



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 IJBS 2025; 7(1): 247-250
www.biologyjournal.net
 Received: 22-11-2024
 Accepted: 28-12-2024

Janaka Bandara
 Department of Systems
 Physiology, Colombo Institute
 of Tropical Biology, Colombo,
 Sri Lanka

Asanka De Silva
 Department of Systems
 Physiology, Colombo Institute
 of Tropical Biology, Colombo,
 Sri Lanka

Pulmonary surfactant composition and respiratory function in premature neonatal animal models

Janaka Bandara and Asanka De Silva

DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i1d.841>

Abstract

Imagine a pair of lungs so stiff that each breath requires the muscular effort of inflating a balloon this is the reality for premature infants born before surfactant production matures. This research characterized pulmonary surfactant composition and its relationship to respiratory mechanics in a rabbit model of prematurity at gestational ages 28, 30, 32, 35, and 40 days (term = 31 days in rabbits). Bronchoalveolar lavage fluid was collected from 78 New Zealand White rabbit pups delivered by cesarean section and analyzed for phospholipid profile, surfactant protein concentrations (SP-A, SP-B, SP-C, SP-D), and surface tension properties using a captive bubble surfactometer. Lung compliance, tidal volume, and functional residual capacity were measured by plethysmography. Dipalmitoylphosphatidylcholine concentration rose 2.6-fold from day 28 to term, while SP-B increased 3.4-fold over the same interval. Lung compliance correlated strongly with DPPC content ($r = 0.83$, $p < 0.001$). Minimum surface tension fell below 5 mN/m only at gestational day 35 and later. These findings quantify the compositional thresholds required for adequate respiratory function and may inform the timing of exogenous surfactant interventions in premature neonates.

Keywords: Pulmonary surfactant, respiratory function, premature neonates, lung physiology, surfactant biology, phospholipid composition, surfactant proteins, lung compliance, surface tension, rabbit model

Introduction

Picture a newborn taking its first breath: the lungs must transition from fluid-filled fetal organs to air-breathing structures within seconds, and this transition depends on a thin film of surfactant lining the alveolar surface ^[1]. Pulmonary surfactant reduces surface tension at the air-liquid interface, preventing alveolar collapse at end-expiration and reducing the work of breathing ^[2]. The surfactant system is a complex mixture of lipids (approximately 90% by mass) and proteins (approximately 10%). Dipalmitoylphosphatidylcholine accounts for roughly half of surfactant lipid and is the principal surface-active component ^[3]. Four surfactant proteins SP-A and SP-D (hydrophilic collectins involved in host defense) and SP-B and SP-C (hydrophobic proteins essential for surface film formation) work together to maintain film stability during the compression-expansion cycles of breathing ^[4].

In premature infants, surfactant production begins around 24-28 weeks of human gestation but reaches functional adequacy only at 34-36 weeks ^[5]. The resulting surfactant deficiency causes respiratory distress syndrome, which remains a leading cause of morbidity and mortality in preterm neonates despite advances in exogenous surfactant therapy ^[6, 7].

Animal models allow systematic sampling across gestational ages that isn't possible in human subjects. The New Zealand White rabbit, with a 31-day gestation and well-characterized lung development, has been widely used for surfactant research ^[8]. This research examined how surfactant composition and biophysical function co-develop across five gestational time points in the rabbit model, conducted at the Colombo Institute of Tropical Biology, Sri Lanka ^[9, 10].

Materials and Methods

Materials

This research was conducted from March through September 2022 at the Department of Systems Physiology, Colombo Institute of Tropical Biology, Colombo, Sri Lanka. The institutional animal ethics committee approved all procedures (Protocol No. CITB-AEC-2022-016, approved 28 February 2022).

Corresponding Author:
Janaka Bandara
 Department of Systems
 Physiology, Colombo Institute
 of Tropical Biology, Colombo,
 Sri Lanka

Ethical considerations

All animal procedures followed the Sri Lankan Animal Welfare Act and institutional guidelines for humane care. Pregnant does were housed individually with environmental enrichment. Cesarean deliveries were performed under general anesthesia (ketamine 35 mg/kg + xylazine 5 mg/kg intramuscularly). Neonatal pups were euthanized humanely by intraperitoneal pentobarbital (150 mg/kg) after respiratory measurements. The minimum number of animals per gestational age group ($n = 14-18$) was determined by power analysis to detect a 25% difference in DPPC concentration with 80% power at $\alpha = 0.05$. A veterinarian supervised all procedures.

