Research article

# Rapid biophysical analyses of gastric aspirates from risk newborns for lung maturity assessment after corticosteroid therapy

Asya Tsanova [a], Albena Jordanova [a,*], Vishnya Stoyanova [a], Elena Tasheva-Terzieva [b], Krasimira Ivanova [c], Zdravko Lalchev [b]

[a] Faculty of Medicine, St. Kl. Ohridski University of Sofia, 1407, Sofia, Bulgaria
[b] Faculty of Biology, St. Kl. Ohridski University of Sofia, 1164, Sofia, Bulgaria
[c] St. Sofia Hospital, 1618, Sofia, Bulgaria

## ARTICLE INFO

## ABSTRACT

*Background:* One of the main causes for the higher mortality among risk newborn children (including preterm infants) is neonatal respiratory distress syndrome (NRDS), which develops as a result of primary deficiency or secondary inactivation of alveolar surfactant (AS). Therefore, fast and early diagnostics of risk newborns lung maturity is crucial for their prompt therapy.

*Materials and methods:* Gastric aspirates (GA) were collected from 77 infants divided into three groups: a control of 38 healthy full-term infants; 16 prematurely newborns with NRDS, and 23 prematurely born infants after *in vitro* fertilization and corticosteroid therapy (CST). Surface parameters: equilibrium ($\gamma_{eq}$), maximal ($\gamma_{max}$) and minimal ($\gamma_{min}$) surface tension, and the shape of hysteresis curves of GA monolayers were measured by axisymmetric drop shape analysis (ADSA) of a pending drop. In addition, the morphology of GA monolayers was studied by Brewster angle microscopy (BAM).

*Results:* Our results showed that only $\gamma_{min}$ values were reliable and were significantly lower in full-term infants, as compared to the risk neonates. The results obtained were proved by the shape of hysteresis curves of GA surface active films. BAM images of GA monolayers from NRDS group showed impaired surface morphology due to the surfactant insufficiency, as compared to the control group. Corticosteroid therapy improved both GA surface characteristics and monolayer morphology.

*Conclusions:* GAs analyses by ADSA and BAM are fast and informative approaches for lung maturity assessment. In addition, the corticosteroid therapy applied improved all GAs surface parameters due to AS maturation.

## 1. Introduction

In the last years as a result of *in vitro* fertilization and multiple pregnancy clear tendency for an increase in the relative percentage of risk newborn children was observed. One of the most severe respiratory dysfunctions in the risk newborns is neonatal respiratory distress syndrome (NRDS) [1, 2, 3, 4, 5]. The disease has complex etiology and many factors are involved in its development, such as gestational age, *in vitro* fertilization usually leading to multiple pregnancy, and therefore prematurely born children before 37 weeks of gestation, sex, antenatal prophylaxis, gestational diabetes, etc. [3, 4, 6]. NRDS develops as a result of deficiency and/or inactivation of alveolar surfactant (AS) components responsible for lung immaturity and it is a leading cause for the neonatal morbidity and mortality. The surfactant is synthesized and secreted by type II pneumocytes, which differentiate between 24 and 34 weeks of gestation in the human [7]. Primary deficiency of AS is due to immaturity of pneumocytes type II which are enable to produce surfactant lipids and proteins [8]. Insufficiency of AS results in decreased pulmonary compliance and in increased surface tension at the air-water interface, which leads to higher risk of alveolar collapse at exhalation followed by reduced total gas exchange surface [9, 10].

For NRDS prevention in prematurely newborns two therapeutic strategies are applied: antenatal administration of corticosteroids, which enhance AS biosynthesis, and prophylactic administration of natural or synthetic exogenous surfactants (ES) immediately after delivery [4, 11, 12, 13]. In the last years surfactant therapy is introduced into the routine practice as lifesaving in the developed countries. Unfortunately, this therapy is too expensive which requires the establishment of fast and

---

* Corresponding author.
  *E-mail address:* albenagj@uni-sofia.bg (A. Jordanova).

adequate methods for lung maturity assessment for optimization of ES administration. On the other hand multiple clinical trials show that antenatal administration of corticosteroids significantly decrease the risk of NRDS development and the prophylactic administration of ES after birth [14, 15]. The antenatal corticosteroid therapy enhances the differentiation of epithelial cells in type II pneumocytes, stimulates the surfactant production by both transcription and post-transcriptional mechanisms, enhancing the rate of phospholipids, fatty acid and specific surfactant proteins biosynthesis in the fetal lung [4, 16, 17].

