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Artigo Original

Teste das microbolhas estáveis no diagnóstico precoce da síndrome do desconforto respiratório do recém-nascido

Stable microbubble test in the early diagnosis of newborn respiratory distress syndrome

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RESUMO

Objetivo: Analisar a importância do Teste das Microbolhas Estáveis (TME) no diagnóstico precoce da Síndrome do Desconforto Respiratório do recém-nascido (SDRRN) em recém-nascidos (RN) com idade gestacional entre 32 e 37 semanas com sintomas respiratórios (SR). **Metodologia:** estudo observacional, de coorte. A coleta de dados ocorreu em um hospital público do sul do Brasil, entre março de 2018 e fevereiro de 2020. **Resultados:** Participaram 23 recém-nascidos e compararam-se os grupos dos pacientes com e sem sintomas respiratórios em relação ao número de microbolhas estáveis. Dentre os RN com SR, 50% apresentaram entre 15 e 50 mbe/mm² e 50% acima de 50 mbe/mm². Já os pacientes sem SR, 18% apresentaram entre 15 e 50 mbe/mm², enquanto 82% apresentaram mais de 50 mbe/mm², sem diferença ou associação significativa. As mães dos RN com SR receberam corticoterapia pré-parto, fator protetor ao desenvolvimento da SDRRN, não necessitando de administração do surfactante exógeno. **Conclusão:** utilizar o TME para auxílio diagnóstico da SDRRN foi um método eficaz, pois não houve RN com menos de 15 mbe/mm² e nenhum RN necessitou surfactante exógeno. Os RN com sintomas respiratórios, cuja quantidade de microbolhas estáveis excede 15mbe/mm², demonstram possuir SDRRN leve, a qual não necessita de administração de surfactante exógeno, como verificado no estudo.

Palavras-chave: Teste das Microbolhas Estáveis; Síndrome do desconforto respiratório; Recém-nascidos; Surfactante exógeno; Corticoterapia.

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ABSTRACT

Objective: To analyze the importance of SMT in the early diagnosis of NRDS in newborns (NB) with gestational age between 32 and 37 weeks with respiratory symptoms (RS). **Methodology:** observational, cohort study. Data collection occurred in a single public hospital in southern Brazil, between March 2018 and February 2020. **Results:** Twenty-three newborns participated, and the groups of patients with and without respiratory symptoms were compared in relation to the number of stable microbubbles. Among the NBs with respiratory symptoms, 50% presented between 15 and 50 SM/mm² and 50% above 50 SM/mm². On the other hand, 18% of the patients without RS presented between 15 and 50 SM/mm², while 82% presented more than 50 SM/mm², with no significant difference or association. The mothers of NBs with RS received pre-delivery corticosteroid therapy, a protective factor for developing NRDS, not requiring the administration of exogenous surfactant. **Conclusion:** using the SMT to aid in the diagnosis of NRDS was an effective method since there was no NB with less than 15 SM/mm² and no NB required exogenous surfactant. Newborns with respiratory symptoms, whose quantity of stable microbubbles exceeds 15 mbe/mm², demonstrate having mild Respiratory Distress Syndrome, which does not require exogenous surfactant administration, as verified in the study.

Keywords: Stable Microbubble Test. Respiratory distress syndrome. Newborns. Exogenous surfactant. Corticosteroid therapy..

INTRODUÇÃO

The newborn respiratory distress syndrome (NRDS) has as its main cause the deficiency of the pulmonary surfactant due to premature birth, immature mechanisms for removing pulmonary fluid, and a small gas exchange area[1]. The syndrome is estimated to affect approximately 1% of all live births[2]. The risk of developing the pathology is inversely proportional to gestational age, with a higher incidence in newborns (NB) younger than 29 weeks[3]. The more premature the newborn, the greater the respiratory symptoms resulting from the disease, starting in the first 24 hours and may increase up to 48 hours after birth[4]. This is because there is no surfactant production until the late stages of pregnancy, between 34 and 36 weeks. Also, other risk factors would be multiple pregnancies, maternal diabetes, and some rare hereditary cases[5].

Lung surfactant is a complex substance composed of lipids and proteins, which has its peak production at 35 weeks of gestation[6], which creates in the air-liquid respiratory interface a film that reduces surface tension and stabilizes the gas exchange of the lung during all cycles of inspiration and expiration. Furthermore, it establishes an innate defense barrier before the entrance of microorganisms[7].