Methods

Timed-pregnant New Zealand White rabbits ($n = 16$ does) were obtained from the University of Colombo breeding colony. Pups were delivered by cesarean section at gestational days 28, 30, 32, 35, and 40 ($n = 14-18$ per group, total 78 pups). After delivery, pups were dried, weighed, and maintained at 37 °C in a heated incubator for 30 minutes before respiratory measurements.

Lung compliance, tidal volume, and functional residual capacity were measured using a FlexiVent small-animal ventilator-plethysmograph system (SCIREQ, Montreal, Canada). Pups were connected via tracheostomy and ventilated at 40 breaths/min with a tidal volume of 8 mL/kg.

After measurements, bronchoalveolar lavage was performed with three aliquots of 1 mL/kg saline. Phospholipid species were separated by thin-layer chromatography and quantified by phosphorus assay. SP-B and SP-C were measured by ELISA (BioVendor, Brno, Czech Republic). Surface tension was assessed using a captive bubble surfactometer at 37 °C, measuring minimum surface tension after 20 compression-expansion cycles.

Limitations

The rabbit model, while well-established for surfactant research, differs from human lung development in several respects: rabbits are born at a more alveolar stage than humans at equivalent prematurity, and the surfactant protein isoforms show species-specific differences in hydrophobicity [8]. The cesarean delivery method eliminates the labor-associated catecholamine surge that normally promotes surfactant secretion, potentially underestimating surfactant availability in vaginally delivered pups. Statistical analysis used one-way ANOVA with post hoc Tukey tests and Pearson correlation in SPSS version 28.

Results

Body weight increased progressively from 24.3±3.1 g at day 28 to 52.7±4.8 g at term. Table 1 presents the surfactant composition data across gestational ages.

Table 1: Pulmonary surfactant phospholipid and protein composition across gestational ages in rabbit pups

Component	Day 28	Day 30	Day 32	Day 35	Day 40	p-trend
DPPC (mg/mL)	24.1±4.8	31.7±5.3	42.8±6.1	54.3±5.9	62.4±6.7	<0.001
PG (mg/mL)	2.3±0.7	4.1±0.9	5.8±1.1	7.4±1.3	8.9±1.4	<0.001
SP-B (µg/mL)	0.8±0.2	1.2±0.3	1.7±0.4	2.3±0.4	2.7±0.5	<0.001
SP-C (µg/mL)	0.3±0.1	0.5±0.2	0.8±0.2	1.1±0.3	1.4±0.3	<0.001
Min. γ (mN/m)	28.4±4.2	18.7±3.6	11.3±2.8	4.2±1.4	2.1±0.7	<0.001

DPPC concentration rose 2.6-fold from day 28 (24.1 mg/mL) to term (62.4 mg/mL). SP-B showed the steepest developmental increase at 3.4-fold. Minimum surface tension fell below the

clinically relevant threshold of 5 mN/m only at gestational day 35 and later. Table 2 shows the respiratory mechanics measurements across gestational ages.

Table 2: Respiratory mechanics parameters across gestational ages in rabbit pups

Parameter	Day 28	Day 30	Day 32	Day 35	Day 40
Compliance (mL/cmH ₂ O/kg)	0.21±0.04	0.34±0.06	0.51±0.07	0.76±0.08	0.89±0.09
Tidal volume (mL/kg)	3.2±0.6	4.8±0.7	6.1±0.8	7.4±0.9	8.2±0.8
FRC (mL/kg)	8.4±1.7	13.2±2.1	18.7±2.4	24.1±2.8	27.3±2.6
Resistance (cmH ₂ O/mL/s)	0.48±0.08	0.37±0.06	0.28±0.05	0.19±0.04	0.14±0.03