The main diagnostic criteria of NRDS include chest radiographic findings and acidosis after delivery with the clinical symptoms like tachypnea, poor feeding, nasal flaring, grunting, cyanosis, intercostal retraction and reduction of respiratory sounds in pulmonary auscultation [18]. However, early diagnostics of lung maturity is crucial for the appropriate lifesaving ES therapy for newborns. Until now lung maturity is determined by laboratory biochemical and biophysical assays of amniotic fluids (AF) from mothers and tracheal aspirates (TA) from newborns. These methods for sample collection are invasive and traumatic. In contrast, gastric aspirates (GA) collection is fast, simple, noninvasive procedure, realized in the first minutes after delivery. During pregnancy the fetus swallows amniotic fluids, as a result characteristics of alveolar surfactant are comparable for AF and GA. However, comparative analyses show differences in their reliability [19, 20, 21]. Therefore, the development and application of noninvasive methods for early and fast diagnostics of NRDS in risk newborns is of a great importance for the clinical practice.

The aim of the present study was to determine the effect of the betamethasone therapy on lung maturity in clinical samples of GA from risk newborns by rapid and informative biophysical methods, such as Axisymmetric Drop Shape Analysis (ADSA) of a pending drop and Brewster Angle Microscopy (BAM).

## 2. Materials and Methods

### 2.1. Gastric aspirates samples

The clinical samples of gastric aspirates were collected from 77 infants divided into three groups: a control group of 38 healthy full-term infants; 16 prematurely newborns (aged 26–32 weeks of gestation) clinically diagnosed with NRDS, and 23 prematurely born infants after *in vitro* fertilization and corticosteroid therapy. Healthy full-term babies are characterized by the following clinical exclusion criteria: gestational age of $38 \pm 2$; body weight $>2800 \pm 184$ g; $SatO_2$ $>90\%$; negative Silverman test; normal X-chest radiography; normal and stable biochemical parameters. Babies with NRDS were diagnosed by the following criteria: gestational age of $29 \pm 3$; body weight of $1050 \pm 226$ g; $SatO2$ $<90\%$; positive Silverman test; abnormal X-chest radiography; unstable biochemical parameters. For collection of GA gastric tube was used immediately after delivery as previously described [22]. For all experiments an informed consent was obtained from all parents for each sample collected. The samples were collected after the approval of the St. Sofia Hospital Ethical Committee.

### 2.2. Determination of surface characteristics of GA by ADSA of a pending drop

The surface tension was determined by tensiometer KSV CAM 101 (KSV Instruments Ltd., Finland) [23]. The setup was computer-controlled by a Windows-integrated program, including the ADSA surface tension calculation algorithm. By an intergrated camera series of pictures were taken. An equilibrium value of the surface tension, $\gamma_{equilibrium}$ ($\gamma_{eq}$, mN/m) in static conditions, as well as maximal surface tension ($\gamma_{max}$, mN/m) at 100% drop surface (corresponding to 30 mm$^2$ drop surface area) and minimal surface tension ($\gamma_{min}$, mN/m) at 20% drop surface (corresponding to 5 mm$^2$ drop surface area) were measured after 10-fold

volume compression and decompression of the GA drop as previously described [22].

### 2.3. Brewster angle microscopy of GA monolayers

Insoluble GA monolayers were formed in a Langmuir trough by spreading 5 μl of GA samples over the subphase of 0.15 M NaCl. After 10 min (for reaching an equilibrium surface tension, hence adsorption of surface active molecules) BAM images were recorded by using Accurion Nanofilm Ultrabram (Accurion GmbH, Goettingen, Germany) as previously described [22].