The surfactant deficiency causes increased alveolar surface tension, progressing to alveolar instability with atelectasis and loss of lung compliance[6,8]. Therefore, some clinical manifestations of NRDS are nasal wing beats, tachypnea, grunting, retractions, and cyanosis with decreased air entry on auscultation[7,9].

The degree of respiratory discomfort can be assessed through the Silverman-Andersen Score, which evaluates respiratory symptoms (thoracoabdominal synchrony, intercostal retractions, xiphoid retraction, nasal wing beats, and grunting) on a scale of 0 to 10. A higher score indicates a more severe condition[10].

The most frequent complications of NRDS are central nervous system hemorrhage, bronchopulmonary dysplasia, patent ductus arteriosus, and retinopathy of prematurity[11]. It is one of the main causes of morbidity and mortality in preterm infants. However, with the appropriate treatment, the prognosis is encouraging. Mortality is less than 10% with adequate ventilation. The production of surfactant begins, and from 4 to 5 days, the NRDS disappears[12].

The main interventions for NRDS include the use of antenatal corticosteroids, optimization of ventilatory support management, administration of surfactant, and general care such as regulation of body temperature, nutrition, electrolyte management, and antibiotic therapy[9]. In milder cases of RDS in the newborn, when the infant is capable of spontaneous breathing with adequate ventilation, requiring an inspired oxygen fraction (FiO2) equal to or less than 40% to maintain Arterial Partial Pressure of Oxygen (PaO2) between 50 and 70 mmHg, there is no need for ventilatory support, and oxygen therapy is sufficient[10]. When the newborn requires an FiO2 greater than or equal to 60% and exhibits moderate to severe respiratory distress, non-invasive mechanical ventilation may be indicated. When there is no response to these therapies, invasive mechanical ventilation is recommended[10].

There are various conditions that present with similar signs and symptoms, such as transient tachypnea of the newborn, air leak syndromes, neonatal pneumonia, meconium aspiration, persistent pulmonary hypertension of the newborn, among others[13]. Therefore, it is necessary to conduct a differential diagnosis through appropriate tests.

The stable microbubble test (SMT) is used to verify lung maturity; therefore, it quantifies the activity and presence of the surfactant in the lung and can be performed by obtaining amniotic fluid and in gastric and tracheal aspirates[14]. The test is fast (5 to 10 minutes), low cost, and easy to operate, and verifies the ability of discharges containing surfactant to form stable bubbles with less than 15 micrometers[15-17]. Some studies of SMT in gastric juice for the diagnosis of NRDS use 10 SM/mm² as a cutoff point to define pulmonary surfactant deficiency[14], while other authors use 15 SM/mm² and 30 SM/mm² [18,19].

Surfactant replacement treatment has been shown to be effective in improving NRDS. Studies have examined the effects of surfactant preparations administered via endotracheal tube within minutes of birth or after symptom onset. In general, they showed improvement in oxygenation and reduced need for ventilatory support[20].

Although the collection of gastric juice is an invasive procedure, the SMT would assist in the diagnosis of the disease while reducing the inappropriate use of the drug and the performance of chest radiography, which is the alternative diagnostic method, in the first hours of life, resulting in economic benefit for the institution. Moreover, there was no similar research in the study hospital, making it important in efficiently managing the syndrome. The gastric aspirate of premature infants has high sensitivity in predicting NRDS, and the SMT presents excellent performance, as well as other more sophisticated tests in the world literature[21].

Also, it is considered necessary that studies of this nature encourage neonatologists, obstetricians, and pediatricians to research the degree of lung maturity in newborns to develop quick and accurate diagnoses. Therefore, this study aims to evaluate the use of the stable microbubble test for early diagnosis of respiratory distress syndrome in newborns with gestational age between 32 and 37 weeks.

METHODOLOGY

An observational, cohort study was conducted between March 2018 and February 2020 in the neonatal intensive care unit (NICU) and the Obstetric Center of a university hospital in southern Brazil.

The research was conducted in newborns with gestational age between 32 and 37 weeks, with and without respiratory symptoms, which included tachypnea, moaning, cyanosis, and nasal wing beat. Gestational age (GA) was established from the date of the last menstruation or confirmation of ultrasound performed before the first 20 weeks.

