Original Article

The Factors Effect on Natural Lung Surfactant Content for the Treatment of Respiratory Distress Syndrome

Marzieh Mokhber Dezfouli[1], Zohre Eftekhari[2, 4*], Sirous Sadeghian Chaleshtori[3, 4]

1. Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.
2. Department of Biotechnology, Pasteur Institute of Iran, Tehran, Iran.
3. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Institute of Biomedical Research, University of Tehran, Tehran, Iran.

Use your device to scan and read the article online

How to Cite This Article Mokhber Dezfouli, M., Eftekhari, Z., & Sadeghian Chaleshtori, S. (2025). The Factors Effect on Natural Lung Surfactant Content for the Treatment of Respiratory Distress Syndrome. *Iranian Journal of Veterinary Medicine, 19*(1), 93-104. http://dx.doi.org/10.32598/ijvm.19.1.1005467

http://dx.doi.org/10.32598/ijvm.19.1.1005467

ABSTRACT

Background: Exogenous surfactants from natural sources help restore normal lung function in premature cases. Pulmonary-surfactant dysfunction can lead to acute lung injury and is characterized by alveolar instability, floating, and collapse. These abnormalities occur in adult respiratory distress syndrome (ARDS) and neonatal respiratory distress syndrome (NRDS).

Objectives: This study aimed to identify the best source of exogenous natural surfactant and its composition.

Methods: Twenty-four healthy Holstein calves were selected in three age groups in both sexes to investigate the impact of sex and age on the surfactant composition. Cell-free bronchoalveolar lavage fluid supernatants were centrifuged at 20000×g for 60 min, allowing separation of crude surfactant pellets. Subsequently, the supernatant was discarded, and crude surfactant pellets was separated into several aliquots and stored at -80°C for further analysis.

Results: It was concluded that bronchoalveolar lavage fluid in female groups was significantly enriched by surfactant protein C and surfactant protein D in comparison with male groups at the same age. Total phospholipids, glycerides, and cholesterols were not age-dependent in the male groups; however, they had a descending manner associated with age in the female groups.

Conclusion: Age and sex could affect the amount of surface tension that increases with aging, and this trend is lower in the female group compared to the male group. Female calves in the younger age group are the best source of natural surfactants required for exogenous surfactant in neonatal respiratory disease due to the highest concentration of dipalmitoylphosphatidylcholin and lowest surface tension.

Keywords: Calf, Factors, Lung, Profile analysis technique, Respiratory distress

Article info:
Received: 25 Jun 2024
Accepted: 20 Jul 2024
Publish: 01 Jan 2025

*** Corresponding Author:**
Zohre Eftekhari, Associate Professor.
Address: *Department of Biotechnology, Pasteur Institute of Iran, Tehran, Iran.*
Phone: *+98 (914) 3126149*
E-mail: *z_eftekhari@pasteur.ac.ir*

Introduction

Lung surfactant is a composite of lipoproteins that are produced, stored, and secreted in type II epithelial cells and cover the lung alveolar epithelial surface in the last weeks of pregnancy (Choi et al., 2020). Natural surfactant components include phospholipids, cholesterol, triglycerides, and four types of proteins (Guzmán & Santini, 2019).

Surfactant comprises ~80%–85% phospholipids, 5%–10% neutral lipids, and 8%–10% protein, with 5%–6% containing four specific surfactant proteins. Eighty-five percent of the phospholipid fraction contains phosphatidylcholines, the most important component (40%) with the highest compaction properties being dipalmitoylphosphatidylcholine (DPPC); 11% consists of phosphatidylglycerol and phosphatidylinositol, which fluidize the lipid monolayer. The remaining fraction comprises various phospholipids with particular functions (Hentschel et al., 2020). DPPC, the main phospholipid of the lung surfactant, decreases surface tension, promotes compliance, and facilitates the lung to expand without complications and reduce the respiratory process (Bae et al., 2019).

Hydrophobic surfactant proteins B and C play a crucial role in natural surfactant structure and cause both adsorption and distribution of phospholipids at the air-liquid interface (Olmeda et al., 2017; Hentschel et al., 2020). Hydrophilic surfactant proteins A and D have a similar structure and participate in down-regulating the inflammatory response of the lung and innate immunity (Sardesai et al., 2017).

Neonatal respiratory distress syndrome (NRDS) is one of the most common problems for preterm infants (Magni et al., 2023). It is common in neonates born before 27 to 32 weeks who need ventilation with the infusion of exogenous lung surfactant from natural sources, helping to restore normal lung function (Han & Mallampalli, 2015; Hockenberry & Wilson, 2018). Destruction of type II alveolar epithelial cells due to adult respiratory distress syndrome (ARDS) increases lung compliance due to dysfunction in surfactant manufacture (Cutts et al., 2017). Common pathogens related to ARDS which can have an effective impact on surfactant quality and its content include *Streptococcus pneumonia*, *Pneumocystis jirovecii*, *Staphylococcus aureus*, and a diversity of respiratory viruses such as H1N1 novel influenza A, novel coronavirus (COVID-19) and respiratory syncytial virus (Al-Abedi et al., 2022; Ashrafi et al., 2020; Mojibi et al., 2022; Rawal et al., 2017; Jamaatia et al., 2020). In addition, different factors affect lung surfactant content and its maturation, such as age, sex, intrinsic cortisol, thyroid hormones, prolactin, epidermal growth factor, diabetic mother, and testosterone levels (Han and Mallampalli, 2015).

Extracting, purifying, and identifying pulmonary surfactants also affects the composition and phospholipid ratio (Christmann et al., 2006). Nielson and Torday (1981) mentioned the biological disparity between fetal sexes in the rabbits as an animal model, which may be the cause for male infants to develop respiratory distress syndrome.

Moreover, some studies indicate that aging could also modify the composition and function of lung surfactant (Christmann et al., 2006). Meanwhile, John Clements reported the first direct measurements of pulmonary surfactant using his homemade Langmuir–Wilhelmy surface balance half a century ago. Subsequently, many more in vitro tensiometric techniques, such as pulsating bubble surfactometry, captive bubble surfactometry, and the constrained sessile drop, have been developed to assess in vitro surfactant function (Stichtenoth et al., 2014).