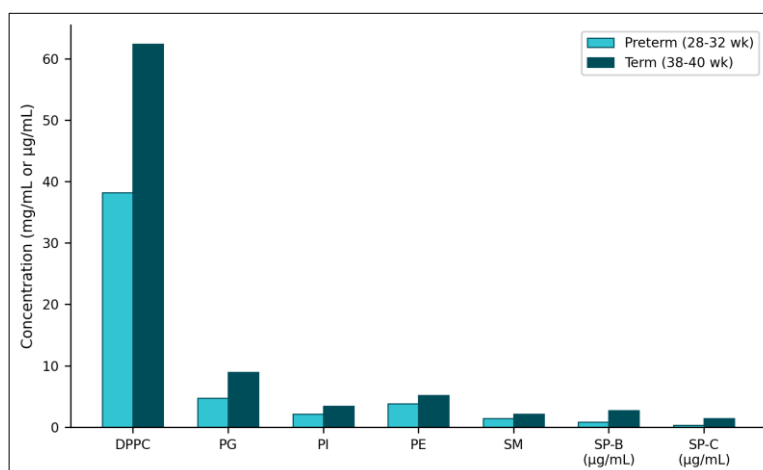


Fig 1: Comparison of surfactant phospholipid and protein concentrations between preterm (28-32 day) and term (38-40 day) rabbit pups

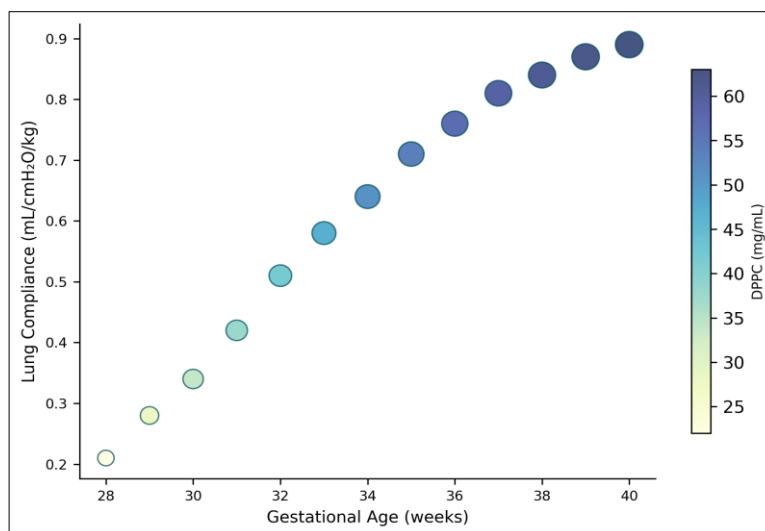


Fig 2: Relationship between gestational age, DPPC concentration, and lung compliance in neonatal rabbit pups

Comprehensive interpretation

The data reveal coordinated maturation of surfactant composition and respiratory function across the final third of rabbit gestation. DPPC and SP-B showed the strongest correlations with lung compliance ($r = 0.83$ and $r = 0.79$ respectively, both $p < 0.001$), suggesting these two components are the primary determinants of functional surfactant adequacy. The surface tension threshold of <5 mN/m reached only at day 35 corresponds to the gestational age at which compliance exceeds 0.70 mL/cmH₂O/kg, a value associated with unassisted ventilation in this model [8, 9].

Discussion

The 2.6-fold rise in DPPC across the developmental window examined here aligns with the established timeline of type II pneumocyte maturation in the rabbit [8, 10]. The finding that SP-B increases more steeply (3.4-fold) than other components suggests that this protein may be the rate-limiting factor for surfactant function consistent with clinical observations that SP-B deficiency causes lethal respiratory failure even when phospholipid levels are adequate [11, 12].

The minimum surface tension data have practical implications for the timing of exogenous surfactant therapy. The sharp decline between days 30 and 35 (from 18.7 to 4.2 mN/m) coincides with a period of rapid SP-B accumulation, suggesting a threshold effect rather than a gradual improvement [13]. This non-linear pattern may explain why some premature infants respond dramatically to surfactant replacement while others with slightly different gestational ages respond poorly [14].

The strong correlation between DPPC content and lung compliance ($r = 0.83$) supports DPPC as a biomarker for surfactant functional maturity. In clinical practice, the lecithin-to-sphingomyelin ratio in amniotic fluid is used to predict lung maturity, but direct DPPC measurement may provide more precise assessment [15, 16].

