Lung ultrasound features and relationships with respiratory mechanics of evolving BPD in preterm rabbits and human neonates

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Lung ultrasound features and relationships with respiratory mechanics of evolving BPD in preterm rabbits and human neonates

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ABSTRACT - Evolving bronchopulmonary dysplasia (BPD) is characterized by impaired alveolarization leading to lung aeration inhomogeneities. Hyperoxia-exposed preterm rabbits have been proposed to mimic evolving BPD; therefore, we aimed to verify if this model has the same lung ultrasound and mechanical features of evolving BPD in human neonates. Semi-quantitative lung ultrasound and lung mechanics measurement were performed in twenty-five preterm rabbits (28 days of gestation) and twenty-five neonates (mean gestational age ≈26 weeks) with evolving BPD. A modified rabbit lung ultrasound score (rLUS) and a validated neonatal lung ultrasound score (LUS) were used. Lung ultrasound images were recorded and evaluated by two independent observers blinded to each other's evaluation. Lung ultrasound findings were equally heterogeneous both in rabbits as in human neonates and encompassed all the classical lung ultrasound semiology. Lung ultrasound and histology examination were also performed in 13 term rabbits kept under normoxia as further control and showed the absence of ultrasound and histology abnormalities compared to hyperoxia-exposed preterm rabbits. The inter-rater absolute agreement for the evaluation of lung ultrasound images in rabbits was very high (ICC: 0.989 (95%CI: 0.975-0.995); $p<0.0001$), and there was no difference between the two observers. Lung mechanics parameters were similarly altered both in rabbits and human neonates. There were moderately significant correlations between airway resistances and lung ultrasound scores in rabbits ($\rho=0.519$; $p=0.008$) and in neonates ($\rho=0.409$; $p=0.042$). In conclusion, the preterm rabbit model fairly reproduces the lung ultrasound and mechanical characteristics of preterm neonates with evolving BPD.

NEW AND NOTHEWHORTY

We have reported that hyperoxia-exposed preterm rabbits and human preterm neonates with evolving BPD are the same lung ultrasound appearance, and that lung ultrasound can be fruitfully applied on this model with a brief training. The animal model and human neonates also presented the same relationship between semiquantitative ultrasound-assessed lung aeration and airway resistances. In conclusion, this animal model fairly reproduce evolving BPD as it is seen in clinical practice.

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is characterized by an impaired lung development due to the aberrant reparative response to both pre- and post-natal lung injury.(1) From a parenchymal perspective, developing BPD is an evolutive and regionally heterogeneous disorder characterized by impaired alveolarization leading to structural lung tissue modifications and lung aeration inhomogeneities.(1) Given its multifactorial nature, mimicking BPD in animal models remains a challenging task. Ideally, an animal model of BPD should include a preterm birth and some possible lung injury triggers, such as invasive ventilation, inflammation or oxidative stress. Additionally, animal care should be as similar as possible to that provided in neonatal intensive care units (NICU).(2) Moreover, animals should be supported for long periods, since the chronic respiratory disease of preterm infants cannot be resumed in a punctual diagnosis at a given time point. In fact, preterm infants are rather affected by a chronic respiratory failure representing a *continuum* evolving over time from early life to infancy and characterized by persistent airway and pulmonary vascular disease: this is expressed by the “chronic pulmonary insufficiency of prematurity” concept.(3) For instance, lambs, pigs, baboons or monkeys are effective to mimic the clinical reality in detail, albeit they may be less cost-effective compared with small laboratory animals.(4, 5) Newborn rodents can be delivered at term during the saccular stage of lung development, which is morphologically similar to the human premature.(6) Nonetheless, these animals are difficult to ventilate and lack the significant impact of preterm birth. Therefore, they cannot reproduce the complex reality of neonatal critical care and the translatability of this model to human BPD can be questioned. Large animals and rodents also have different survival, with the former surviving for longer times, which impacts the type of studies that can be performed and their costs. Rabbits represent an excellent compromise to model BPD since they have many offspring, can be delivered prematurely and allow some perinatal manipulations, making the model closer to the actual neonatal care.(2, 7) For instance, preterm rabbits are fed artificially with milk formula and can be exposed to hyperoxia to induce the alveolarization arrest similar to what occurs in infants with evolving BPD.(8, 9)

From the imaging point of view, the heterogeneous parenchymal abnormalities of BPD can be accurately described by lung ultrasound, which is characterized by a steady loss of lung aeration over time.(10) By this means, early lung ultrasound performed at 7-14 days of post-natal age can predict the occurrence of BPD at 36 weeks post-menstrual age.(10) From a mechanical point of view, BPD is characterized by a mixed pattern (obstructive and restrictive) with heterogeneous distribution and a certain variability according to clinical severity.(11) The lung ultrasound features of hyperoxia-exposed preterm rabbits are unknown. Therefore, the present study compared the lung ultrasound and mechanical features of preterm rabbits and human neonates with evolving BPD to validate the hyperoxia-exposed preterm rabbit as a reliable model of BPD.

MATERIALS AND METHODS

This project was divided into two phases: 1) an experimental model of evolving BPD in hyperoxia-exposed preterm rabbits, and 2) data collection in extremely preterm infants with evolving BPD.

Experimental model and animal care

Time-mated New Zealand White female rabbits were purchased from Charles River Laboratories (Miserey, France). Pregnancy was confirmed with ultrasounds at 12-14 days after the artificial insemination. Subsequently, they were maintained with *ad libitum* food and water until caesarean section was performed. At 28 days of gestation, which is equivalent to the saccular stage of human lung development,(2, 7) does (3.7±0.4 kg of body weight) were sedated with medetomidine 2 mg/kg (Domitor®, Orion, Espoo, Finland). Ten minutes later, the animals received 25 mg/kg of ketamine (Imalgene 1000®, Merial, France) and 5 mg/kg of xylazine (Rompun®, Bayer, Leverkusen, Germany). When adequate sedation was reached, the abdomen was shaved and does were euthanized with pentothal sodium (50 mg/kg; MSD Animal Health, Madison-NJ, USA). All drugs were given intramuscularly. The abdomen was immediately opened through a low midline abdominal incision, the uterus was exposed, and pups were extracted. They were dried, stimulated and placed in the

incubator at 32°C, with 95% oxygen and 50-60% relative humidity. After 1h, the surviving pups were weighed, numbered and placed on soft bedding for 7 days with no treatment. Housing material was changed daily. Pups were fed twice a day through 3.5 Fr esophageal tubes (Vygon, Ecouen, France) transiently introduced for the feeding which lasted few minutes. A milk replacer (Day One®, Protein 30%, Fat 50%; FoxValley, Aurora-IL, USA) dissolved in water (250 mg/mL) was given. The volume of each feed was increased from 40 mL/kg/day on the day of birth, to 50 mL/kg/day on day 1, 75 mL/kg/day on day 2, and 100 mL/kg/day from day 3 to day 7. Pups were stimulated twice daily to urinate before feeding. A mixture of pre- (fructo-oligosaccharide and acacia) and pro-biotics (*Saccharomyces cerevisiae)* were added for all 7 days (Bio-Lapis®; Probiotics International, UK; 25 mg/mL), whereas immunoglobulins (15 mg/mL) were added only for the first 3 days (Col-o-Cat®, SanoBest, Hertogenbosch, Netherlands). On day 2, vitamin K was intramuscularly given (0.25 mg/kg, Izokappa®, Brescia, Italy). These rabbits generally open eyelids around 7-10 days of life, which is similar to what happens in extremely preterm human neonates.(12, 13)

A control group of newborn rabbits without lung injury was also included. This consisted of term rabbits naturally delivered on the 31st day of gestation, which is their physiological term of pregnancy and correspond to human lung development's alveolar stage .(12) After birth, pups were maintained with their mothers at room air in individual cages until day 4 in order to reach the age equivalence with preterm pups at day 7. At the end of all experiments, newborn rabbits were euthanatized as described above. The protocol was approved by the Italian Ministry of Health review board (n°744/2017) and met all the local and European regulations on animal research. This study phase followed ARRIVE guidelines.(14)

Semi-quantitative lung ultrasound in rabbits

On days 7 and 4 in preterm and term rabbits, respectively, the animals were anesthetized with isofluorane (3% in pure oxygen). The lung ultrasound procedure in adult rabbits has been already described.(15) Here, we adapted the technique to apply it to newborn rabbits and particularly to preterm rabbits. Each lung was scanned using a hand-held ultrasound tablet equipped with a "hockey

stick" and a micro-linear, high-resolution probe (18 MHz, 2.5 cm length, Sonoscanner U-LITE® EXP, Sonoscanner, Paris, France). Since the probe was relatively large compared to the rabbit chest, the evaluation was done on the whole hemithorax (i.e. without division in smaller chest areas) as performed by Raimondi et al. in neonates scanned with a broadband linear probe.(16) A simplified rabbit lung ultrasound score (rLUS) was adapted from the previously validated neonatal lung ultrasound score (LUS).(17) rLUS and LUS were based on the same lung ultrasound semiology, and all images were evaluated according to the classical lung ultrasound scoring system assigning 0-to-3 points.(17, 18) If the rabbit lung appeared homogenous, a single picture (300 dpi) per lung was captured (one picture for the left and one for the right lung); if the lung appearance was not homogenous, additional pictures were recorded to capture all ultrasound features, which were averaged for each rabbit. Ultrasound scans were performed both transversally and longitudinally, with automatic gain adjustment, using the dedicated software feature of the ultrasound device; no harmonics were used. Depth and focus were adjusted according to chest size and the sign of interest.(19) Illustrative pictures of lung ultrasound procedure and findings, as well as rLUS calculation, are shown in **Fig.1**. All images per each rabbit were independently evaluated by the investigator (CCas) performing the ultrasound and animal care, who received a short formal lung ultrasound training, as described elsewhere (20), and by a skilled neonatologist with several years of lung ultrasound experience (DDL), who did not participate in animal care and performed the evaluation blinded to any animal data.