The Research Ethics Committee (REC) approved this study, under CAAE: 86220218.0.0000.5346. Resolution No. 466, of December 12, 2012, of the National Health Council[22], which addresses guidelines and regulatory standards for research involving human beings, was considered. The Informed Consent Form (ICF) was used, which was signed by the parents, to whom the researcher previously contacted and explained it verbally and in person.

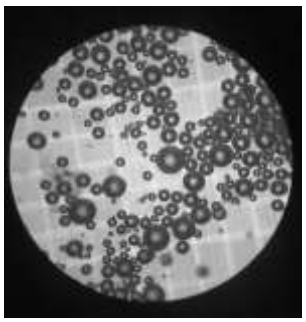
The performed procedure followed the protocol outlined by Pattle and colleagues (1979)[23]. Two milliliters of gastric fluid were collected from the newborns using a syringe through an oro-gastric tube within their first minutes of life. Notably, the aspirate was part of the hospital routine protocol and post-birth care of the institution where the study was conducted. If the responsible physician was present, the content was analyzed immediately. Otherwise, the material was frozen at a temperature below -1°C for further analysis within 24 hours. For thawing, the sample was removed from the freezer and kept at room temperature for up 20 minutes approximately. After this period, the material was analyzed.

For analysis, a minimum quantity of 40 microliters of fluid was placed in a Neubauer counting chamber. Using a Pasteur pipette adapted with a 2ml rubber bulb, the fluid was tapped vertically onto the slide, rapidly aspirating and expelling the content around 20 times. The chamber was immediately inverted onto an empty slide, forming a suspended droplet. After four minutes, the chamber was examined under the 10x objective of a microscope. The number of microbubbles with a diameter of less than 15 micrometers was counted, and subsequently, the average quantity of bubbles per mm² was calculated. Counts were performed in five regions within four quadrants and the center of the bubble field[23].

Black and non-spherical bubbles were discarded, and the count results were expressed in mbe/mm²[20,23]. The test indicates surfactant deficiency when observing a quantity of up to 15 stable microbubbles/mm², and above this value, surfactant deficiency is considered absent[23,24].

Upon completion of observation and analysis, the results were recorded on a sheet, a photograph was taken as shown in Figure 1, and the information was archived in a designated folder on the computer. The collected material was immediately discarded after the TME procedure. At the time of admission to the NICU, a chest X-ray was performed in some patients, on average 4 to 6 hours after birth, depending on the baby's conditions, which served as a way to reinforce the diagnosis and support the decision to administer surfactant.

Figure 1. Collection of the first patient with respiratory symptoms.



Source: Withdrawal of microscopic observation performed by the researcher.

A descriptive analysis of each variable studied was performed to characterize the sample. The comparison of quantitative data was performed using the Mann-Whitney U test and, for qualitative data, Fisher's exact test or G test. Statistical analyses were performed using the Statistica Software 9.1, considering a significance level of 5% ($\alpha<0.05$).

RESULTS

The study participants were divided into two groups, with and without respiratory symptoms, and compared gender, weight, the relationship between weight and adequacy for gestational age, microbubble count, and need for ventilatory support in the first six hours of life. The absolute and relative frequencies, as well as the p-value, are shown in Table 1.

Table 1. Comparison between the groups with and without respiratory symptoms regarding gender, weight, the relationship between weight and adequacy for gestational age, microbubble count, and need for ventilatory support in the first six hours of life (absolute and relative frequencies).

		With respiratory symptoms	Without respiratory symptoms	p-value
Number of individuals	(n=23)	(n=12; 52.2%)	(n=11; 48.2%)	
Gender				
Female	15 (65.2)	5 (41.7)	10 (90.9)	
Male	8 (34.8)	7 (58.3)	1 (9.1)	0.0272†
Weight				
SGA	1 (4.3)	0 (0.0)	1 (9.1)	
AGA	21 (91.4)	12 (100.0)	9 (81.8)	
LGA	1 (4.3)	0 (0.0)	1 (9.1)	0.2061††
Microbubble count				
< 15 SM/mm²				
Between 15 and 50 SM/mm²	8 (34.8)	6 (50.0)	2 (18.2)	
>50 SM/mm²	15 (65.2)	6 (50.0)	9 (81.8)	0.1930†
Ventilatory support in the first 6 hours of life				
No				
Yes/CPAP	14 (60.9)	3 (25.0)	11 (100.0)	
	9 (39.1)	9 (75.0)	0 (0.0)	0.0003†

† Fisher's exact test. †† G test. SGA – small for gestational age; AGA – adequate for gestational age; LGA – large for gestational age; CPAP - continuous positive airway pressure.