Although some research has been conducted on lung surfactant composition, there is still insufficient data to evaluate the effect of age and gender on lung surfactant content, especially in calves, as an appropriate source of exogenous surfactant consumed in respiratory diseases. This study aimed to determine the effect of age and sex on the quality and quantity of lung surfactants and measure their functionality by new methods, which potentially can support finding the best source to obtain natural surfactants for respiratory distress syndrome (RDS) or other respiratory disorders.

Materials and Methods

Study animals

Twenty-four healthy Holstein calves were collected from the Tehran University of Medical Sciences farm, Tehran City, Iran, and divided into the following three age groups: 0-4 months, 4-8 months, and 8-12 months (n=8 in both sexes in each group). All calves' characteristics, such as age, weight, and gender, were registered. The calves' general health was assessed through clinical exams, and blood samples were taken to assess their complete blood count and confirm their health. The clinical criteria for admission to the study included the absence of eye and nasal discharge, normal body tem-

perature, and absence of respiratory sounds or cough. The calves were kept under controlled conditions for approximately 12 hours before the procedure (Fozouni & Tahaei, 2023). All experimental procedures followed the guidelines on ethical standards for experimental processes in animals, according to a protocol approved by the Animal Ethics Committee, University of Tehran, Iran. This study adheres to internationally accepted standards for animal research, following the 4Rs principle. All animal experiments adhere to the ARRIVE guidelines (2.0 version) and are conducted in compliance with the U.K.

Bronchoalveolar lavage method (BAL)

BAL was performed in the anesthetized calves with propofol (Fresenius Kabi, USA) (Diprivan® 5 mg/kg) using a sterilized and flexible catheter with a 3-5 mL balloon cuff (Supa Co, Iran). The head and neck of the calf were extended to facilitate the passage of the sterile BAL catheter. The BAL catheter was introduced into the trachea via a tracheal tube, and its positioning was confirmed by repeated coughing. The balloon cuff was then inflated with 3 mL of air, and subsequently, 5 aliquots of 200-300 mL pre-warmed sterile saline solution (37 ˚C) were infused. Immediately after infusion, the lavage fluid was aspirated by applying negative pressure (Danlois et al., 2000). The lavaged fluid was mixed and pooled in a sterile tube maintained on ice and immediately transferred to the biochemistry laboratory. The calves were under critical care support after the BAL procedure to prevent bronchial complications. To obtain a cell-free supernatant, BALF was centrifuged at 400×g for 15 min.

Cytology

For cytological evaluation of BALF, the cells were precipitated by centrifuging and stained using Wright-Giemsa staining and direct smear preparation (Allen et al., 1992).

Crude surfactant extraction

Cell-free BALF supernatants were centrifuged at 20000×g for 60 min at 4 ˚C, allowing separation of crude surfactant pellets (CSP). Subsequently, the supernatant was discarded, and CSP was separated into several aliquots and stored at -80 ˚C for further analysis.

Calf lung surfactants extract analysis

The obtained pellets were re-suspended in $CaCl_2$-NaCl (Merck, Whitehouse Station, NJ) solution, and the lipid part of the calf lung surfactant was extracted by modified Bligh and Dyer method (Bligh & Dyer, 1959). The lower phase-separated and concentrated with a rotary evaporator (IKA Co, rv10 digital) and stored at -20 ˚C. For the triglyceride assay, the dried extract was redissolved in isopropanol and vortexed, and then the saponification reagent was added. After mixing, the periodate solution was added and kept at room temperature for 5 min.

Subsequently, acetylacetone reagent was added, and samples were heated at 65 ˚C for 15 min in a water bath. Finally, the optical density (OD) was read at 410 nm vs blank (Neri & Fring, 1973). To evaluate total lipid content, sulfuric acid was added to a test tube containing the sample, mixed well, and heated in a boiling water bath for 10 minutes. After cooling the samples, the phosphovanillin reagent was added and mixed. Finally, tubes were incubated at 37 ˚C for 15 minutes, cooled at room temperature, and OD was read at 540 nm.

The analysis of total cholesterol was carried out using the total cholesterol assay mentioned before by Loeffler and McDougald (Loeffler & McDougald, 1963). Briefly, isopropanol was added to the conical test tube containing the sample, mixed well, and then put at room temperature for 5 min, later centrifuged for 5 min.

Subsequently, clear supernatant was transferred to a clean test tube, and $FeCl_3.6H_2O$ was added to each tube; after mixing, sulfuric acid was added, and OD read at 550 nm using blank.

The aqueous phase was collected and stored at -20 ˚C to analyze the hydrophilic proteins. The phospholipids classes distribution was determined by thin-layer chromatography (TLC) on silica gel plates (Merck, Whitehouse Station, NJ; 60 F 254) using a mobile phase containing chloroform/methanol/2-propanol/triethylamine/H_2O (Touchstone et al., 1983). The samples and standards include L-α-phosphatidylcholine from Soy (CAS No.=840054C, Avanti polar lipids Inc) and 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (CAS No.=850355C, Avanti polar lipids Inc) and 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (CAS No.=850468C, Avanti polar lipids Inc) were placed on plates and after drying, the TLC plate was put in the mobile phase containing tank. Then, the samples were stained with 10% H_2SO_4 and 8% H_3PO_4 and incubated at 150 ˚C (oven) for 15 minutes. Finally, the phospholipids were determined using Power Scan 2017 software (Mokra et al., 2016).

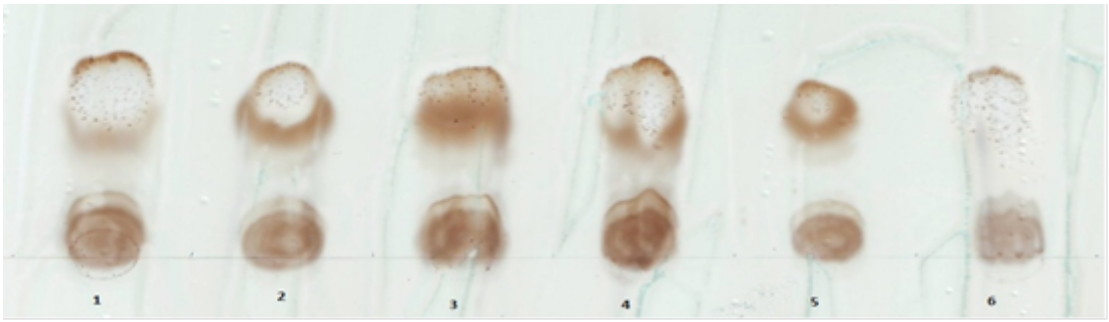

Figure 1. Phospholipids composition of extracted surfactant on thin-layer chromatography

Notes: 1) 1,2-dipalmitoyl-sn-glycero-3-phosphocholine standard; 2) Sample; 3) L –α-phosphatidylcholine standard; 4) Sample; 5) 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine standard; 6) Sample.