### 2.4. Statistical analysis

Descriptive statistics were applied for the surface tension parameters in the group, treated antenatally with corticosteroid therapy (CST). Sample limits, mean, standard error of mean, standard deviation and median were calculated for the equilibrium, minimal and maximal surface tension. The data were represented by box plots. Shapiro-Wilk test for normality was used. Comparisons with healthy infants born at term (control) and premature infants developing clinical signs of NRDS (NRDS) were made by one way analysis of variance (ANOVA) or Kruskal-Wallis test (ANOVA on ranks) at a significant level $\alpha = 0.05$. The multiple comparison procedure for ANOVA on ranks followed Dunn's method. The statistical analysis was performed by SigmaPlot 12 for Windows.

## 3. Results and discussion

Almost half a century ago a study demonstrated the positive effect of a single course of antenatal corticosteroid therapy [24]. Corticosteroids were introduced into the neonatal practice in the developed countries after 1994 and their administration proved that CST may enhance fetal maturation before preterm birth and decrease the rate of NRDS, intraventricular haemorrhage and neonatal death with no complications for both babies and mothers [25, 26]. Nowadays it was proved that the use of antenatal steroid is most effective in reducing the incidence of NRDS in pregnancies that deliver 24 h after and up to 7 days after administration of the second dose of antenatal corticosteroids [27, 28].

In our study we demonstrated for the first time the effect of a single course of CST on surface properties of gastric aspirates samples, taken from prematurely born infants, and compared with a control group of healthy full term babies, and infants developing clinical signs of NRDS. For this purpose we used axisymmetric drop shape analysis of a pending drop. The pending drop method allows the analysis of the surface behavior of small amounts (50 μl) of GA samples. ADSA method is based on the numerical fit between the shape of experimental drops and the mathematical model given by the classical Laplace equation of capillarity. By this approach we measured the following surface characteristics: $\gamma_{eq}$ measured at static conditions, the dynamic parameters, $\gamma_{max}$ and $\gamma_{min}$, and the shape of the hysteresis curves of surface tension vs. surface area during compression-decompression cycling.

Our results compared by ANOVA test showed no statistically significant difference ($p > 0.05$) between $\gamma_{eq}$ values of GA samples from the three groups studied: mean $\gamma_{eq}$ varied from $36.5 \pm 5.6$ mN/m for NRDS

**Table 1.** Surface tension ($\gamma$, mN/m) values (mean $\pm$ standard deviation) of GA samples of the three groups studied and the results from group comparisons (*F*-statistics by ANOVA and *H*-statistics by Kruskal-Wallis test).

| GA samples | $\gamma_{equilibrium}$ | $\gamma_{max}$ | $\gamma_{min}$ |
|---|---|---|---|
| Control group | $37.5 \pm 4.8$ | $52.0 \pm 5.0$ | $6.9 \pm 2.3$ |
| NRDS group | $36.5 \pm 5.6$ | $47.0 \pm 6.5$ | $19.6 \pm 6.1$ |
| CST group | $39.1 \pm 7.0$ | $49.3 \pm 5.1$ | $13.8 \pm 4.9$ |
| Statistics value | $F = 1.04$ | $H = 7.9$ | $H = 40.5$ |
| *p* value | $p = 0.359$ | $p = 0.019$ | $p < 0.0001$ |

group to $39.1 \pm 7.0$ mN/m for the CST group (Table 1, Figure 1). These findings confirmed our previous data that the equilibrium surface tension was not a reliable parameter for lung maturity assessment, since *in vivo* the mechanism of the alveolar function was not performed in static conditions [22].