Lung mechanics in preterm rabbits

Preterm rabbits also underwent pulmonary function tests immediately after lung ultrasound. Pulmonary function was studied with the forced oscillation technique, using the FlexiVent™ system equipped with module 2 (Scireq, Montreal, Canada), as we previously described.(21) A tracheostomy was performed and an 18-gauge needle was inserted in the trachea and tied. Pups were connected to the FlexiVent ventilator (tidal volume: 10 mL/kg, frequency: 120 breaths/min, positive end-expiratory pressure (PEEP): 3 cmH_2O).(21) In addition to total inspiratory capacity, airway resistance

(Raw), dynamic compliance (Cdyn), lung tissue damping and elastance were measured using the Prime-8 and Snapshot-120 perturbation programs, respectively, as previously described.(22) All measurements were performed until three consistent values (with a minimum R^2>0.95) were obtained and averaged.

Histology

Right after the sacrifice, the trachea was cannulated with a 20-gauge catheter. Lungs were removed and fixed with 10% buffered formalin (Sigma-Aldrich, St.Louis-MI, USA) for at least 4 hours under constant pressure (25 cmH_2O) using a custom-made fixation device to ensure homogenous fixation pressures. Subsequently, the lungs were first left in formalin for at least 24h, then transferred to 70% ethanol, embedded in paraffin, and stained with eosin and hematoxylin (H&E) following standard histology protocols. A section for each lung was used to evaluate the mean linear intercept (Lm).(23) Lm was evaluated in 40 fields per section to cover the entire section area by calculating the number of intersections between the reference line and the alveolar tissue using a micrometric scale; Lm value of the right and left lung were then averaged and used in the analysis.(24, 25)

Patients

Neonates were enrolled between 2018 and 2020 in a prospective cohort study performed in an academic tertiary referral NICU if they fulfil all the following criteria: 1) gestational age $\leq 28^{+6}$ weeks; 2) invasive ventilation and oxygen supplementation at 14^{th} day of postnatal life for ongoing hypoxemic respiratory failure; 3) invasive ventilation for at least 10 days in the first 2 weeks of life. Exclusion criteria were: 1) severe hemodynamic instability (defined as any need for inotropes); 2) need for surgery; 3) air leaks; 4) grade III-IV intraventricular hemorrhage; 5) congenital lung anomalies; 6) major congenital malformations or chromosomal abnormalities. In addition, all enrolled neonates had to qualify for BPD diagnosis at 36 weeks post-menstrual age according to the National Institute for Child Health and Development criteria(26) otherwise, they would have been excluded *a posteriori*. Data were collected in real-time by attending physicians in charge of patients, and no procedure was performed solely for the study participation. In fact, lung

ultrasound and mechanics evaluation are part of our routine care and are serially performed in long-term ventilated neonates with evolving BPD.(10, 27) Data were anonymously collected in a dedicated and secured database, respecting all local and European privacy regulations. The protocol was approved by the local ethical committee (SRLF16/58), and written informed consent was obtained from parents/guardians upon NICU admission. This study phase followed STROBE guidelines.(28)

Semi-quantitative lung ultrasound in preterm neonates

We performed lung ultrasound on the 14th day of postnatal life (while all neonates were invasively ventilated) as lung ultrasound score peaks and has the highest diagnostic accuracy to predict BPD at this time-point.(10) We calculated the classical neonatal LUS on 6 chest areas (3 per each side), assigning 0-to-3 points to each area (total score going from 0 to 18), based on lung ultrasound semiology patterns, as previously described.(17) Transversal and longitudinal scans were performed on all chest areas with a "hockey stick", micro-linear, high-resolution probe (15 MHz; CX-50 Philips Healthcare, Eindhoven, The Netherlands), as previously reported.(18) Exams were accomplished in a standardized manner when ventilation was optimized and infants were in a calm state, using the same technical settings described above for rabbits. No sedation was needed to perform lung ultrasound. Clinicians performing ultrasound had at least 1 year of experience in daily use of lung ultrasound,(19) as this is the first-line imaging technique in our NICU.(27)

Lung mechanics in preterm neonates

Neonates were intubated with appropriately sized endotracheal tubes, lightly sedated as previously described.(29) Time-cycled, pressure-regulated, assisted-controlled ventilation was provided with the following targets: 5-6 ml/kg tidal volume, 7.4 pH, and 35-65 mmHg $PaCO_2$. PEEP was set between 5 and 8 cmH_2O. A maximal peak pressure of 22 cmH_2O was allowed, FiO_2 was as low as possible to guarantee a peripheral hemoglobin saturation between 90% and 95% and PaO_2 between 50 and 70 mmHg. The flow trigger was set at the maximal possible sensitivity without auto-triggering. Inspiratory time and flow were set looking at flow and pressure tracings and aiming to avoid any gas-

trapping and optimize synchrony. All patients were on continuous flow neonatal ventilators equipped with a low dead-space, hot-wire anemometers. Airway pressure and flow were both measured at the Y-piece (30) to maximize the accuracy of lung mechanics assessment. Ventilators were subjected to serial technical quality controls.(31) Sensors were calibrated before each use and manufacturers' recommendations were always followed. Lung mechanics was evaluated within 1h from lung ultrasound, considering Raw and Cdyn shown by a breath-to-breath analysis on the ventilator screen. Measurements were performed when neonates were stable, after airway suctioning, and following the previously described technique.(32) In detail, spontaneous breathing was temporally avoided by increasing mechanical rate, while the flow was decreased to 5L/min to reach a quasi-static situation. In these conditions, when we observed gas leaks to be <5% and normal-appearing pressure-volume and flow-volume loops, Raw and Cdyn were averaged on 10 mechanical breaths showing identical tracing.

Statistics

We enrolled 25 preterm rabbits as in previous studies describing lung mechanics and pathology of this experimental model.(33) To be consistent, we aimed to enroll the same number of neonates with evolving BPD in the second study phase. We also enrolled 13 term rabbits as further controls. Data were tested for normality with the Shapiro-Wilk test and expressed as mean (standard deviation) or median [interquartile range], as appropriate. Data sets were compared with Student or Mann-Whitney test, respectively. The conformity of lung ultrasound interpretation, that is, the inter-rater absolute agreement between the two independent observers, was evaluated using the intra-class correlation coefficient (ICC).(34) rLUS and LUS were correlated with lung mechanics measures in preterm rabbits and neonates, respectively, using Spearman correlation coefficient (ρ). Results were also graphically shown using scatter plots analyzed with local (smoother) regression with 95% Epanechnikov kernel; $p<0.05$ values were considered statistically significant. Analyses were performed using SPSS version 27.

RESULTS

Details of preterm rabbits and human neonates recruited in the first and second project phase are summarized in **Tab.1**. All enrolled neonates were invasively ventilated at the fourteenth day of life with a mean airway pressure of 15 (1.8) cmH_2O. They were ventilated for ongoing respiratory failure due to evolving BPD, and no infant had other concomitant reasons to need invasive ventilation: the total duration of invasive ventilation was 13 [11-16] days. During their NICU stay, 6 (24%) neonates needed postnatal steroids to facilitate extubation, and 17 (68%) were treated at least once with nebulized salbutamol for spells due to bronchospasm. All neonates qualified for BPD diagnosis at 36 weeks' gestation and were eventually discharged from the NICU: eight (32%) infants had mild, eight (32%) had moderate, and nine (36%) had severe BPD. **Tab.2** shows lung mechanics variables: Raw and Cdyn were respectively above and below the normal values, both in preterm rabbits and neonates.