The phospholipid contents of the extracted samples were confirmed using high-performance liquid chromatography with C8 reversed-phase column 150×4.6 mm 5 µm (waters) along with refractive index detection. The mobile phase was prepared by combining 500 mL acetonitrile, 450 mL methanol, and 100 mL 50 mM acetic acid to a final ratio of 50:45:10. The mobile phase was degassed for 10 minutes. The extracted samples were injected into the column containing a mobile phase of acetonitrile, methanol, and acetic acid (50:45:10) at a 1 mL/min flow rate.

The total protein of the surfactant was measured by the Micro-Bradford method (Bradford, 1976). Besides, surfactant protein (SP)-A, -B, -C, and -D contents were analyzed using enzyme-linked immune sorbent assay (ELISA) technique by commercial kits (CAS No.: E0890b, U1622b and U1623b and E1039b).

A profile analysis tensiometer method was used (PAT1, Sin-interface Technology, Germany) to evaluate extracted lung surfactant in situ. Measurements of dynamic interfacial tension and dilatational viscoelasticity at the water-lipid interface were performed by the drop profile analysis tensiometer (Vatanparast et al., 2017). Briefly, the measurement process is based on image achievement of drop profile calculated by the Gauss-Laplace equation in which all the experiments are conducted at 25 ˚C and atmospheric pressure.

Data analysis

Statistical analysis was conducted using SPSS software, version 21. Significance levels were set at the $P<0.05$ using a one-way analysis of variance (ANOVA) and independent t-test. All experimental procedures involving animals were approved by the Ethics Committee of the Faculty of Veterinary Medicine of the University of Tehran, Tehran, Iran.

Results

Clinical parameters and BALF cytology

Table 1 presents that general hematological parameters were recorded in the normal range in all groups. The data showed no significant difference between the cytological content of BALF at different ages and sex (Tables 1 and 2) ($P>0.05$).

Phospholipids and protein contents

The results showed that sex and age did not affect SP-A, SP-B, SP-D, and the total protein contents of the extracted surfactant ($P>0.05$). However, BALF in female groups was significantly enriched by SP-C compared to male groups at the same age ($P=0.003$). On the other hand, the amount of SP-C increased considerably in group A in females compared to those of similar sex in other groups. Furthermore, SP-C decreased in the males of group C compared with the males in group A ($P=0.04$).

In addition, a decreasing trend by aging was observed in the amount of SP-C and SP-D in

both female and male groups. As Figure 1 shows, the phospholipids composition of the extracted surfactant was confirmed by TLC (Figure 1). No significant differences were found in age and sex on the number of total cholesterols and glycerides ($P>0.05$).

Total phospholipids in the female groups were significantly higher than the male groups in the same age group ($P=0.002$). Additionally, total lipids in group A were significantly higher than the other groups for males and females ($P=0.003$) (Table 3). It can be inferred from the present data that all measured factors except total glycerides have a descending trend by aging in both sexes.

Table 1. Complete blood count and plasma fibrinogen concentration evaluation for calves' healthy conditions before initializing bronchoalveolar lavage

Parameter	No.	Mean±SEM	Normal Range
Platelets (×10³ µL)	24	4.29±1.1	1-8
Eosinophil (per/µL)	24	1532±53.4	0-2400
Monocyte (per/µL)	24	230.4±15.45	25-800
Lymphocyte (per/µL)	24	6450.23±70.65	2500-7500
Neutrophil (×10³ µL)	24	13.20±7.34	0-120
WBC (per/µL)	24	10430±874.5	4000-12000
Fibrinogen (mg/L)	24	408.78±21.4	200-700
Total protein (g/L)	24	63. 3±3.99	30-70
MCHC (g/L)	24	320.62±11.33	300-360
MCH (pg)	24	12.42±1.05	11-17
MCV (fL)	24	48.67±3.14	40-60
RBC (×10⁶ µL)	24	7.63±0.8	5-10
Hemoglobin (g/L)	24	9.42±0.82	8-15
Hematocrit (%)	24	32.07±2.5	24-46

WBC: White blood cell; RBC: Red blood cell; MCHC: Mean corpuscular hemoglobin concentration; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume.

Surface tension measurement results

Based on the obtained results, the surface tension of male samples was recorded at 44.76±0.38 mN/m in 0-4 months, 45.97±1.25 mN/m in 4-8 months, and 55.06±0.45 mN/m in 8-12 months old animals (Figure 2). It was construed from the results that the surface tension of female samples was recorded 24. 85±1.30 mN/m in 0-4 months, 25.02±1.05 mN/m in 4-8 months and 50.36±0.8 mN/m in 8-12 months old animals (Figures 3, 4, and 5).

Discussion

Lung surfactant is a composite of lipoproteins that are produced, stored, and secreted in type II epithelial cells and cover the lung alveolar epithelial surface in the last weeks of pregnancy. It reduces the surface tension at the air-water interface, improves alveolar ventilation exchanges of respiratory gases, prevents pulmonary edema formation, and finally prevents the alveoli from collapsing (Khawar & Marwaha, 2023; Singh et al., 2021). The

Table 2. Cytological analysis of bronchoalveolar lavage fluids collected from calves of three different ages

Groups		Epithelial	Neutrophil	Lymphocyte	Macrophage	Number of Cells
A (0-4 m)	Male	10.5±2.85	9.52±3.61	19.24±3.47	68.74±6.45	686.8±48.25
	Female	9.45±1.95	9.26±2.48	20.45±2.99	63.55±5.28	735±29.84
B (4-8 m)	Male	9.15±2.75	8.98±2.82	18.43±4.26	66.13±7.23	740.26±41.58
	Female	9.06±3.23	9.33±3.47	18.55±3.47	67.42±5.64	666.08±44.29
C (8-12 m)	Male	8.87±2.39	8.65±1.89	19.58±2.43	69.25±4.6	729.25±32.66
	Female	8.38±2.49	9.26±2.69	19.24±1.86	65.29±5.87	650.25±42.01