The dynamics surface tension values proved to be appropriate parameters for AS functionality, since they represent the changes in alveolar surface area during breathing. Our results showed that the values of maximal surface tension, achieved at 100% drop area, were higher in the control group ($52.0 \pm 5.0$ mN/m), as compared to the infants with NRDS ($47.0 \pm 6.5$ mN/m). The administration of CST lead to a decrease in mean $\gamma_{max}$ ($49.3 \pm 5.1$ mN/m) in comparison to data for the control group. This value was intermediate between the groups tested (Table 1, Figure 2), showing an improvement in this parameter. Moreover, the comparison of the groups by pairs revealed statistically significant difference (Kruskal-Wallis test) only between the control and NRDS groups ($p < 0.05$). It is known that maximal surface tension is detected at the end of inhalation where maximal surface area is achieved. This parameter reflects the degree of re-adsorption and re-arrangement of the surface active molecules of the alveolar surfactant at air-water interface during decompression of the monolayers: higher $\gamma_{max}$ value indicates that more surface active molecules are squeezed out from the interface during compression forming reservoir of lipids and possibly proteins into the subphase [29, 30].

Among the surface tension values determined, the most important and informative about the composition-function relationship of AS is $\gamma_{min}$. The classical model for surfactant function explains that AS in *in vivo* conditions reaches $\gamma_{min}$ values near zero during exhalation, i.e. at compression of the monolayer. The main function of the surfactant is to prevent alveolar collapse at exhalation due to the dense packaging of the surface active molecules at the air-water interface, which is manifested by the low $\gamma_{min}$ values [31]. The comparison of the groups studied by pairs indicated that the minimal surface tension values of GA of healthy full-term children (control group) differs from the other two groups studied. The test of Kruskal-Wallis showed that only this parameter gave statistical significance ($p < 0.001$). CST group had an intermediate position between the other two groups: $13.8 \pm 4.9$ mN/m, as compared to $6.9 \pm 2.3$ mN/m for the control, and $19.6 \pm 6.1$ mN/m for NRDS groups. These results indicated an improvement of AS composition after the therapy applied (Table 1, Figure 3). Our data confirmed that the induction of type II pneumocytes by corticosteroids increases the biosynthesis of surfactant proteins and enzymes necessary for phospholipid synthesis [16, 32, 33].

