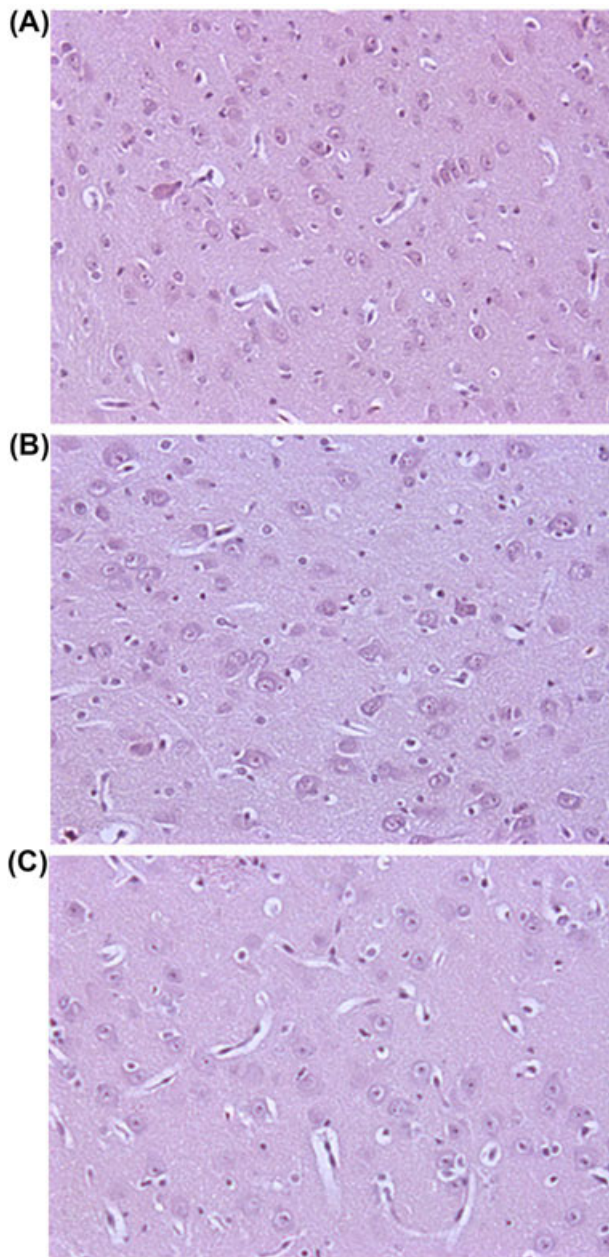


FIGURE 5 Photomicrographs (200× magnification) of representative brain sections from INSURE (A), LISA-NT (B) and LISAcath® (C) groups. Panels obtained from the striatum region of the brain

maintaining adequate cerebral oxygenation,¹⁹ as this SF administration method requires appropriate placement of the NT in the trachea using Magill forceps and the SF is administered to a spontaneously breathing patient maintained on nCPAP. On the other hand, if SF was administered using a LISAcath® catheter no significant alteration was observed in any of aforementioned parameters. In accordance with this, Bao et al¹⁷ observed less fluctuation in F_{IO_2} and SpO_2 in infants receiving LISA than those in the INSURE group, during the whole SF administration procedure, the improvement in SpO_2 being faster during the INSURE procedure.

The LISAcath® catheter has been specifically designed to be placed in the trachea without the need for Magill forceps, the handling

and insertion of the catheter being straightforward due to its greater thickness and the ease of double checking the position, thanks to two sets of marks, and its placement in the trachea has been perceived to be less traumatic.²⁶ The present study is the first to evaluate the cerebral effect of the LISA procedure using a LISAcath® catheter, and hence, these results cannot be compared to previous studies. In looking for an explanation of why an effect on cerebral oxygenation was observed when LISA was applied using an NT but not a LISAcath®, we hypothesize that the maneuver requiring NT placement in the trachea with Magill forceps may in some way disturb cerebral oxygenation, as the NT and LISAcath® catheter size (5Fr), medication used, animal weight and SF dose and duration of administration were similar in the two LISA groups.

The INSURE and LISA techniques require the use of a laryngoscope to visualize the vocal cords. Hence, both involve procedures that can be difficult and traumatic, especially if performed by untrained individuals. Previous articles have indicated that LISA and INSURE procedures need well-developed skills and it is really important that they are performed after adequate training by clinicians with sufficient expertise.^{26,32} As previously reported, longer training periods lead to better results in terms of avoiding intubation, and mechanical ventilation, changes in hemodynamic parameters, among other factors, possibly because the technique is better executed.³³

Using our pathological injury score, the LISAcath procedure seems to result in higher values of atelectasis, alveolar inflammation and total lung injury than INSURE and LISA-NT procedures. Nevertheless, as occurs when physiological variables are analyzed, it should be borne in mind that there is a range of values between which it cannot be considered that there is relevant lung damage. Zimmermann et al²³ described that total lung injury scores of 12 can be considered quite severe lung injury. Unpublished data from our group using this animal model suggest that mean values higher than eight may be related to mild lung injury, and hence, irrespective of whether SF was administered, in the current study, it was not associated with poor histological lung injury outcomes.

In brief, the INSURE method and LISA, using either an NT or a LISAcath® catheter, are different ways of delivering SF that all provide effective therapies. Further, although a one-off perturbation of cerebral oxygenation was observed in INSURE and LISA groups during SF administration, it was short, and no significant changes in the brain were detected in histological analysis.^{34,35}

4.1 | Limitations

Limitations of this study include the use of newborn piglets (2-4 days old), which require SF washout lavage to induce lung injury. The SF washout lavage model has been frequently used in adult and juvenile animals to implement successful animal models of acute pulmonary failure in the context of RDS.^{21,22,36,37} The newborn piglet model was chosen because the brain maturation, lung volume, and birth weight resemble those of newborn infants. Moreover, the feasibility of using nCPAP and SF strategies in this model of SF-deficient lung injury has been previously demonstrated.^{21,36,37} Nevertheless, 2- to 4-day-old

piglets have a greater level of brain development than a 32-week human fetus,³⁸ The effects of INSURE and LISA procedures on the brain need to be interpreted with caution, as effects on the term piglet brain may not be the same as those on the vulnerable premature brain of newborn infants.

Another limitation is that we did not have a control group that was not administered SF in this study. Only two studies have evaluated the impact of spontaneous breathing during³⁹ or after SF administration⁴⁰ on SF distribution, with somewhat conflicting and inconclusive results. It remains uncertain how much SF is the ideal amount that should be deposited and how much SF actually reaches the lung with the use of INSURE and LISA techniques. Factors influencing SF distribution, including the optimal dose, rate of administration and/or nCPAP level, need to be explored.

5 | CONCLUSION

In spontaneously breathing SF-deficient newborn piglets, INSURE and LISA procedures improved pulmonary outcomes without changes in hemodynamic parameters. On the other hand, short-lasting decreases in cerebral oxygenation are associated with SF administration by the INSURE method or LISA using a nasogastric tube, while the LISA procedure using a LISAcath® was not associated with any changes in cerebral oxygenation. Notably, none of treatments studied seem to have a negative impact on the neonatal brain.

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Fabrizio Salomone is an employee at Chiesi Farmaceutici.

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