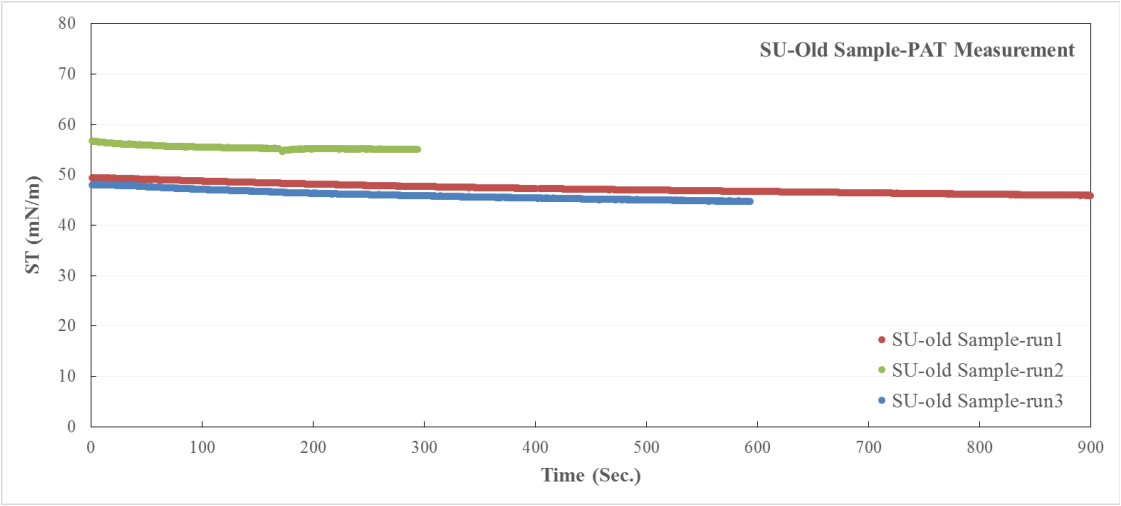

Figure 2. Profile analysis tensiometer (PAT) measurement of male samples in different ages

Notes: Run 1: 4-8 months in the male group, run 2: 0-4 months in the male group, and run 3: 8-12 months in the male group. Based on PAT results, the surface tension of male samples was recorded 44.76±0.38 mN/m in 0-4 months, 45.97±1.25 mN/m in 4-8 months and 55.06±0.45 mN/m in 8-12 months old.

qualitative and quantitative lung surfactant change due to infectious and noninfectious diseases. One of the more significant findings from this study is that the quality of isolated lung surfactant content in female calves under 4 months of age is higher than in other groups, which can be potentially used as the best source to obtain natural surfactant to prescribe for respiratory diseases. On the other hand, it probably rationalizes the high incidence of infectious diseases such as RDS due to viral and bacterial pneumonia in the elderly because of the reduction in quantity and functionality of natural lung surfactants.

The analysis of BALF undertaken in this study demonstrated that SP-C, SP-D, total phospholipid, total lipid content, and functionality in BALF depended on gender, and in female groups, it was significantly higher than in the male groups. Additionally, it would be interesting that the only compositions changed by aging are SP-C and SP-D, and the other surfactant contents were not dependent on it. It can be inferred from the PAT data that both age and sex could affect the amount of surface tension as increased by aging and decreased in the female group in comparison to the male group. It possibly reflects the high incidence and case fatality rate of

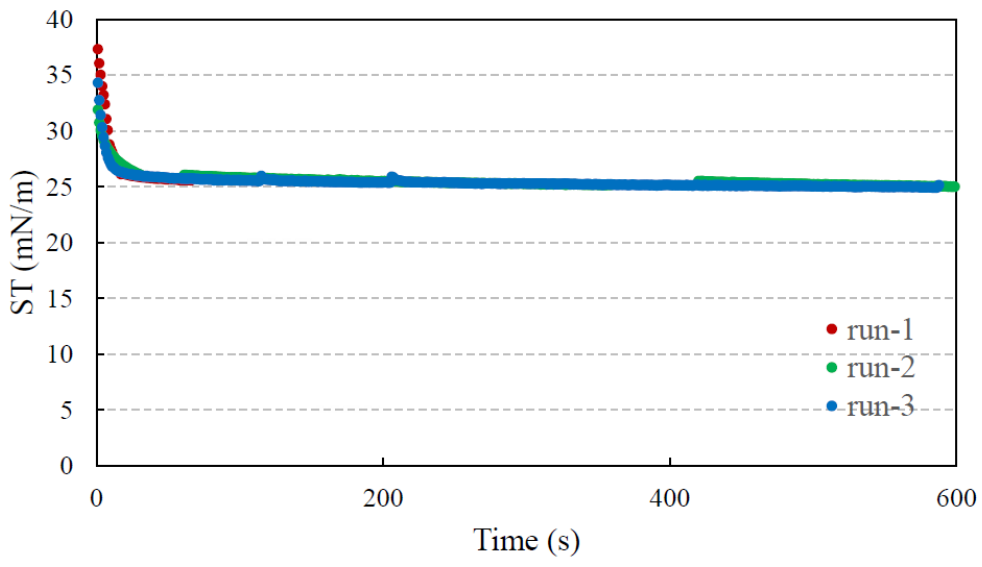

Figure 3. PAT measurement of female samples in 0-4 months old

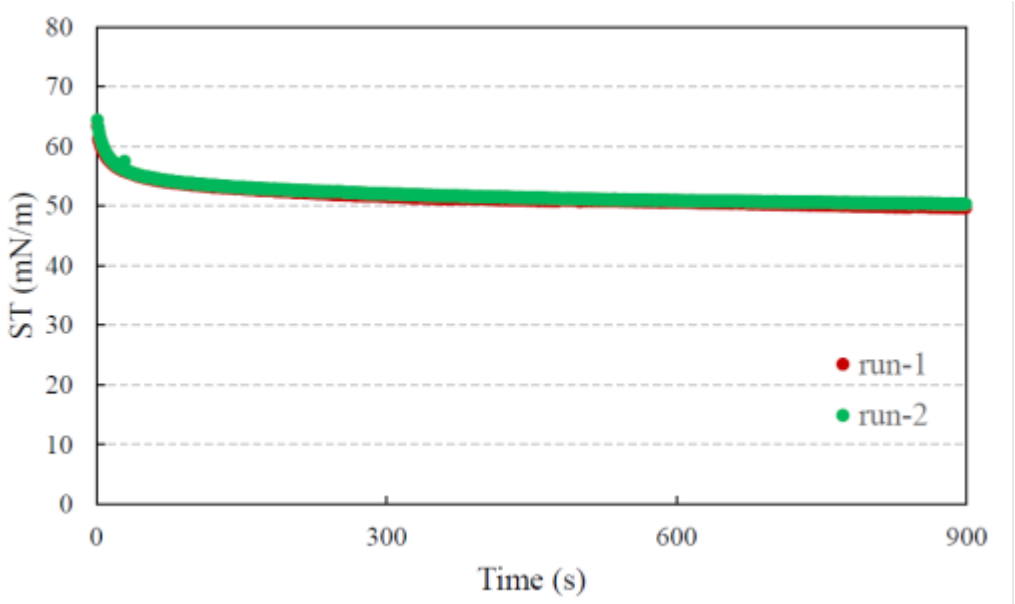

Figure 4. PAT measurement of female samples in 4-8 months old

infectious diseases such as COVID-19 among males in comparison to females (Jamaatia et al., 2020).