In addition to surface tension measurements by ADSA method, the hysteresis curves shape was compared between the three groups studied (Figure 4, Panel A). The graphs represent the dynamic changes in surface
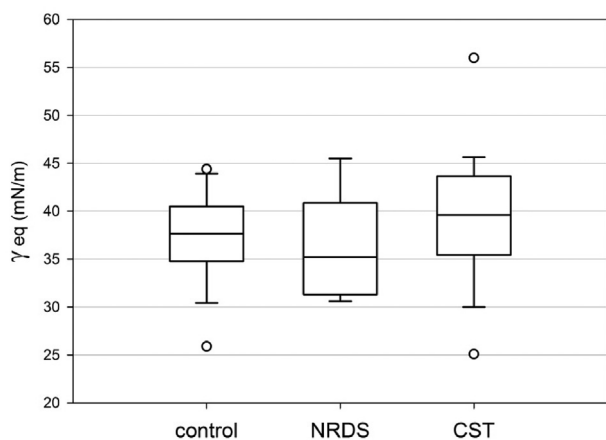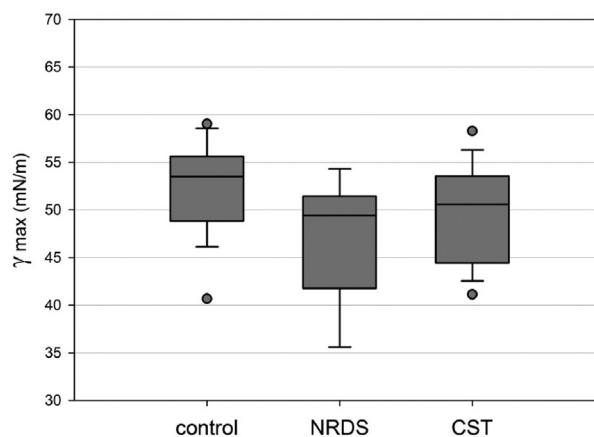
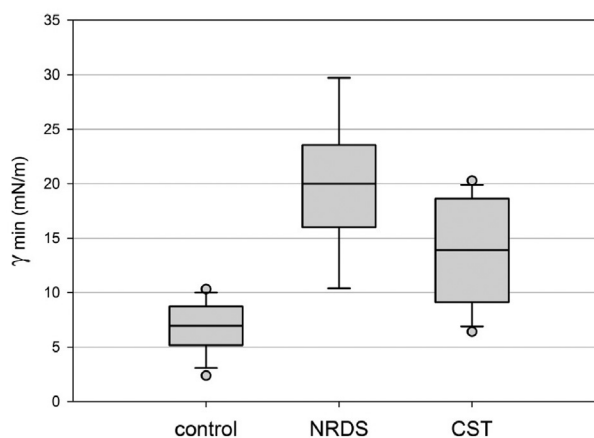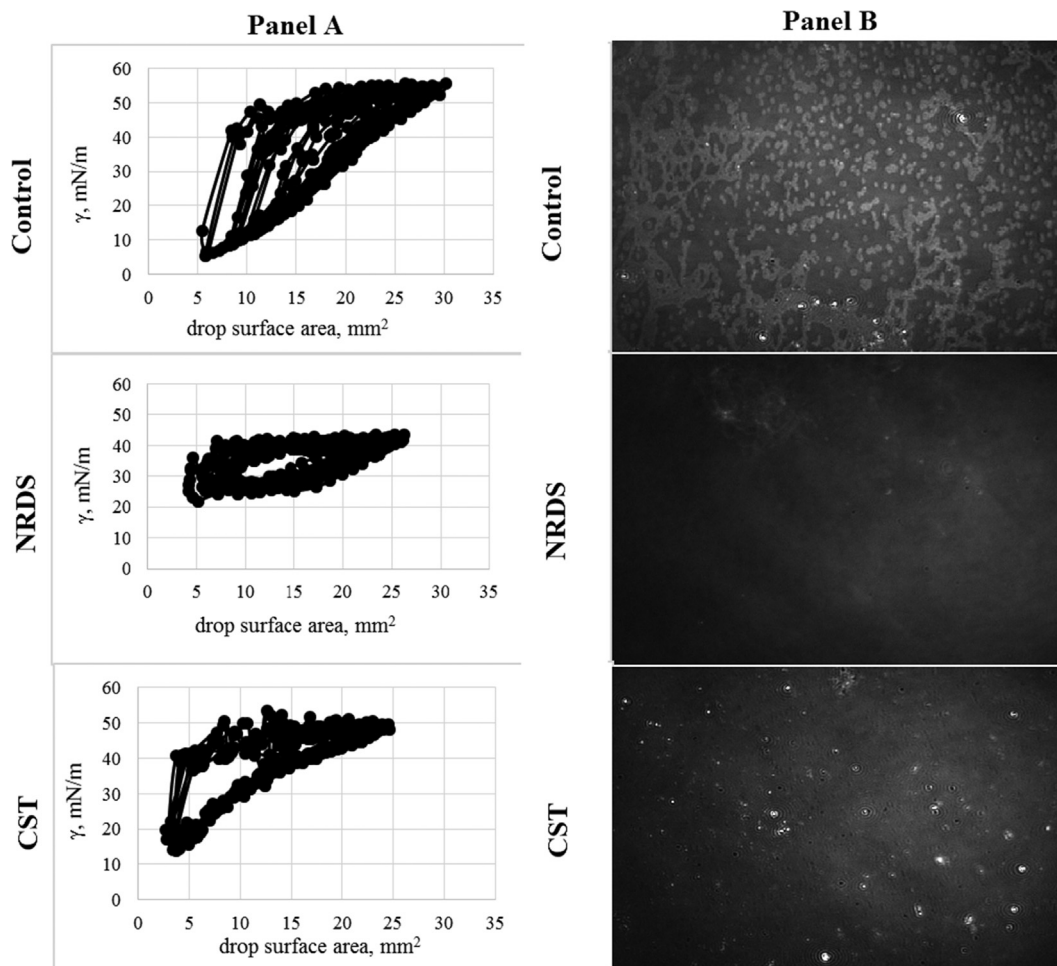**Figure 2.** Values of the maximal surface tension ($\gamma_{max}$, mN/m) of control, NRDS and CST groups.