Among the 23 participants, 52.2% presented respiratory symptoms at birth. In this group, the prevalence was male (58.3%). In the group of NB without RS, 90.9% were female (p =0.0272). Regarding the relationship between weight and adequacy for gestational age, newborns with RS were all adequate for gestational age (AGA). Among the NBs without RS, 81.8% were AGA, 9.1% were small for gestational age (SGA), and 9.1% were large for gestational age (LGA) (p=0.2061).

The groups were also compared in relation to the number of stable microbubbles. Three cutoff points were used: less than (<) 15 SM/mm², between 15 and 50 SM/mm², and greater than (>) 50 SM/mm². In both groups, there were no newborns with an amount < 15 SM/mm². Among newborns with respiratory symptoms, 50% presented between 15 and 50 SM/mm² and 50% above 50 SM/mm². In patients without respiratory symptoms, 18.2% presented between 15 and 50 SM/mm², while 81.8% presented more than 50 SM/mm². There was no significant difference between the groups.

Notably, the mothers of NBs with RS received a pre-partum corticosteroid therapy scheme, a protective factor for developing NRDS. This practice was routine in the obstetrics service in pregnant

women in preterm labor. Another comparison variable between the groups was the need for ventilatory support in the first 6 hours of life. It was divided into 4 groups: patients who did not require oxygen support; required a nasal cannula; used nasal continuous positive airway pressure (nasal CPAP); and required mechanical ventilation. In the group of patients without respiratory symptoms, all did not require ventilatory support. In the group of NBs with RS, 25% did not need it, nor did they need resuscitation maneuvers, but 75% used nasal CPAP ($p<0.003$). It is observed that the group that presented the greatest need for ventilatory support was the one in which most patients had microbubbles between 15-50 SM/mm². Thus, the lower the number of stable microbubbles, the greater the need for ventilatory support and respiratory symptoms.

In this study, the groups were also compared in relation to gestational age, Capurro, weight at birth, and 1st-minute and 5th-minute Apgar. The means with standard deviation and p-value are described in Table 2.

Table 2. Comparison between the groups in relation to gestational age, Capurro, weight at birth, and 1st-minute and 5th-minute Apgar.

	Mean (±DP†)	With respiratory symptoms	Without respiratory symptoms	p-value††
Number of individuals	(n=23)	(n=12)	(n=11)	
Gestational age	34.3 (±1.9)	34.0 (±1.6)	35.0 (±2.1)	0.3793
Capurro	33.5 (±6.7)	34.3 (±1.8)	32.6 (±9.7)	0.1693
Weight at birth	2.229,6(±717.3)	1,930.8(±535.3)	2,555.5(±770.0)	0.0439
1st-minute Apgar	8.1 (±1.5)	7.5 (±1.8)	8.7 (±0.6)	0.0792
5th-minute Apgar	9.4 (±0.7)	9.1 (±0.8)	9.8 (±0.4)	0.0317

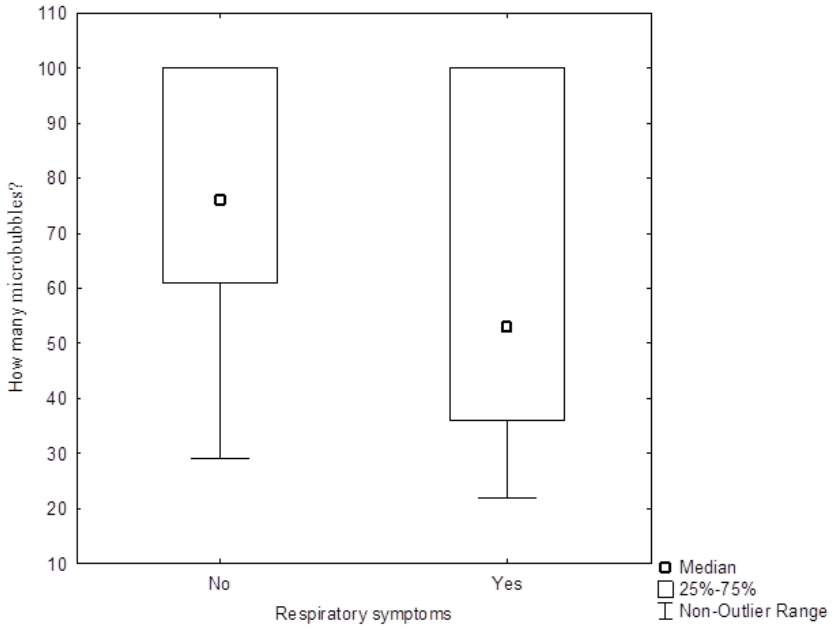
† SD = standard deviation. †† Mann-Whitney U test