SP-B is an important component of surfactant substitute mixtures that can alter PL membrane association, enhancing the surfactant-like properties and the uptake of phospholipids (PLs) vesicles by type II cells in vitro while resisting surface tension by increasing the lateral stability of the phospholipid's monolayer (Hockenberry & Wilson, 2018).

Exogenous surfactant prepared from natural sources or synthetic form is mainly used to treat NRDS and meconium aspiration. In addition to respiratory distress syndrome, surfactant deficiency is observed in many other clinical situations in term and preterm infants and adults. So, scientists should be encouraged to develop different methods of extraction and recognize the best sources for exogenous surfactants (Han & Mallampalli, 2015; Sardesai et al., 2017).

Total lipids and phospholipids in the lung surfactant are responsible for the surface active function of pulmonary surfactant by substituting interfacial water molecules, and its ultimate objective is to reduce surface tension at the water-air interface (Han & Mallampalli, 2015; Cañadas et al., 2020). Pulmonary surfactant proteins, particularly SP-B and SP-C, show a strong affinity for in-

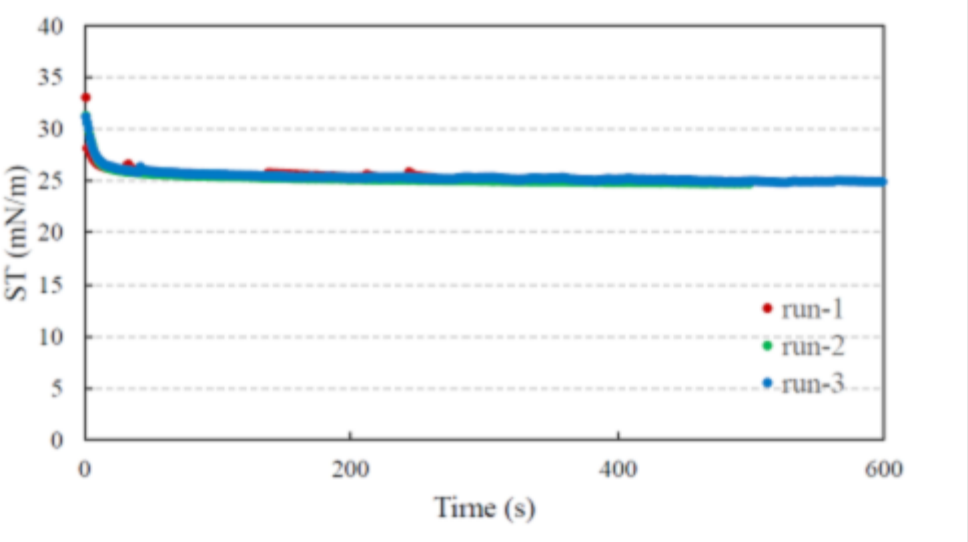

Figure 5. PAT measurement of female samples in 8-12 months old

Table 3. Different composition of surfactant content

Groups		Total Protein (mg/mL)	SP-A (ng/mL)	SP-B (ng/mL)	SP-C (ng/mL)	SP-D (ng/mL)
A (0-4 m)	Male	0.011±0.001	96.67±8.7	10.13±0.57	1.23±0.13[a]	47.62±6.36
	Female	0.012±0.005	109.52±16.43	11.45±1.66	1.95±0.26	56.84±6.31
B (4-8 m)	Male	0.011±0.002	106.67±14.71	11.4±0.34	0.86±0.03[a]	38.77±4.12
	Female	0.013±0.005	93.57±8.11	11.87±1.57	1.51±0.28[b]	51±4.27
C (8-12 m)	Male	0.012±0.005	101.2±14.81	12.38±0.4	0.56±0.07[a, b]	35.17±6.17
	Female	0.011±0.004	96.92±14.07	12.55±1.61	0.95±0.08[b]	44.96±6.31

Groups		Total Phospholipids (mg/mL)	Total Cholesterols (mg/mL)	Total Lipids (mg/mL)	Total Triglycerides (µg/mL)
A (0-4 m)	Male	0.557±0.074[a]	0.112±0.009	0.742±0.102	10.13±0.57
	Female	0.805±0.109	0.115±0.010	0.727±0.083	11.45±1.66
B (4-8 m)	Male	0.447±0.061[a]	0.105±0.012	0.58±0.055[b]	11.4±0.34
	Female	0.667±0.045	0.12±0.019	0.615±0.024	11.87±1.57
C (8-12 m)	Male	0.417±0.033[a]	0.095±0.012	0.522±0.017[b]	12.38±0.4
	Female	0.61±0.014	0.11±0.013	0.512±0.056[b]	12.55±1.61

[a] Sex comparison in the same age group (P<0.05), [b] Age comparison with the group A between the same sex (P<0.05).

terfaces and ensuing surface-active properties indicated by many researchers as key constituents in achieving the optimal dynamic and mechanical properties of surfactant membranes (Sardesai et al., 2017).

The study results by Torday et al. (1981) revealed that male infants had a higher risk for RDS in comparison to females, and the reason for this phenomenon is a delay in surfactant production in response to the inhibitory effect by testes-derived hormones or diminishing the response of the male to corticosteroids stimulating surfactant synthesis. Another study showed differences in the two genders by proteomic analysis, which may be due to varying expression of specific proteins in which the level of SP-C and SP-D in the female was higher than the male; this can be the cause of more respiratory abnormalities in males (Sardesai et al., 2017; Rahmanian et al., 2014). Seaborn et al. (2010) found that by blocking lung maturation, testicular hormones can contribute to higher morbidity and mortality rates in male infants.