**Figure 3.** Values of the minimal surface tension ($\gamma_{min}$, mN/m) of control, NRDS and CST groups.

parameters during compression/decompression. It was obvious that the administration of corticosteroids led to repeatable and intermediate values of the dynamic surface parameters between the control and NRDS groups during 10-fold cycling. There are two main indicative parameters from the hysteresis curves shape, (Figure 4, Panel A): $\Delta\gamma$ - the difference of $\gamma$ between initial maximal drop area (30 mm$^2$) and minimal drop area (5 mm$^2$) and the total area of the hysteresis loop (A) of the three groups. Both parameters logically decrease in the order: ($\Delta\gamma$, A of control group) > ($\Delta\gamma$, A of CST group) > ($\Delta\gamma$, A of NRDS group).

To confirm our observation we analyzed the morphology of the same samples by Brewster Angle Microscopy (Figure 4, Panel B). BAM provides direct visualization of ultra-thin surface films at the air-water interface in the absence of any fluorescent dye. The contrast in BAM images is due to local differences in the monolayer refractive index caused by differences in local molecular density or packing. Without surface film there is no light reflection from the clean interface and the image is black. However, in presence of surface active material the light is reflected by the film giving contrasting brighter image. One of the main advantages of this method is that it requires minimal volume of the sample and the short time for the observation of the AS monolayer. As it is seen from Figure 4, Panel B the images showed thick and dense surface film with clearly visible network of contrast brighter domains in healthy full-term children, without black areas (control), as a result of the optimal composition of AS indicating lung maturity. In NRDS group the film observed was extremely thin, loose, consisting of large black and dark grey areas because of the lack of enough surface active material. The image

**Figure 1.** Values of the equilibrium surface tension ($\gamma_{eq}$, mN/m) of control, NRDS and CST groups.

**Figure 4.** Panel A: Hysteresis curves (γ, mN/m vs. drop surface area, mm$^2$) and Panel B: BAM images of GA samples from the group studied.

representing the effect of CST confirmed the results for surface tension measurements taking an intermediate position between the other two groups: the film formed was thicker and more contrast, with less black areas than NRDS group but without so large network of contrast brighter area found in the control. As a result of GST larger bright AS domains were observed due to higher surface active molecules concentration at the air-water interface as compared to the samples from prematurely newborns with NRDS.

## 4. Conclusions

Our results showed that several surface parameters of GA from the pending drop method and BAM images differed between healthy full-term infants, prematurely born children with NRDS, and risk neonates after *in vitro* fertilization and corticosteroid therapy. The results revealed some conclusion as follows:

(1). The values of the equilibrium surface tension ($\gamma_{eq}$) between the control, NRDS and CST groups showed no statistically significant difference ($p > 0.05$).

(2). Maximal surface tension ($\gamma_{max}$) of CST takes an intermediate position between the control and NRDS groups showing an improvement of AS properties as a result of the therapy applied.

(3). The comparison of the groups studied indicated that only $\gamma_{min}$ showed statistically significant difference ($p < 0.001$) between the values of GA of healthy full-term children (control group) and other two groups as well as between NRDS and CST groups showing the positive effect of the corticosteroid therapy.

(4). The hysteresis curves after 10-fold cycling of the drop illustrated the potential of two parameters for lung maturity assessment: Δγ - the difference of γ between maximal and minimal drop area during compression/decompression and A - hysteresis loop area, both decreasing in the order: (Δγ, A of control group) > (Δγ, A of CST group) > (Δγ, A of NRDS group).