Regarding gestational age (GA), a mean of 33.7 (±1.6) was found in patients with respiratory symptoms, while in neonates who did not present respiratory symptoms, the mean GA was 34.9 (±2.1), and 34.3 (±1.9) was the mean GA of the sample. The groups were homogeneous in relation to gestational age ($p=0.3793$). The Capurro of the sample presented a mean of 33.5 (±6.7) weeks. In the group of NB with RS, the mean was 34.3 (±1.8) weeks, while in patients who did not present RS, the mean was 32.6 (± 9.7) weeks. The groups were homogeneous in relation to the Capurro ($p=0.1693$).

Regarding weight at birth, the mean in the group of NBs with respiratory symptoms was 1,930.8 (±535.3)kg, while in the group without RS, it was 2,555.5 (±770.0)kg ($p=0.0439$). The weights of the participants who presented respiratory symptoms were lower compared to those who did not present RS, with a statistical difference. Concerning the measures of Apgar scores, it was found that, in patients who presented RS, the mean Apgar score in the first and fifth minutes, respectively, were 7.5 (±1.8) and 9.1 (±0.8), while in the group without respiratory symptoms, it was 8.7 (±0.6) and 9.8 (±0.4). There was no statistically significant difference between the groups regarding the measures of the 1st-minute Apgar score ($p=0.0792$). On the other hand, there was a difference between the groups in the 5th-minute Apgar measurements ($p=0.0317$).

The number of bubbles in patients with and without respiratory symptoms was analyzed. The result is shown in Figure 2.

Figure 2. Box plot of the number of bubbles among patients with and without respiratory symptoms.



The number of bubbles in the sample ranged between 55.3 and 80.2 SM/mm², with a mean of 67.7 (± 28.7) SM/mm². In patients with respiratory symptoms, the mean was 62.2 (±31.5) SM/mm², with a minimum of 42.2 and a maximum of 82.2 SM/mm², while in patients who did not have respiratory symptoms, the mean was 73.8 (± 25.5) SM/mm², ranging from 56.6 to 90.9 SM/mm². The groups were similar regarding the number of microbubbles per mm² (p=0.5658).

The groups were compared in relation to maternal history. The absolute and relative frequencies, as well as the p-value, are described in Table 3. It should be noted that there was no difference between the groups in all variables.

Table 3. Characterization of maternal history in patients with respiratory symptoms and patients without respiratory symptoms

		With respiratory symptoms	Without respiratory symptoms	p-value†
Number of individuals	(n=23)	(n=12; 52.2%)	(n=11; 48.2%)	
Birthway				
Cesarean birth	21 (91.3)	11 (91.7)	10 (90.9)	1.0000
Vaginal birth	2 (8.7)	1 (8.3)	1 (9.0)	
Smoking				
No	21 (91.3)	12 (100.0)	9 (81.8)	0.2174
Yes	2 (8.7)	0 (0.0)	2 (18.2)	
Alcoholism				
No	23 (100.0)	12 (100.0)	11 (100.0)	1.0000
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Gestational diabetes				
No	11 (47.8)	5 (41.7)	7 (63.6)	0.4136
Yes	12 (52.2)	7 (58.3)	4 (36.4)	
Pre-eclampsia				
No	17 (73.9)	4 (33.3)	2 (18.2)	0.6404
Yes	6 (26.1)	8 (66.7)	9 (81.8)	
Eclampsia				
No	21 (91.3)	11 (91.7)	10 (90.9)	1.0000
Yes	2 (8.7)	1 (8.3)	1 (9.1)	
Maternal Infection				
No	21 (91.3)	11 (91.7)	10 (90.9)	1.0000
Yes	2 (8.7)	1 (8.3)	1 (9.1)	

† Fisher's exact test.