Lung ultrasound findings were heterogeneous in hyperoxia-exposed preterm rabbits and encompassed normally aerated zones, zones with interstitial-alveolar pattern and others with total loss of aeration. More than one picture per lung was recorded in 17 (68%) preterm rabbits (an average of 2.9 (0.8) lung ultrasound images were taken per rabbit), as the lung appearance was not homogenous (**Fig.1-2**). The inter-rater absolute agreement for the evaluation of lung ultrasound images in preterm rabbits (ICC: 0.989 [95%CI: 0.975-0.995]; $p<0.0001$) was very high, and there was no difference when rLUS was calculated by the animal lab investigator (1.6 [0.9-2]) or the independent expert rater (1.6 [1-2]; $p=0.462$; **Fig.2**). All preterm rabbits completed the experimental protocol; lung ultrasound lasted 2-3 minutes per rabbit and no issue was noticed during the procedure. Lung ultrasound findings were also heterogeneous in neonates with evolving BPD: **Fig.3** shows the similarities between the main lung ultrasound abnormalities in hyperoxia-exposed preterm rabbits and neonates with evolving BPD.

Fig.4 shows the relationship between lung ultrasound scores and Raw or Cdyn in preterm rabbits and neonates. Raw significantly correlated with rLUS (ρ=0.519, p=0.008, R^2=0.20) and LUS (ρ=0.409, p=0.042, R^2=0.29) (**Fig.4, Panel A and C**). There was no significant correlation between rLUS and any other mechanics parameter in rabbits (Cdyn: ρ=0.067, p=0.750; inspiratory capacity: ρ=0.463, p=0.259; tissue damping: ρ=0.327, p=0.190; elastance: ρ=0.067, p=0.750), nor between LUS and Cdyn (ρ=0.205, p=0.325) in neonates.

Fig.5 reports the differences between hyperoxia-exposed preterm rabbits and control rabbits delivered at term and treated in normoxia. Typical histological findings of experimental BPD were observed in preterm rabbits with extensive inflammation and airspace simplification (**Fig.5A-D**). This is confirmed by Lm, which was higher in hyperoxia-exposed preterm rabbits compared to term animals (**Fig.5G**). Illustrative images show that the lungs of preterm rabbits were poorly aerated, compared to control animals (**Fig.5E-F**), and this is confirmed by rLUS, which was always zero in term rabbits and significantly higher than in hyperoxia-exposed preterm rabbits.

DISCUSSION

Our study adds novel findings to the knowledge about experimental BPD models: we show that the hyperoxia-exposed preterm rabbit model has similar lung ultrasound features and relationships with lung mechanics (that is, correlation between lung ultrasound scores and Raw, lack of correlation with other parameters) of evolving BPD in human neonates.

To our knowledge, this is the first use of lung ultrasound in an experimental model of BPD. Evolving BPD in neonates is characterized by a non-homogeneous pattern composed of consolidated, tissue-like zones with significant loss of aeration, zones with a more or less severe interstitial-alveolar pattern, and zones with a normal aeration (10, 35). These features were also found in rabbits with experimental BPD. Interestingly, these ultrasound findings were coherent with those obtained by

micro-CT scan demonstrating altered lung aeration in preterm rabbits,(36) and this is fully consistent with data showing a close correlation between lung ultrasound score and CT-measured lung aeration.(37, 38) The similarity can be attributed to the shared pathophysiology, such as impaired alveolarization and airway development typical of both human BPD and the experimental BPD-like phenotype generated in the rabbit model.(39, 40) Of note, lung ultrasound appearance looks similar in preterm rabbits and human neonates, while lung development is also known to be comparable (41) and we can estimate to have a similar developmental stage in preterm rabbits and human neonates studied at 7 and 14 days of postnatal age, respectively. (12, 13, 41)

Semi-quantitative ultrasound assessment of lung aeration has been successfully applied in small preterm rabbits, with similar probes, frequencies and procedures used in preterm neonates, confirming the reliability of our findings. Interestingly, relatively novice and experienced operators achieved similar lung ultrasound scores, exactly as observed in NICU care when operators of different degrees of expertise performed lung ultrasound.(19) These data expand those showing the feasibility of lung ultrasound in adult rabbit models of acute respiratory distress syndrome (15) or in preterm rabbits with primary surfactant deficiency at birth (42) and represent the first example of ultrasound-assessed lung aeration in a model of chronic, evolutive lung injury with a mixed obstructive/restrictive pattern.

Our findings also show consisting similarities in the relationship between ultrasound-assessed lung aeration and mechanics. Lung ultrasound scores correlated with Raw both in preterm rabbits and human neonates, while no other significant correlation was found with any other lung mechanics parameter. Infants with BPD have variably decreased compliance and increased resistances, leading to non-uniform ventilation and time-constant (43) and this inhomogeneity is well described by our heterogeneous lung ultrasound findings. In addition, established BPD seems to be particularly characterized by small airway obstruction, (44) and this may explain the observed relationship

between Raw and the lung ultrasound scores. When resistances are higher due to small airway obstruction, lung parenchyma is less aerated, resulting in higher lung ultrasound scores. Small airway obstruction may also explain the lack of correlation between lung ultrasound scores and Cdyn as significantly obstructed lung zones are less ventilated and cannot contribute to the Cdyn calculation. The lack of correlation between rLUS and other mechanical parameters in rabbits may be due either to the non-homogeneous lung injury pattern and the relatively low resolution of lung ultrasound, which cannot provide further details in these small animals. Correlation findings are analogous in the experimental model and in neonates with evolving BPD, confirming the equally impaired lung mechanics (33, 36) as results of similar histology abnormalities.(39, 40) In fact, all lung mechanics parameters showed in **Tab.2** are significantly impaired compared to term rabbits with no lung disease.(33) The analyses performed in term rabbits as further control corroborate our findings. In fact, histology clearly shows typical features of lung injury mimicking BPD in hyperoxia-exposed preterm,(33) while these were absent in term rabbits. Also, lung ultrasound findings were heterogeneous in preterm rabbits as in human neonates with evolving BPD,(10) while lung ultrasound appearance was always normal in term rabbits, exactly as it is always observed in term neonates without any respiratory disorder.(45)

This study has some limitations. The rabbit model is suitable but obviously cannot reproduce all the features of evolving BPD. The effect of invasive ventilation is not considered, since preterm rabbits were spontaneously breathing while the neonates were intubated. Other injuries such as fetal inflammation or infections, that may add to the complexity of BPD development, are also not considered in this model. Moreover, rabbits are not exposed to prenatal steroids, while hyperoxia is avoided in neonates as much as possible. Studying a cohort of infants with evolving BPD serves as "control" of the experimental model, further to the control rabbits delivered at term and unexposed to hyperoxia.(33) The studied sample size is relatively small but is represented by a well-described experimental model and by a homogeneous population of infants with severe ongoing respiratory

failure due to evolving BPD. The choice of BPD definition and a given time-point to perform LUS may be seen as limitations. However, we only used the classical BPD definition since recent analyses performed using multiple definitions have yielded similar diagnostic accuracy for LUS to predict BPD.(35) Also, we only performed LUS at 14 days of postnatal age, because LUS predictivity for BPD is best, while rabbit and human lung development are quite similar at this time-points.(10, 12) Lung ultrasound has been applied for the first time in this experimental model and its use could be improved with experience or smaller probes with higher resolution, albeit using the same devices and protocols as in neonatal lung ultrasound guaranteed a fair comparison of resulting images. The application of a given mean airway pressure by invasive ventilation during lung mechanics measurements in rabbits can provide a certain alveolar recruitment and improve the lung aeration compared to spontaneous breathing: as lung ultrasound and mechanics measurements in rabbits are performed during spontaneous breathing and invasive ventilation, respectively, this may have partially influenced results. However, this is unavoidable to perform lung mechanics measurements and ventilation has been applied with low positive end-expiratory pressure: therefore, its influence, if any, should have been minimal. Our population consisted of BPD neonates who survived and obviously those who died during the NICU hospitalization might have worse lung mechanics, which might influence results. However, mortality directly due to BPD is uncommon in newborn populations similar to ours (46) and we wanted to mimic the majority of patients with evolving BPD.

CONCLUSIONS

Lung ultrasound features are similar in the preterm rabbit model of experimental BPD and in human neonates with evolving BPD. Lung ultrasound is easy to be used even in small animals and with short training. We found an inverse correlation between airway resistance and ultrasound-assessed lung aeration both in preterm rabbits and neonates. The preterm rabbit model fairly reproduces the lung ultrasound and mechanical characteristics of preterm neonates with evolving BPD.