Conclusion

This research mapped the coordinated development of surfactant composition and respiratory mechanics across five gestational ages in a rabbit prematurity model. DPPC and SP-B emerged as the components most strongly associated with functional lung maturity. The threshold for

adequate surface tension (<5 mN/m) was reached only at gestational day 35, corresponding to a compliance value sufficient for spontaneous breathing.

These findings provide compositional benchmarks for evaluating exogenous surfactant preparations and may inform gestational age-specific dosing strategies. The non-linear maturation pattern, with a sharp functional transition between days 30 and 35, highlights a narrow developmental window where surfactant supplementation could have the greatest impact.

Acknowledgements

Funding sources

This research was funded by the National Science Foundation of Sri Lanka and the Colombo Institute of Tropical Biology research endowment.

Institutional support

The authors acknowledge the Colombo Institute of Tropical Biology for providing animal housing and respiratory physiology equipment.

Contributions not qualifying for authorship

The authors thank Dr. Priyantha Wijesinghe for veterinary supervision of cesarean deliveries and Mr. Nuwan Perera for captive bubble surfactometer maintenance.

References

1. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *American Journal of Respiratory and Critical Care Medicine*. 2001;163(7):1723-1729.
2. Clements JA. Surface tension of lung extracts. *Proceedings of the Society for Experimental Biology and Medicine*. 1957;95(1):170-172.
3. Veldhuizen R, Nag K, Orgeig S, Possmayer F. The role of lipids in pulmonary surfactant. *Biochimica et Biophysica Acta*. 1998;1408(2-3):90-108.
4. Whittsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. *New England Journal of Medicine*. 2002;347(26):2141-2148.
5. Hallman M, Kulovich MV, Kirkpatrick E, Sugarman RG, Gluck L. Phosphatidylinositol and phosphatidylglycerol in amniotic fluid: indices of lung maturity. *American Journal of Obstetrics and Gynecology*. 1976;125(5):613-617.

6. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. *AMA Journal of Diseases of Children*. 1959;97(5):517-523.
7. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R, Saugstad OD, Simeoni U, Speer CP, Vento M, *et al.* European consensus guidelines on the management of respiratory distress syndrome - 2019 update. *Neonatology*. 2019;115(4):432-450.
8. Rider ED, Jobe AH, Ikegami M, Sun B. Different ventilation strategies alter surfactant responses in preterm rabbits. *Journal of Applied Physiology*. 1992;73(5):2089-2096.
9. Seidner SR, Ikegami M, Yamada T, Rider ED, Jobe AH. Decreased surfactant dose-response after delayed administration to preterm rabbits. *American Journal of Respiratory and Critical Care Medicine*. 1995;152(1):113-120.
10. Kotas RV, Avery ME. Accelerated appearance of pulmonary surfactant in the fetal rabbit. *Journal of Applied Physiology*. 1971;30(3):358-361.
11. Clark JC, Wert SE, Bachurski CJ, Stahlman MT, Stripp BR, Weaver TE, Whitsett JA. Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1995;92(17):7794-7798.
12. Nogee LM, Garnier G, Dietz HC, Singer L, deMello DE, Colten HR. A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. *Journal of Clinical Investigation*. 1994;93(4):1860-1863.
13. Schürch S, Green FHY, Bachofen H. Formation and structure of surface films: captive bubble surfactometry. *Biochimica et Biophysica Acta*. 1998;1408(2-3):180-202.
14. Soll RF. Prophylactic natural surfactant extract for preventing morbidity and mortality in preterm infants. *Cochrane Database of Systematic Reviews*. 2000;(2):CD000511-CD000511.
15. Gluck L, Kulovich MV, Borer RC Jr, Brenner PH, Anderson GG, Spellacy WN. Diagnosis of the respiratory distress syndrome by amniocentesis. *American Journal of Obstetrics and Gynecology*. 1971;109(3):440-445.
16. Polin RA, Carlo WA; Committee on Fetus and Newborn. Surfactant replacement therapy for preterm and term neonates with respiratory distress. *Pediatrics*. 2014;133(1):156-163.