(5). BAM images of GAs monolayers from the control group showed thick and dense films with surface domains as a result of better surface characteristics and AS composition. In contrast, the monolayers from NRDS group were thin, homogenous without surface aggregates due to the lung immaturity. As a result of corticosteroid therapy phospholipids biosynthesis was increased and visualized by BAM images as larger bright AS domains than in NRDS group.

(6). The effect of the corticosteroid therapy applied to prematurely born babies after *in vitro* fertilization and multiple pregnancy showed activation of alveolar surfactant biosynthesis in risk newborn children.

(7). The results proved that Brewster angle microscopy, along with ADSA of a pending drop give reliable information on lung maturation using GAs as clinical samples from neonates.

### Declarations

*Author contribution statement*

Asya Tsanova, Albena Jordanova: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the

data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Vishnya Stoyanova: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Elena Tasheva-Terzieva: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Krasimira Ivanova: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Zdravko Lalchev: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

## Competing interest statement

The authors declare no conflict of interest.

## Additional information

No additional information is available for this paper.

## References

[1] P. Krafft, P. Fridrich, T. Pernerstorfer, R.D. Fitzgerald, D. Koc, B. Schneider, A.F. Hammerle, H. Steltzer, The acute respiratory distress syndrome: definitions, severity and clinical outcome. An analysis of 101 clinical investigations, Intensive Care Med. 22 (1996) 519–559.

[2] J. Floros, D.S. Phelps, U. Pison, R. Spragg, Molecular biology and clinical implications, Curr. Respir. Med. Rev. 1 (2005) 77–84.

[3] P.O. Nkadi, T.A. Merritt, D.M. Pillers, An overview of pulmonary surfactant in the neonate: genetics, metabolism, and the role of surfactant in health and disease, Mol. Genet. Metab. 97 (2009) 95–101.

[4] Лалчев З, Христова Е, Алвеоларен сърфактант и неонатален респираторен дистрес синдроM, Университетско издателство „Св. КлиМент Охридски“, 2010.

[5] M.A. Matthay, L.B. Ware, G.A. Zimmerman, The acute respiratory distress syndrome, J. Clin. Investig. 122 (2012) 2731–2740.

[6] C.P. Tallo, B. Vohr, W. Oh, L.P. Rubin, D.B. Seifer, R.V. Haning Jr., Maternal and neonatal morbidity associated with in vitro fertilization, J. Pediatr. 127 (5) (1995) 794–800.

[7] A.H. Jobe, Neonatal-Perinatal Medicine, Mosby, St Louis, 2002, pp. 973–977. Lung development and maturation.

[8] P.H. Burri, Fetal and postnatal development of the lung, Annu. Rev. Physiol. 46 (1984) 617–628.

[9] J.A. Clements, Surface tension of lung extracts, Proc. Soc. Exp. Biol. Med. 95 (1957), 170-1722.

[10] M.E. Avery, J. Mead, Surface properties in relation to atelectasis and hyaline membrane disease, AMA J. Dis. Child. 97 (1959) 517–523.

[11] B. Robertson, Corticosteroids and surfactant for prevention of neonatal RDS, Ann. Med. 25 (1993) 285–288.

[12] E. Herting, O. Gefeller, M. Land, L. Van Sonderen, K. Harms, B. Robertson, Surfactant treatment of neonates with respiratory failure and group B streptococcal infection, Pediatrics 106 (2000) 957–964.

[13] M. Luchetti, F. Ferraro, C. Gallini, A. Natale, A. Pigna, L. Tortorolo, G. Marraro, Multicenter, randomized, controlled study of porcine surfactant in severe respiratory syncytial virus-induced respiratory failure, Pediatr. Crit. Care Med. 3 (2002) 261–268.

[14] D. Roberts, J. Brown, N. Medley, S.R. Dalziel, Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth, Cochrane Database Syst. Rev. 21 (3) (2017). CD004454.

[15] M.W. Kemp, J.P. Newnham, J.G. Challis, A.H. Jobe, S.J. Stock, The clinical use of corticosteroids in pregnancy, Reprod. Update 22 (2016) 240–259.