DISCLOSURE

DDL received research and educational grants from Chiesi Pharmaceuticals spa and ABBVIE inc. He served as lecturer for, Chiesi Pharmaceuticals spa, ABBVIE inc and Getinge. Finally, has been a member of advisory boards for Chiesi Pharmaceuticals spa, ABBVIE inc, Airway Therapeutics and a consultant for OPHIREX inc. These companies produce surfactants or surfactant components but had no role at all in this project.

NY received travel grants from Chiesi Pharmaceuticals spa. This company produce surfactants or surfactant components but had no role at all in this project.

CCas, CC, MS and FS are employees of Chiesi Pharmaceuticals spa.

ABBREVIATION LIST

BPD: broncho-pulmonary dysplasia; Cdyn: dynamic compliance; CRIB-II: critical risk index for babies; ICC: Intra-class correlation coefficient; LUS: neonatal lung ultrasound score; NICU: neonatal intensive care units; PEEP: positive end-expiratory pressure; Raw: airway resistance; rLUS: rabbit lung ultrasound score.

REFERENCES

1. **Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, Aschner JL, Davis PG, McGrath-Morrow SA, Soll RF, Jobe AH**. Bronchopulmonary dysplasia. *Nat Rev Dis Primers* 5: 78, 2019. doi: 10.1038/s41572-019-0127-7.

2. **Salaets T, Gie A, Tack B, Deprest J, Toelen J.** Modelling Bronchopulmonary Dysplasia in Animals: Arguments for the Preterm Rabbit Model. *Curr Pharm Des* 23: 5887–5901, 2018. doi: 10.2174/1381612823666170926123550.

3. **Steinhorn R, Davis JM, Göpel W, Jobe A, Abman S, Laughon M, Bancalari E, Aschner J, Ballard R, Greenough A, Storari L, Thomson M, Ariagno RL, Fabbri L, Turner MA.** Chronic Pulmonary Insufficiency of Prematurity: Developing Optimal Endpoints for Drug Development. The *J Pediatr* 191: 15-21.e1, 2017. doi: 10.1016/j.jpeds.2017.08.006.

4. **Albertine KH.** Utility of large-animal models of BPD: chronically ventilated preterm lambs. *Am J Physiol Lung Cell Mol Physiol* 308: L983–L1001, 2015. doi: 10.1152/ajplung.00178.2014.

5. **Yoder BA, Coalson JJ.** Animal models of bronchopulmonary dysplasia. The preterm baboon models. *Am J Physiol Lung Cell Mol Physiol* 307: L970–L977, 2014. doi: 10.1152/ajplung.00171.2014.

6. **Remesal A, De Luca D, San Feliciano L, Isidoro-Garcia M, Minucci A, Pocino K, Casas J, Fabrias G, Capoluongo ED, de la Cruz DL.** Effect of prenatal steroidal inhibition of sPLA2 in a rat model of preterm lung. *Pulm Pharmacol Ther* 36: 31–36, 2016. doi: 10.1016/j.pupt.2015.12.001.

7. **D'Angio CT, Ryan RM.** Animal models of bronchopulmonary dysplasia. The preterm and term rabbit models. *Am J Physiol Lung Cell Mol Physiol* 307: L959–L969, 2014. doi: 10.1152/ajplung.00228.2014.

8. **Lorenzo AV.** The Preterm Rabbit: A Model for the Study of Acute and Chronic Effects of Premature Birth. *Pediatr Res* 19: 201–205, 1985. doi: 10.1203/00006450-198502000-00011.

9. **Mascaretti RS, Mataloun MMGB, Dolhnikoff M, Rebello CM**. Lung morphometry, collagen and elastin content: changes after hyperoxic exposure in preterm rabbits. *Clinics (Sao Paulo)* 64: 1099-1104, 2009. doi: 10.1590/S1807-59322009001100010.

10. **Loi B, Vigo G, Baraldi E, Raimondi F, Carnielli VP, Mosca F, De Luca D,** On behalf of the LUSTRE (Lung UltraSound To pReterm nEonates) study group. Lung Ultrasound to Monitor Extremely Preterm Infants and Predict BPD: Multicenter Longitudinal Cohort Study. *Am J Respir Crit Care Med* 2020 Dec 22. doi: 10.1164/rccm.202008-3131OC

11. **Kalikkot Thekkeveedu R, Guaman MC, Shivanna B**. Bronchopulmonary dysplasia: A review of pathogenesis and pathophysiology.
Respir Med 132: 170–177, 2017. doi: 10.1016/j.rmed.2017.10.014.

12. **Dutta S, Sengupta P.** Rabbits and men: relating their ages.
J Basic Clin Physiol Pharmacol 29: 427–435, 2018. doi: 10.1515/jbcpp-2018-0002.

13. **Duerksen KM, Barlow WE, Stasior OG.** Fused Eyelids in Premature Infants.
Ophthalmic Plastic Reconstr Surg 10: 234–240, 1994. doi: 10.1097/00002341-199412000-00002.

14. **Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H.** The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research.
PLoS Biol 18: e3000410, 2020. doi: 10.1371/journal.pbio.3000410.

15. **Li D, Liu D, Long Y, Wang X.** Use of Lung Ultrasound to Assess the Efficacy of an Alveolar Recruitment Maneuver in Rabbits With Acute Respiratory Distress Syndrome.
J Ultrasound Med 34: 2209–2215, 2015. 34: 2209–2215, 2015. doi: 10.7863/ultra.14.11051.

16. **Raimondi F, Migliaro F, Sodano A, Umbaldo A, Romano A, Vallone G, Capasso L.** Can neonatal lung ultrasound monitor fluid clearance and predict the need of respiratory support?
Crit Care 16: R220, 2012. doi: 10.1186/cc11865.

17. **Brat R, Yousef N, Klifa R, Reynaud S, Shankar Aguilera S, De Luca D.** Lung Ultrasonography Score to Evaluate Oxygenation and Surfactant Need in Neonates Treated With Continuous Positive Airway Pressure.
JAMA Pediatr 169: e151797, 2015. doi: 10.1001/jamapediatrics.2015.1797.

18. **De Martino L, Yousef N, Ben-Ammar R, Raimondi F, Shankar-Aguilera S, De Luca D.** Lung Ultrasound Score Predicts Surfactant Need in Extremely Preterm Neonates.
Pediatrics 142(3). pii: e20180463. doi: 10.1542/peds.2018-0463, [date unknown].

19. **Gomond-LeGoff C, Vivalda L, Foligno S, Loi B, Yousef N, De Luca D.** Effect of different probes and expertise on the interpretation reliability of point-of-care lung ultrasound. *Chest* 157: 924-931, 2020. doi: 10.1016/j.chest.2019.11.013.

20. **Mazmanyan P, Kerobyan V, Shankar-Aguilera S, Yousef N, De Luca D.** Introduction of point-of-care neonatal lung ultrasound in a developing country. *Eur J Pediatr* 179:1131-1137, 2020. doi: 10.1007/s00431-020-03603-w.

21. **Salaets T, Gie A, Jimenez J, Aertgeerts M, Gheysens O, Vande Velde G, Koole M, Murgia X, Casiraghi C, Ricci F, Salomone F, Villetti G, Allegaert K, Deprest J, Toelen J.** Local pulmonary drug delivery in the preterm rabbit: feasibility and efficacy of daily intratracheal injections. *Am J Physiol Lung Cell Mol Physiol* 316: L589–L597, 2019. doi: 10.1152/ajplung.00255.2018.

22. **Gie AG, Regin Y, Salaets T, Casiraghi C, Salomone F, Deprest J, Vanoirbeek J, Toelen J.** Intratracheal budesonide/surfactant attenuates hyperoxia-induced lung injury in preterm rabbits. *Am J Physiol Lung Cell Mol Physiol* 319: L949–L956, 2020. doi: 10.1152/ajplung.00162.2020.

23. **Campbell H, Tomkeieff SI.** Calculation of the internal surface of a lung. *Nature* 170: 116–117, 1952.

24. **Cardini S, Dalli J, Fineschi S, Perretti M, Lungarella G, Lucattelli M.** Genetic Ablation of the Fpr1 Gene Confers Protection from Smoking-Induced Lung Emphysema in Mice. *Am J Respir Cell Mol Biol* 47: 332–339, 2012. doi: 10.1165/rcmb.2012-0036OC.

25. **Fineschi S, De Cunto G, Facchinetti F, Civelli M, Imbimbo BP, Carnini C, Villetti G, Lunghi B, Stochino S, Gibbons DL, Hayday A, Lungarella G, Cavarra E**. Receptor for Advanced Glycation End Products Contributes to Postnatal Pulmonary Development and Adult Lung Maintenance Program in Mice. *Am J Respir Cell Mol Biol* 48: 164–171, 2013. doi: 10.1165/rcmb.2012-0111OC.