DISCUSSION

Male newborns are more likely to present RS and surfactant deficiency, similar to what has been verified in other studies[20,21,25]. A study including 63 newborns with gestational age between 24 and 34 weeks, demonstrated a significantly lower value of SMT in gastric aspirate in the group of patients with NRDS, bringing as the best cutoff point 14 SM/mm^2 [26]. A similar outcome was demonstrated in a study that evaluated 64 newborns using SMT with a gastric and oral aspirate, with cutoff points of 15 SM/mm^2 and 25 SM/mm^2, respectively, in which newborns with symptoms of respiratory distress presented lower values in the SMT. Another study performed SMT through amniotic fluid, demonstrating high sensitivity (100%) to predict NRDS[21].

The mothers of NBs with RS received a pre-partum corticosteroid therapy scheme, a protective factor for developing NRDS. It reflected in the lack of need to use the exogenous surfactant in any study participant, as none of them presented less than 15 mbe/mm^2, as ratified in research that reports the drug's action in the fetal lungs' maturity, which is important for producing surfactant[27].

Another comparison variable between the groups was the need for ventilatory support in the first 6 hours of life. The lower the number of stable microbubbles, the greater the need for ventilatory support and respiratory symptoms. A study found that, of 20 patients with NRDS, 17 required nasal CPAP, 15 invasive mechanical ventilation, and 19 received exogenous surfactant administration[21]. Another study found that all patients with NRDS required nasal CPAP and/or invasive mechanical ventilation after hospitalization in the NICU[25].

Regarding weight at birth, the weights of the participants who presented respiratory symptoms were lower compared to those who did not present RS, with a statistical difference. These findings are

similar to those of other studies, highlighting that, in one of them, only seven neonates weighed more than 1500g out of a total of 114 patients eligible for SMT[21,25-27]. Concerning the measures of Apgar scores, there was a difference between the groups in the 5th-minute Apgar measurements. Another study observed a significant difference between the groups in relation to Apgar scores, with 7/8 and 9/9 being the means in the groups with and without RS, respectively[21].

Pulmonary surfactant is fundamental in the processes of pulmonary mechanics. Therefore, pulmonary maturity tests among newborns with gestational age below 37 weeks are indispensable when providing exogenous surfactant. This would differentiate early from newborns with pulmonary immaturity and surfactant deficiency before presenting a clinical condition of severe respiratory distress. It would prevent all newborns with gestational age below 37 weeks with an adequate amount of pulmonary surfactant from undergoing surfactant supplementation, reducing costs and operationalizing neonatology units as much as possible, which would focus mainly on those patients who require it.

The Stable Microbubbles Test, or SMT, proved to be efficient in predicting Newborn Respiratory Distress Syndrome, thus facilitating the diagnosis of whether or not surfactant is needed in preterm newborns. The test, which can be done by analyzing gastric aspirate, has a high sensitivity for prediction and a low cost. However, further studies on the best cutoff point of the test still need to be developed.

This comparative study found that patients who presented respiratory symptoms had lower counts of stable microbubbles compared to the group without RS. This finding is decisive when providing surfactant in patients who presented results < 15 SM/mm². It was possible to identify the newborns who did not need to receive exogenous surfactant in this study since none of the 23 patients analyzed had microbubbles < 15 SM/mm², making it the safe test for the diagnosis of newborn respiratory distress syndrome in premature patients between 32-37 weeks of gestational age.

From the analysis performed, it was possible to verify that respiratory symptoms is associated with the gender of the NB and the need for ventilatory support in the first hours of life. It was also verified that NBs with respiratory symptoms have lower weight and lower 5th-minute Apgar scores. Antenatal corticosteroid administration is associated with increased stable microbubbles in premature patients, decreasing the need for exogenous surfactant administration.

Contribuição dos autores: Todos os autores participaram na concepção e no planejamento do estudo, na obtenção, análise e interpretação dos dados, na redação ou revisão crítica do manuscrito, e na aprovação de sua versão final.

Conflito de interesse: Os autores declaram não possuir conflito de interesse.

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