Another important factor that can change the surfactant content is aging due to reduced alveolar, alveolar-capillary, and lung parenchyma surface area. An age-related decrease in surfactant in monkeys could be explained by a lower number of type II alveolar cells per unit lung volume with aging and decreased alveolar surface tension produced by surfactants (Shimura et al., 1986; Dezfouli et al., 2022; Vanstapel et al., 2021). Furthermore, the decreases occur with the alveolar septal area and the total surface area of the lung parenchyma, which can cause a decrease in surfactant composition (Pruthi & Multani, 2012).

Christmann et al. (2006) showed that surface tension and phospholipid composition of surfactants in neonatal foals were significantly different compared to adult horses, which may influence the biophysical and immunologic functions of surfactants. However, studies on the whole-lung tissue extracted surfactant from humans, rats and rhesus monkeys revealed no significant alterations in the content of disaturated phosphatidylcholine with aging (Egberts et al., 1987; Ghidoni et al., 2015).

Conclusion

Finally, owing to the importance of surfactant replacement therapy in preterm foals, lambs, calves, and babies, obtaining the best source with new method identifications to acquire the natural surfactant to produce exogenous surfactant is necessary. Based on the obtained results, female BALF was more enriched in SP-C and total PL contents than differences in surfactant content;

besides, PAT analysis between neonate males and females may be attributed to surfactant phosphatidylcholine synthesis in late pregnancy, but further investigation is necessary by longer age intervals.

Ethical Considerations

Compliance with ethical guidelines

All experimental procedures followed the guidelines on ethical standards for experimental processes in animals, according to a protocol approved by the Animal Ethics Committee, University of Tehran, Tehran, Iran. This study adheres to internationally accepted standards for animal research, following the 4Rs principle. All animal experiments adhere to the ARRIVE guidelines (2.0 version) and are conducted in compliance with the UK.

Funding

This research was financially supported by the Institute of Biomedical Research of Veterinary Medicine, the University of Tehran, Tehran, Iran and Persian Darou Alborz Research and Technology, Tehran, Iran.

Authors' contributions

Supervision and project administration: Zohre Eftekhari and Sirous Sadeghian Chaleshtori; Conceptualization, methodology, formal analysis, data curation, resources, review and editing: All authors; Investigation and writing the original draft: Marzieh Mokhber Dezfouli; Validation: Marzieh Mokhber Dezfouli and Sirous Sadeghian Chaleshtori.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the financial support from the Institute of Biomedical Research of Veterinary Medicine, the University of Tehran, Tehran, Iran and Persian Darou Alborz Research and Technology fund.

References

Al-Abedi, G. J. K., Al-Eodawee, E. M. M., Khalili, S., & Gharban, H. A. J. (2022). First molecular genotyping of cryptosporidium felis in Cattle, Iraq. *Archives of Razi Institute, 77*(6), 2345–2352. [DOI:10.22092/ARI.2022.358621.2271] [PMID]

Allen, J. W., Viel, L., Bateman, K. G., Rosendal, S., & Shewen, P. E. (1992). Cytological findings in bronchoalveolar lavage fluid from feedlot calves: Associations with pulmonary microbial flora. *Canadian Journal of Veterinary Research, 56*(2), 122–126. [PMID]

Ashrafi, F., Azari, A. A., & Fozouni, L. (2022). Prevalence and antibiotic resistance pattern of mannheima haemolytica and pasteurella multocida isolated from cattle lung samples from an industrial abattoir: A study from Northeastern Iran. *Iranian Journal of Veterinary Medicine, 16*(4), 414-422. [Link]

Bae, C. W., Kim, C. Y., Chung, S. H., & Choi, Y. S. (2019). History of pulmonary surfactant replacement therapy for neonatal respiratory distress syndrome in Korea. *Journal of Korean Medical Science, 34*(25), e175. [DOI:10.3346/jkms.2019.34.e175] [PMID]

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911–917. [DOI:10.1139/o59-099] [PMID]

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72,* 248–254. [DOI:10.1016/0003-2697(76)90527-3] [PMID]

Cañadas, O., Olmeda, B., Alonso, A., & Pérez-Gil, J. (2020). Lipid-protein and protein-protein interactions in the pulmonary surfactant system and their role in lung homeostasis. *International Journal of Molecular Sciences, 21*(10), 3708. [DOI:10.3390/ijms21103708] [PMID]

Christmann, U., Livesey, L. C., Taintor, J. S., Waldridge, B. M., Schumacher, J., & Grier, B. L., et al. (2006). Lung surfactant function and composition in neonatal foals and adult horses. *Journal of Veterinary Internal Medicine, 20*(6), 1402–1407. [DOI:10.1892/0891-6640(2006)20[1402:lsfaci]2.0.co;2] [PMID]

Choi, Y., Jang, J., & Park, H. S. (2020). Pulmonary surfactants: A new therapeutic target in asthma. *Current Allergy and Asthma Reports, 20*(11), 70. [DOI:10.1007/s11882-020-00968-8] [PMID]

Cutts, S., Talboys, R., Paspula, C., Prempeh, E. M., Fanous, R., & Ail, D. (2017). *Annals of the Royal College of Surgeons of England, 99*(1), 12–16. [DOI:10.1308/rcsbull.2017.12] [PMID]

Danlois, F., Zaltash, S., Johansson, J., Robertson, B., Haagsman, H. P., & van Eijk, M., et al. (2000). Very low surfactant protein C contents in newborn Belgian White and Blue calves with respiratory distress syndrome. *Biochemical Journal, 351*(Pt 3), 779–787. [DOI:10.1042/bj3510779] [PMID]

Dezfouli, M. R. M., Zarghami, F., Rahbari, S., Abkooh, E. E., Lotfolahzadeh, S., & Chaleshtori, S. S., et al. (2022). Protection of calves against cryptosporidiosis by hyperimmunization of pregnant cattle colostrum using oocyst whole antigens. *Iranian Journal of Veterinary Medicine, 16*(4), 380-389. [Link]

Egberts, J., Beintema, A., & de Boers, A. (1987). Phosphatidylinositol and not phosphatidylglycerol is the important minor phospholipid in Rhesus-monkey surfactant. *Biochimica et Biophysica Acta, 919*(1), 90-92. [DOI:10.1016/0005-2760(87)90221-9]