26. **Jobe AH, Bancalari E.** Bronchopulmonary Dysplasia. *Am J Resp Crit Care Medicine* 163: 1723–1729, 2001. doi: 10.1164/ajrccm.163.7.2011060.

27. **Escourrou G, De Luca D.** Lung ultrasound decreased radiation exposure in preterm infants in a neonatal intensive care unit. *Acta Paediatr* 105: e237–e239, 2016. doi: 10.1111/apa.13369.

28. **von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative**. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 61: 344–349, 2008. doi: 10.1016/j.jclinepi.2007.11.008.

29. **Dell'Orto V, Bourgeois-Nicolaos N, Rouard C, Romain O, Shankar-Aguilera S, Doucet-Populaire F, De Luca D.** Cell Count Analysis from Nonbronchoscopic Bronchoalveolar Lavage in Preterm Infants. *J Pediatr* 200:30-37.e2, 2018. doi: 10.1016/j.jpeds.2018.04.074.

30. **Cappa P, Sciuto SA, Silvestri S.** Experimental evaluation of errors in the measurement of respiratory parameters of the newborn performed by a continuous flow neonatal ventilator. J Med Eng Technol 30:31-40, 2006. doi: 10.1080/03091900500040295.

31. **Ottaviani V, Veneroni C, Dell'Orto V, Lavizzari A, Ventura ML, Tossici M, Maroszynska I, Mosca F, Tagliabue P, De Luca D, Dellacà RL.** Accuracy of volume and pressure delivery by mechanical ventilators in use in neonatal intensive care units: A quality control study. *Pediatr Pulmonol* 55: 1955–1962, 2020. doi: 10.1002/ppul.24852.

32. **De Luca D, Piastra M, Conti G.** Technical problems with dynamic compliance evaluation in neonates and infants. *Intensive Care Med* 38: 1082–1083, 2012. doi: 10.1007/s00134-012-2522-3.

33. **Richter J, Toelen J, Vanoirbeek J, Kakigano A, DeKoninck P, Verbeken E, Deprest J.** Functional assessment of hyperoxia-induced lung injury after preterm birth in the rabbit. *Am J Physiol Lung Cell Mol Physiol* 306: L277–L283, 2014. doi: 10.1152/ajplung.00315.2013.

34. **McGraw KO, Wong SP.** Forming inferences about some intraclass correlation coefficients. *Psychol Methods* 1: 30–46, 1996. doi: 10.1037/1082-989X.1.1.30.

35. **Liu X, Lv X, Jin D, Li H, Wu H.** Lung ultrasound predicts the development of bronchopulmonary dysplasia: a prospective observational diagnostic accuracy study. *Eur J Pediatr* 2021 Mar 23. doi: 10.1007/s00431-021-04021-2.

36. **Salaets T, Aertgeerts M, Gie A, Vignero J, de Winter D, Regin Y, Jimenez J, Vande Velde G, Allegaert K, Deprest J, Toelen J.** Preterm birth impairs postnatal lung development in the neonatal rabbit model. *Respir Res* 21: 59, 2020. doi: 10.1186/s12931-020-1321-6.

37. **Chiumello D, Mongodi S, Algieri I, Vergani GL, Orlando A, Via G, Crimella F, Cressoni M, Mojoli F.** Assessment of Lung Aeration and Recruitment by CT Scan and Ultrasound in Acute Respiratory Distress Syndrome Patients. *Crit Care Med* 46: 1761–1768, 2018. doi: 10.1097/CCM.0000000000003340.

38. De Luca D. Semiquantititative lung ultrasound scores are accurate and useful in critical care, irrespective of patients' ages: The power of data over opinions. J Ultrasound Med 39:1235-1239, 2020. doi: 10.1002/jum.15195.

39. **Manzano RM, Mascaretti RS, Carrer V, Haddad LB, Fernandes AR, Reyes AMA, Rebello CM.** A Hyperoxic Lung Injury Model in Premature Rabbits: The Influence of Different Gestational Ages and Oxygen Concentrations. *PLoS One* 9: e95844, 2014. doi: 10.1371/journal.pone.0095844.

40. **Coalson JJ.** Pathology of Bronchopulmonary Dysplasia. *Semin Perinatol* 30: 179–184, 2006. doi: 10.1053/j.semperi.2006.05.004.

41. **Kovar J, Sly PD, Willet KE.** Postnatal alveolar development of the rabbit. *J Appl Physiol* 93: 629–635, 2002. doi: 10.1152/japplphysiol.01044.2001.

42. **Cattarossi L, Copetti R, Poskurica B, Miserocchi G.** Surfactant administration for neonatal respiratory distress does not improve lung interstitial fluid clearance: echographic and experimental evidence. *J Perinat Med* 38: 557-563, 2010. doi: 10.1515/jpm.2010.096.

43. **Baraldi E, Filippone M, Trevisanuto D, Zanardo V, Zacchello F.** Pulmonary function until two years of life in infants with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 155: 149–155, 1997. doi: 10.1164/ajrccm.155.1.9001304.

44. **Robin B, Kim Y-J, Huth J, Klocksieben J, Torres M, Tepper RS, Castile RG, Solway J, Hershenson MB, Goldstein-Filbrun A.** Pulmonary function in bronchopulmonary dysplasia. *Pediatr Pulmonol* 37: 236–242, 2004. doi: 10.1002/ppul.10424.

45. **Raimondi F, Yousef N, Migliaro, F, Capasso L, De Luca D.** Point-of-care lung ultrasound in neonatology: classification into descriptive and functional applications. *Pediatr Res* 2018 Jul 20:1–8. doi: 10.1038/s41390-018-0114-9.

46. **Park JH, Chang YS, Sung S, Ahn SY, Park WS.** Trends in Overall Mortality, and Timing and Cause of Death among Extremely Preterm Infants near the Limit of Viability. *PLoS One* 12(1):e0170220, 2017. doi: 10.1371/journal.pone.0170220.

Figure 1. Lung ultrasound procedure in newborn rabbits. Both lungs were entirely scanned in each animal (**Panel A**) and, since the probe was relatively large compared to the animal chest, the evaluation was done on the whole hemithorax (i.e., without any division in chest areas (16)) both transversally (**Panel B_1**) and longitudinally (**Panel B_2**). rLUS was calculated according to the classical semiology pattern used for the neonatal lung ultrasound score (17), that is, assigning 0-to-3 points (**Panel C-F**). A 0 indicates A-pattern (defined by the presence of A-lines only, white triangles, representative of normally aerated zones); 1, B-pattern (defined as the presence of ≥3well-spaced B-lines, white arrows, indicative of zones with interstitial edema); 2, severe B-pattern (defined as the presence of crowded and coalescent B-lines, white arrows with parentheses, with or without consolidations limited to the subpleural space, that is zones with alveolar edema); and 3, extended consolidations (that is zones with total loss of aeration; white star). If the lung appearance was not homogenous, several pictures were registered to capture all lung ultrasound features and their evaluation was averaged. Pictures refer to a hyperoxia-exposed preterm rabbit, but the same technique was used both in preterm and term rabbits.