Fozouni, L., & Tahaei, M. (2023). Anticandidal effects of zinc oxide nanoparticles on fluconazole-resistant candida isolates causing diarrhea in calves, in vitro. *Archives of Razi Institute, 78*(1), 499-504. [PMID]

Ghidoni, R., Caretti, A., & Signorelli, P. (2015). Role of sphingolipids in the pathobiology of lung inflammation. *Mediators of Inflammation, 2015,* 487508. [DOI:10.1155/2015/487508] [PMID]

Guzmán, E., & Santini, E. (2019). Lung surfactant-particles at fluid interfaces for toxicity assessments. *Current Opinion in Colloid and Interface Science, 39,* 24-39. [DOI:10.1016/j.cocis.2019.01.003]

Han, S., & Mallampalli, R. K. (2015). The role of surfactant in lung disease and host defense against pulmonary infections. *Annals of the American Thoracic Society, 12*(5), 765–774. [DOI:10.1513/AnnalsATS.201411-507FR] [PMID]

Hentschel, R., Bohlin, K., van Kaam, A., Fuchs, H., & Danhaive, O. (2020). Surfactant replacement therapy: From biological basis to current clinical practice. *Pediatric Research, 88*(2), 176-183. [DOI:10.1038/s41390-020-0750-8] [PMID]

Hockenberry, M. J., & Wilson, D. (2018). *Wong's nursing care of infants and children-E-book*. Amesterdam: Elsevier Health Sciences. [Link]

Jamaatia, H .,Dastana, F., Tabarsic, P., Marjanic, M., Saffaeid, A., & Hashemiana, M. R. (2020). A fourteen-day experience with coronavirus disease 2019(covid-19) induced Acute Respiratory Distress Syndrome (ARDS): An Iranian Treatment Protocol. *Iranian Journal of Pharmaceutical Research , 19* (1): 31-36. [PMID]

Khawar, H., & Marwaha, K. (2023). Surfactant. In StatPearls [Internet]. StatPearls Publishing. [PMID]

Loeffler, H.H., McDougald, C.H. (1963). Estimation of cholesterol inserum by means of improved techniques. In: J.S. Annino and R.W. Giese (Eds), Clinical chemistry principles and procedures. Boston: Little Brown and Company Boston Press.

Magni, T., Ragni, C., Pelizzi, N., Sharma, S., Perez-Kempner, L., & Turkstra, E., et al. (2023). Health economic studies of surfactant replacement therapy in neonates with respiratory distress syndrome: A systematic literature review. *PharmacoEconomics - Open, 7*(3), 359–371. [DOI:10.1007/s41669-023-00399-x] [PMID]

Mojibi, R., Mehrzad, J., Sharifzadeh, A., & Nikaein, D. (2022). Apoptotic effects of caffeic acid phenethyl ester and matricaria chamomilla essential oil on A549 non-small cell lung cancer cells. *Iranian Journal of Veterinary Medicine, 16*(4), 390-399. [Link]

Mokra, D., Mikusiakova, L. T., Mikolka, P., Kosutova, P., Jurcek, M., & Kolomaznik, M., et al. (2016). High-frequency jet ventilation against small-volume conventional mechanical ventilation in the rabbit models of neonatal acute lung injury. *Advances in Experimental Medicine and Biology, 912,* 83–93. [DOI:10.1007/5584_2016_230] [PMID]

Neri, B. P., &Fring, C. S. (1973). Improved method for determination of triglycerides in serum. *Clinical Chemistry, 19*(10), 1201–1202. [PMID]

Olmeda, B., Martínez-Calle, M., & Pérez-Gil, J. (2017). Pulmonary surfactant metabolism in the alveolar airspace: Biogenesis, extracellular conversions, recycling. *Annals of Anatomy, 209,* 78-92. [DOI:10.1016/j.aanat.2016.09.008] [PMID]

Pruthi, N., & Multani, N. K. (2012). Influence of age on lung function tests. *Journal of Exercise Science and Physiotherapy, 8*(1), 1-6. [DOI:10.18376//2012/v8i1/67598]

Rahmanian, S. D., Wood, K. L., Lin, S., King, M. A., Horne, A., & Yang, S., et al. (2014). Gender differences in pulmonary function, respiratory symptoms, and macrophage proteomics among HIV-Infected smokers. *Scientifica, 2014,* 613689. [DOI:10.1155/2014/613689] [PMID]

Rawal, G., Kumar, R., Yadav, S., & Sujana, R. (2017). H1N1 influenza induced acute respiratory distress syndrome rescued by extracorporeal membrane oxygenation: A case report. *Journal of Translational Internal Medicine, 5*(3), 182-185. [DOI:10.1515/jtim-2017-0018] [PMID]

Sardesai, S., Biniwale, M., Wertheimer, F., Garingo, A., & Ramanathan, R. (2017). Evolution of surfactant therapy for respiratory distress syndrome: past, present, and future. *Pediatric Research, 81*(1), 240-248. [DOI:10.1038/pr.2016.203] [PMID]

Seaborn, T., Simard, M., Provost, P. R., Piedboeuf, B., Tremblay, Y. (2010). Sex hormone metabolism in lung development and maturation. Trends in Endocrinology & Metabolism. Dec 1;21(12):729-738. [DOI: 10.1016/j.tem.2010.09.001] [PMID]

Shimura, S., Boatman, E. S., & Martin, C. J. (1986). Effects of aging on the alveolar pores of Kohn andon the cytoplasmic components of alveolar type II cells in monkey lungs. *The Journal of Pathology, 148*(1), 1–11. [DOI:10.1002/path.1711480103] [PMID]

Singh, J., Jaffe, A., Schultz, A., & Selvadurai, H. (2021). Surfactant protein disorders in childhood interstitial lung disease. *European Journal of Pediatrics, 180*(9), 2711–2721. [DOI:10.1007/s00431-021-04066-3] [PMID]

Stichtenoth, G., Walter, G., Lange, R., Raith, M., Bernhard, W., & Herting, E. (2014). Surface tension of airway aspirates withdrawn during neonatal resuscitation reflects lung maturity. *Pediatric Pulmonology, 49*(8), 751-756. [DOI:10.1002/ppul.22885] [PMID]

Torday, J. S., & Nielsen, H. C. (1981). Surfactant phospholipid ontogeny in fetal rabbit lung lavage and amniotic fluid. *Biology of the Neonate, 39*(5-6), 266-271. [DOI:10.1159/000241447] [PMID]

Torday, J. S., Nielsen, H. C., Fencl, M.deM., & Avery, M. E. (1981). Sex differences in fetal lung maturation. *The American Review of Respiratory Disease, 123*(2), 205–208. [PMID]

Touchstone, J.C., Levin, S. S., Dobbins, M. F., Matthews, L., Beers, P. C., & Gabbe, S. G. (1983). (3-sn-Phosphatidyl)cholines (lecithins) in amniotic fluid. *Clinical Chemistry, 29*(11), 1951–1954. [DOI:10.1093/clinchem/29.11.1951] [PMID]

Vatanparast, H., Javadi, A., & Bahramian, A. (2017). Silica nanoparticles cationic surfactants interaction in water-oil system. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 521,* 221-230. [DOI:10.1016/j.colsurfa.2016.10.004]

Vanstapel, A., Weynand, B., Kaes, J., Neyrinck, A. P., Ceulemans, L. J., & Vanaudenaerde, B. M., et al. (2021). Interalveolar pores increase in aging and severe airway obstruction. *American Journal of Respiratory and Critical Care Medicine, 204*(7), 862-865. [DOI:10.1164/rccm.202102-0530LE] [PMID]

مقاله پژوهشی

اثر سن و جنس بر عملکرد سورفکتانت طبیعی ریه گوساله به عنوان یک داروی با ارزش برای درمان سندرم دیسترس تنفسی

مرضیه مخبر دزفولی[1]، *زهره افتخاری[2,4]، سیروس صادقیان چالشتری[3,4]

۱. مرکز تحقیقات قلب و عروق شهید رجایی ، مرکز آموزشی ، تحقیقاتی و درمانی قلب و عروق شهید رجایی، دانشگاه علوم پزشکی ایران ، تهران، ایران.
۲. بخش بیوتکنولوژی، انستیتو پاستور ایران، تهران، ایران.
۳. گروه بیماری‌های داخلی، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران.
۴. پژوهشکده تحقیقات زیست پزشکی، دانشگاه تهران، تهران، ایران.

Use your device to scan and read the article online

How to Cite This Article Mokhber Dezfouli, M., Eftekhari, Z., & Sadeghian Chaleshtori, S. (2025). The Factors Effect on Natural Lung Surfactant Content for the Treatment of Respiratory Distress Syndrome. *Iranian Journal of Veterinary Medicine, 19*(1), 93-104. http://dx.doi.org/10.32598/ijvm.19.1.1005467

http://dx.doi.org/10.32598/ijvm.19.1.1005467

چکیده

زمینه مطالعه: سورفکتانت اگزوژن تهیه شده از منابع طبیعی به بازیابی عملکرد طبیعی ریه در نوزادان نارس کمک می کند. اختلال عملکرد سورفکتانت ریوی می تواند منجر به آسیب حاد ریه شود که معمولا با بی ثباتی آلوئول ها ، شناور شدن و کلاپس مشخص می شود. نشان داده شده است که این ناهنجاری ها در سندرم زجر تنفسی حاد (ARDS) و سندرم دیسترس تنفسی نوزادان (NRDS) رخ می دهند.

هدف: شناسایی بهترین منبع سورفکتانت طبیعی اگزوژن و ترکیب آن. **روش کار:** بیست و چهار گوساله هلشتاین سالم در سه گروه سنی در هر دو جنس برای بررسی تأثیر جنسیت و سن بر ترکیب سورفکتانت انتخاب شدند. مایعات رویی بدون سلول تهیه شده از لاواژ ریه ی گوساله ها به مدت ۶۰ دقیقه در دور g ۲۰۰۰۰ سانتریفیوژ شدند و رسوب سورفکتانت خام (CSP) به دست آمد. پس از آن، مایع رویی دور ریخته شد و CSP به چند بخش جدا شد و برای تجزیه و تحلیل بیشتر در دمای ۸۰- درجه سانتیگراد نگهداری شد.

روش کار: بیست و چهار گوساله هلشتاین سالم در سه گروه سنی در هر دو جنس انتخاب شدند و برای بررسی تاثیر جنس و سن بر ترکیب سورفکتانت. برونش آلوئولار بدون سلول، مایع رویی مایع لاواژ در ۲۰۰۰۰×g به مدت ۶۰ دقیقه سانتریفیوژ شد تا جداسازی مواد خام ایجاد شود.

سپس مایع رویی دور انداخته شد و گلوله‌های سورفکتانت خام به چند بخش جدا شده و برای تجزیه و تحلیل بیشتر در دمای ۸۰- درجه سانتیگراد نگهداری می شود.

نتایج: نتیجه‌گیری شد که محتوای SP-C و SP-D در گروه‌های ماده به‌طور معنی‌داری با در مقایسه با گروه‌های نر در همان سن بیشتر بود. محتوای کل فسفولیپیدها، گلیسریدها و کلسترول ها در گروه های نر وابسته به سن گزارش نشد. با این حال، محتوای این مواد با افزایش سن در گروه های ماده ، روند کاهشی را نشان داد.

نتیجه‌گیری نهایی: می‌توان چنین استنباط کرد که هم سن و هم جنس می‌توانند بر میزان کشش سطحی اثر گذار باشد به طوری که با افزایش سن میزان کشش سطحی افزایش یافته و در گروه ماده نسبت به گروه نر کاهش یافت. گوساله‌های ماده در گروه سنی جوان‌تر بهترین منبع سورفکتانت‌های طبیعی مورد نیاز برای سورفکتانت اگزوژن در بیماری‌های تنفسی نوزادان به دلیل بالاترین غلظت دی پالمیتوئیل فسفاتیدیل کولین و کمترین کشش سطحی هستند.

کلیدواژه‌ها: تکنیک تجزیه و تحلیل مشخصات، دیسترس تنفسی گوساله، ریه، فاکتور

تاریخ دریافت: ۰۵ تیر ۱۴۰۳
تاریخ پذیرش: ۳۰ تیر ۱۴۰۳
تاریخ انتشار: ۱۲ دی ۱۴۰۳

* **نویسنده مسئول:**
زهره افتخاری
نشانی: تهران، انستیتو پاستور ایران، بخش بیوتکنولوژی.
تلفن: ۳۱۲۶۱۴۹ (۹۱۴) ۹۸+
رایانامه: *z_eftekhari@pasteur.ac.ir*

This Page Intentionally Left Blank