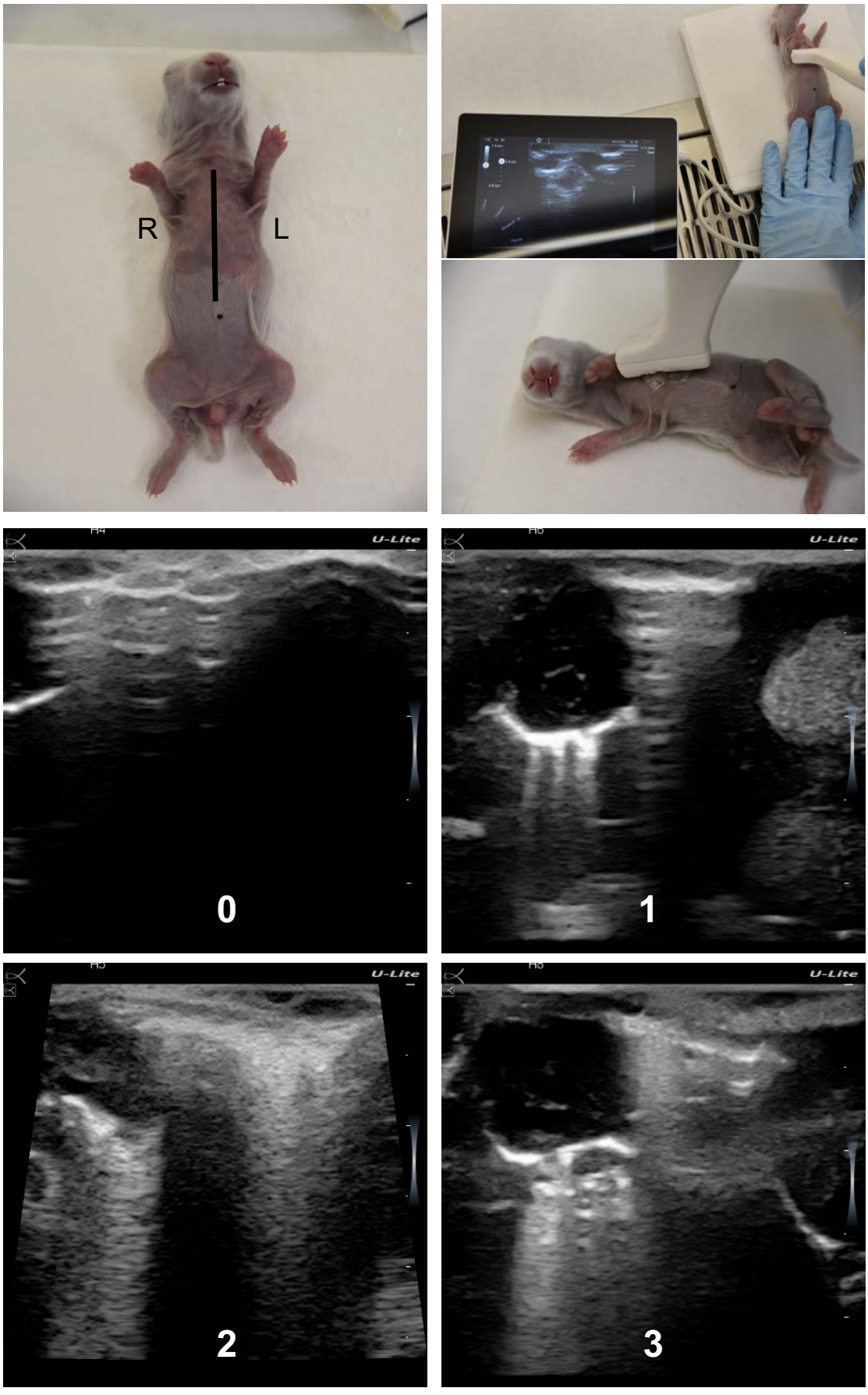
Figure 2. Conformity of lung ultrasound features interpretation to calculate rLUS. Observer 1 and 2, respectively, indicate the investigator performing animal care and the neonatologist expert in lung ultrasound who independently evaluated the lung ultrasound images. Horizontal lines depict medians and 25^{th}-75^{th} percentiles and analyzed with intra-class correlation coefficient and Mann-Whitney test. Experiments were performed in 25 hyperoxia-exposed preterm rabbits. rLUS is a dimensionless number. **Abbreviations**: rLUS: rabbit lung ultrasound score.

Figure 3. Illustrative pictures of abnormal lung ultrasound features in hyperoxia-exposed preterm rabbits and human neonates with evolving BPD. Pictures on the left- and right-side have been taken from rabbits and infants, respectively. Panels A and B show a B-pattern (interstitial); panels C and D show a severe B-pattern (alveolar) and panels E and F show extended consolidation

with loss of lung aeration. Lung ultrasound findings were very and equally heterogeneous both in preterm rabbits and in neonates.

Figure 4. Relationship between lung ultrasound scores and lung mechanics measurements in hyperoxia-exposed preterm rabbits (n=25; panel A-B) and neonates with evolving BPD (n=25; panel C-D). Spearman correlation coefficients and their *p*-values are shown. The hatched curve shows the tendency and represents the best fitting data curve generated with local smoother regression. Panel A and B represent the correlation between rLUS and airway resistance or dynamic lung compliance, respectively. Panel C and D represent the correlation between the classical neonatal LUS and airway resistance or dynamic lung compliance, respectively. LUS and rLUS are dimensionless numbers. **Abbreviations**: Cdyn: dynamic lung compliance; Raw: airway resistance; LUS: lung ultrasound score; rLUS: rabbit lung ultrasound score.

Figure 5. Histology and lung ultrasound features in hyperoxia-exposed preterm rabbits (left side; n=25) and term rabbits kept in normoxia (right side; n=13). Illustrative hematoxylin and eosin staining of a sagittal section of the right lung from one animal per group are shown with 2x (panel A-B) and 10x magnification (panel C-D). There are clear differences between the groups, with hyperoxia-exposed preterm rabbits showing inflammatory cell infiltration, larger alveoli, and thickened septa. Panel E-F show illustrative lung ultrasound images from one animal per group: the hyperoxia-exposed preterm rabbit has an alveolar pattern with confluent B-lines, while the control rabbit has a normal lung appearance with only A-lines. Panel G shows morphometric and lung ultrasound score data in the two groups. rLUS is a dimensionless number. **Abbreviations**: Lm: mean linear intercept; rLUS: rabbit lung ultrasound score.


A
R
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B1
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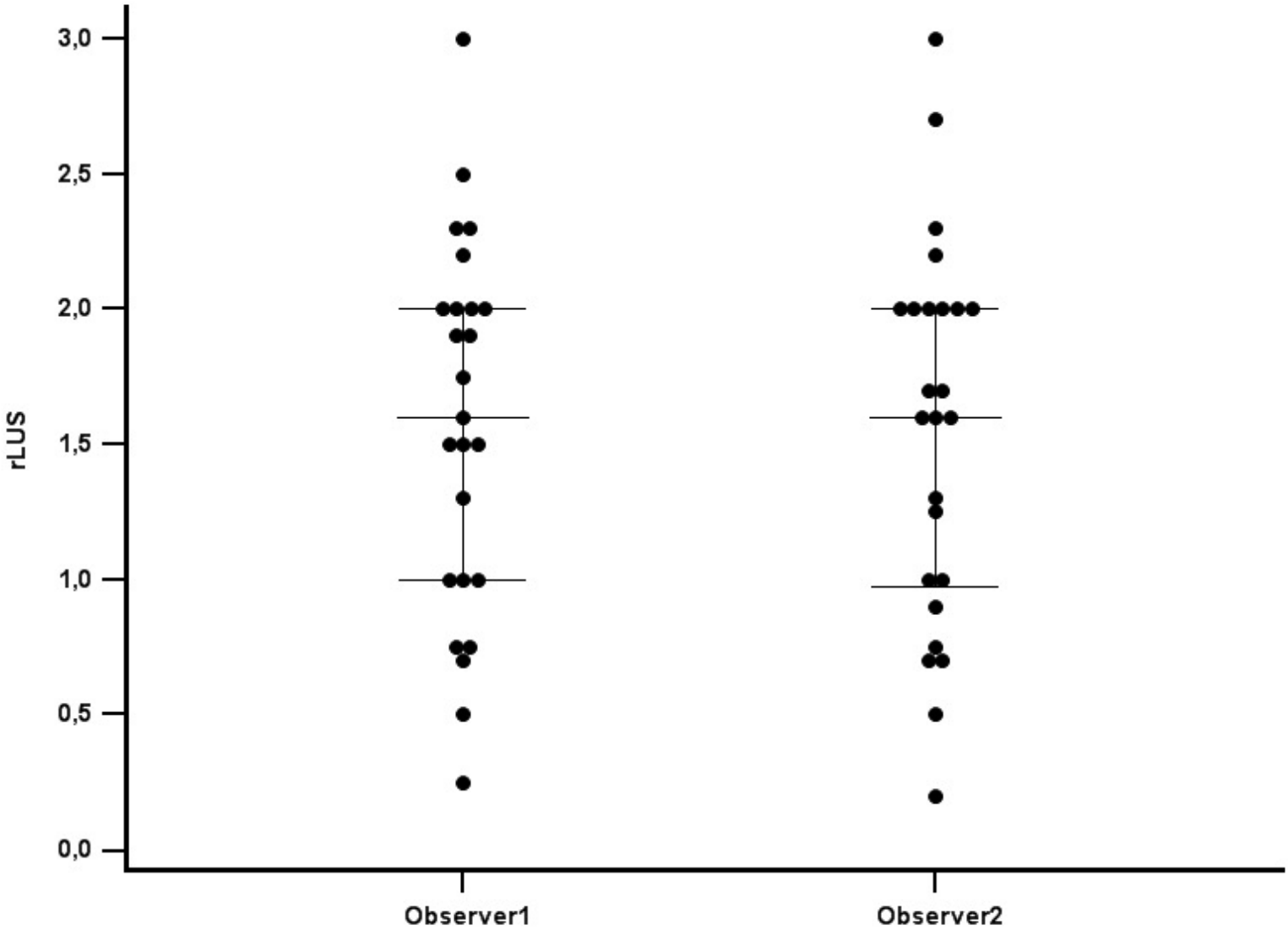
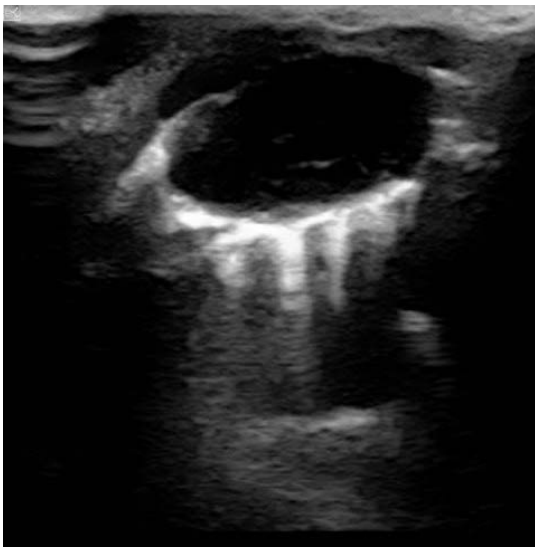
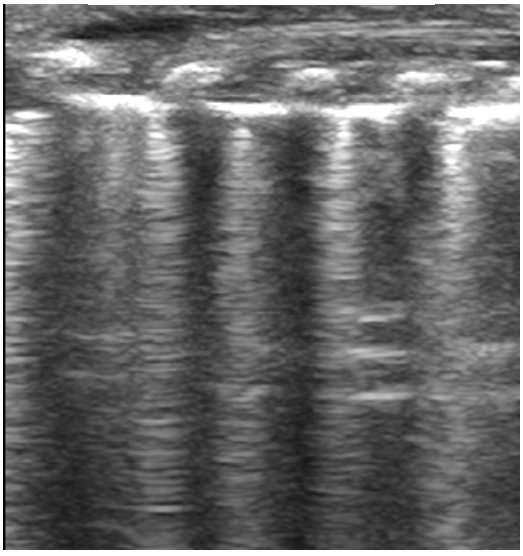

3,0
2,5
2,0
1,5
1,0
0,5
0,0
rLUS
Observer1
Observer2

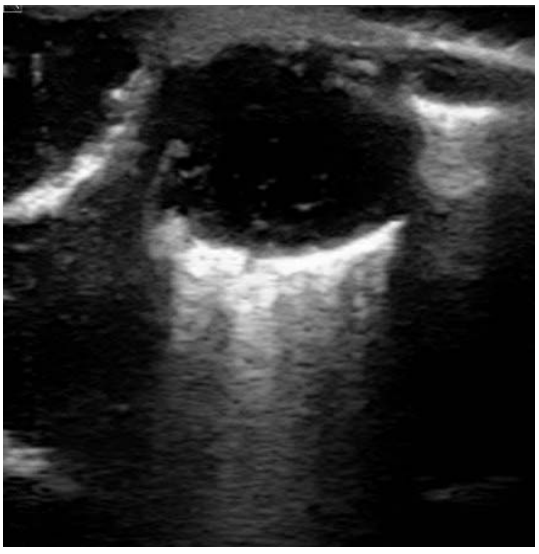
A

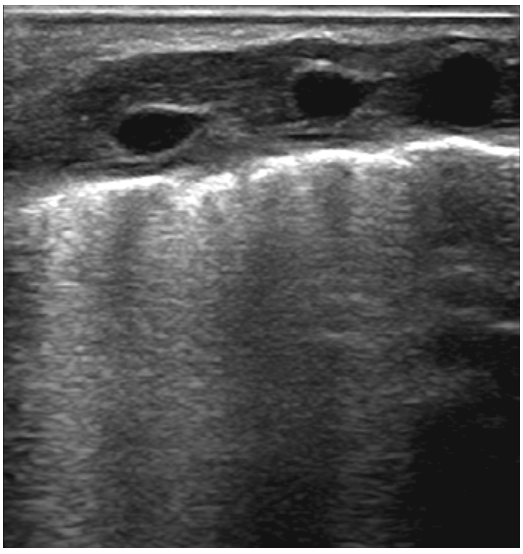




B

C





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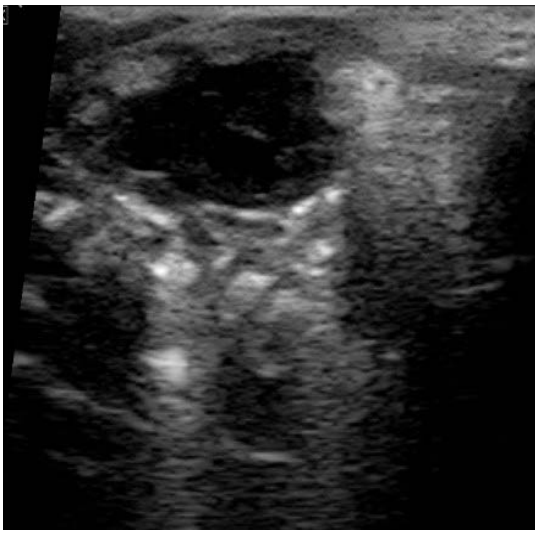

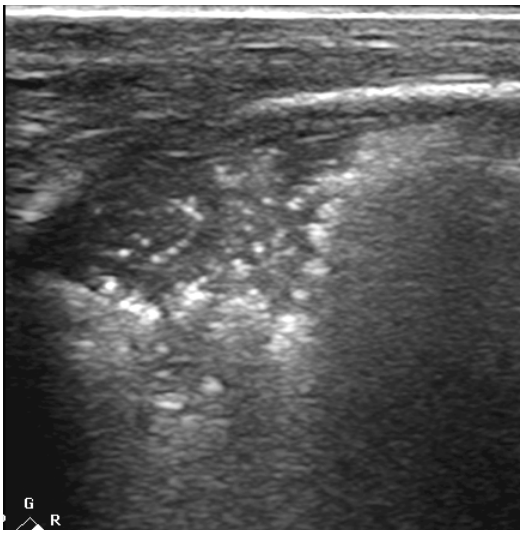

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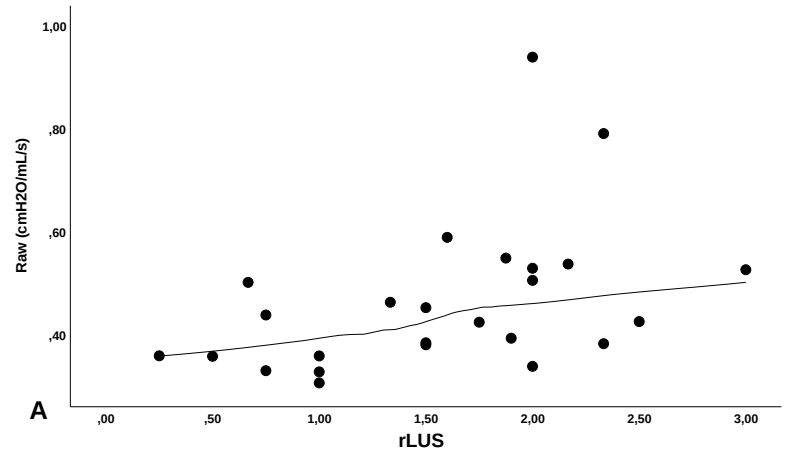
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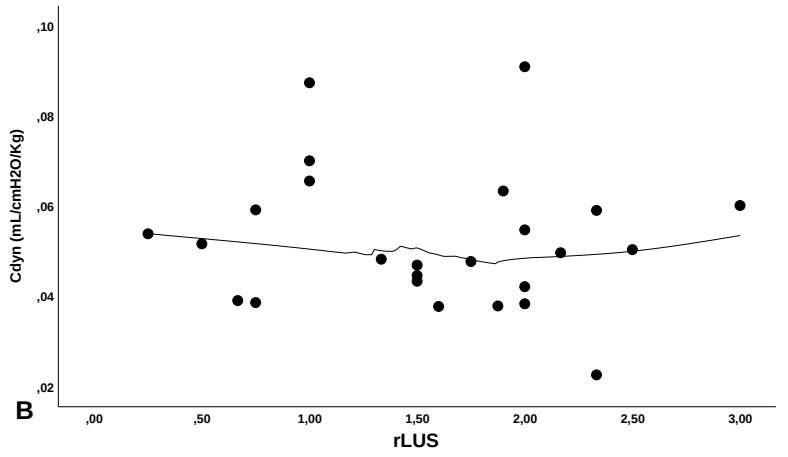
,60
,40
,00
,50
1,00
1,50
2,00
2,50
3,00
rLUS
A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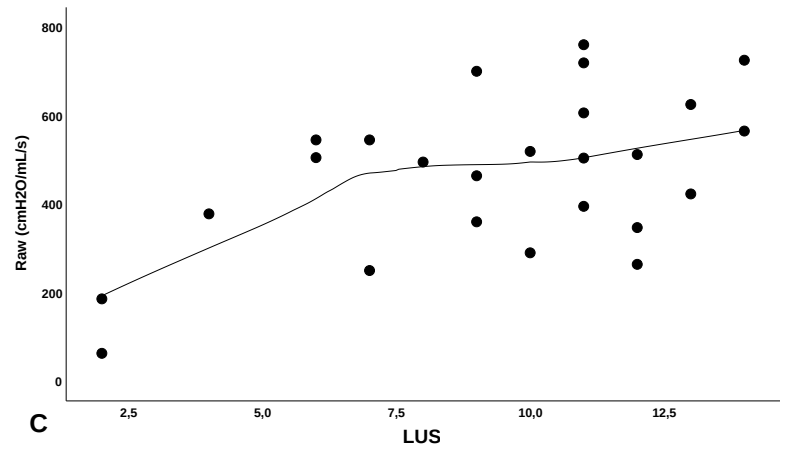
,02
,00
,50
1,00
1,50
2,00
2,50
3,00
rLUS
B


Raw (cmH2O/mL/s)
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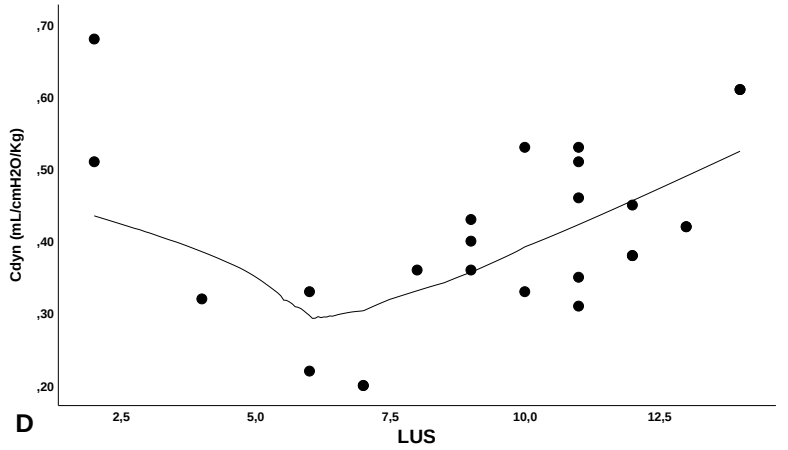
600
400
200
0
2,5
5,0
7,5
10,0
12,5
LUS
C


Cdyn (mL/cmH2O/Kg)
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,30
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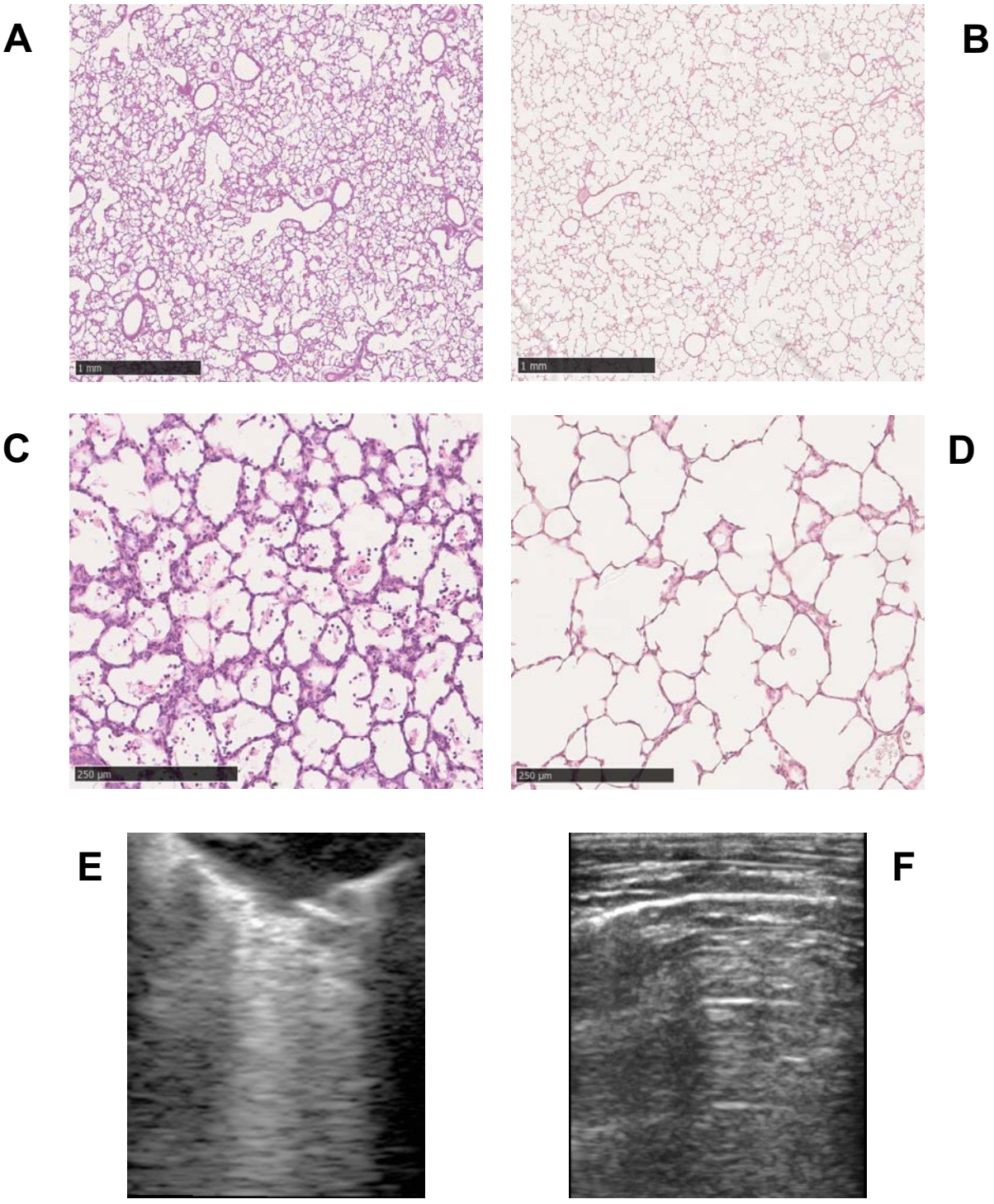
10,0
12,5
LUS
D



G	Preterm (hyperoxia-exposed)	Term (normoxia)	*p*
Lm (µm)	84 (4.9)	72 (5.5)	<0.0001
rLUS	1.6 [1-2]	0 [0-0]	<0.0001

Table 1. Characteristics of hyperoxia-exposed preterm rabbits and human neonates. Data are expressed as mean (standard deviation), median [interquartile range] and number (%). Weight-$_{D7}$ and Weight-$_{D14}$ indicate the weight at the 7th and 14th day of life, respectively. rLUS-$_{D7}$ and LUS-$_{D14}$ indicate the rabbit and the neonatal lung ultrasound score at the 7th and 14th day of life, respectively. Apgar, CRIB-II, LUS and rLUS are dimensionless numbers. Prenatal steroids prophylaxis is considered if complete, that is, if two 12mg betamethasone doses were administered 24h apart and within 7 days before the delivery.

Abbreviations: CRIB-II: critical risk index for babies; LUS: neonatal lung ultrasound score; NICU: neonatal intensive care units; rLUS: rabbit lung ultrasound score.

Preterm rabbits (n=25)	
Birth weight (grams)	37 (1.01)
Weight-$_{D7}$ (grams)	49.3 (1.44)
rLUS-$_{D7}$	1.5 [0.25-3]
Preterm neonates (n=25)	
Gestational age (weeks)	25.8 (1.01)
Birth weight (grams)	759 (115)
Weight-$_{D14}$ (grams)	898 (125)
Prenatal steroids prophylaxis	17 (68%)
Male Sex	14 (63.6%)
5'Apgar score	9 [5.5-8.5]
CRIB-II score	12 [11-13]
LUS-$_{D14}$	7 [8-11]
NICU stay (days)	101 [96-130]

Table 2. Lung mechanics variables in preterm rabbits and neonates. Data are expressed as mean (standard deviation). **Abbreviations**: Cdyn: dynamic compliance, Raw: airway resistance.

Preterm rabbits (n=25)	
Raw (cmH_2O/mL/sec)	0.5 (0.1)
Cdyn (mL/cmH_2O/kg)	1.07 (0.3)
Inspiratory capacity (mL/kg)	20.6 (6.2)
Tissue damping (cmH_2O/mL)	3.1 (0.7)
Elastance (cmH_2O/mL)	12 (2.9)
Preterm neonates (n=25)	
Raw (cmH_2O/L/sec)	470 (176)
Cdyn (mL/cmH_2O/kg)	0.32 (0.1)