[16] H.G. Liley, R.T. White, B.J. Benson, P.L. Ballard, Glucocorticoids both stimulate and inhibit production of pulmonary surfactant protein A in fetal human lung, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 9096–9100.

[17] M.R. Garbrecht, J.M. Klein, T.J. Schmidt, J.M. Snyder, Glucocorticoid metabolism in the human fetal lung: implications for lung development and the pulmonary surfactant system, Biol. Neonate 89 (2006) 109–119.

[18] J. Wang, X. Liu, T. Zhu, C. Yan, Analysis of neonatal respiratory distress syndrome among different gestational segments, Int. J. Clin. Exp. Med. 8 (9) (2015) 16273–16279.

[19] D. Armstrong, D.E. Van Wormer, W.P. Gideon, Predicting respiratory distress by thin-layer chromatography of the newborn gastric aspirate, Obstet. Gynecol. 48 (1976) 35–39.

[20] L. Brandell, W. Sepúlveda, H. Araneda, Clements' test in gastric aspirate of newborn infants for the prediction of pulmonary maturity, Rev. Chil. Pediatr. 61 (1990) 299–302.

[21] S. Chida, T. Fujiwara, M. Konishi, H. Takahashi, M. Sasaki, Stable microbubble test for predicting the risk of respiratory distress syndrome: II. Prospective evaluation of the test on amniotic fluid and gastric aspirate, Eur. J. Pediatr. 152 (1993) 152–156.

[22] M. Bangyozova, A. Jordanova, A. Tsanova, V. Stoyanova, E. Tasheva, K. Ivanova, R. Todorov, E. Hristova, Z. Lalchev, Application of axisymmetric drop shape analysis and Brewster angle microscopy for assessment of clinical samples from prematurely born infants with NRDS, Colloid. Surf. Physicochem. Eng. Asp. 519 (2017) 187–191.

[23] Y. Rotenberg, L. Boruvka, A.W. Neumann, Determination of surface tension and contact angle from the shapes of axisymmetric fluid interfaces, J. Colloid Interface Sci. 93 (1983) 169–183.

[24] G.C. Liggins, R.N. Howie, A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants, Pediatrics 50 (1972) 515–525.

[25] S. Cuosens, T. Hansen, J. Lawn, et al., Antenatal steroids in preterm labour for the prevention of neonatal deaths due to complications of preterm birth, Int. J. Epidemiol. S39 (2010) 122–133.

[26] J.P. Newnham, A.H. Jobe, Should we be prescribing repeated courses of antenatal corticosteroids? Semin. Fetal Neonatal Med. 14 (2009) 157–163.

[27] W. Carlo, S. McDonald, M. Fanaroff, et al., Association of antenatal corticosteroids with mortality and neurodevelopmental outcomes among infants born at 22 to 25 weeks' gestation, J. Am. Med. Assoc. 306 (2011) 2348–2358.

[28] Royal College of Obstetricians and Gynaecologists, Antenatal Corticosteroids to Reduce Neonatal Morbidity and Mortality, Green-Top Guideline No7, 2010.

[29] E.M. Scarpelli, The alveolar surface network: a new *anatomy* and its physiological significance, Anat. Rec. 251 (1998) 491–527.

[30] E.J.A. Veldhuizen, H.P. Haagsman, Role of pulmonary surfactant components in surface film formation and dynamics, Biochim. Biophys. Acta Biomembr. 1467 (2000) 255–270.

[31] Y.Y. Zuo, F. Possmayer, How does pulmonary surfactant reduce surface tension to very low values? J. Appl. Physiol. 102 (2007) 1733–1734.

[32] P.L. Ballard, R.A. Ballard, Scientific basis and therapeutic regimens for use of antenatal glucocorticoids, Am. J. Obstet. Gynecol. 173 (1995) 254–262.

[33] A.C. Vidaeff, S.M. Ramin, L.C. Gilstrap, J.L. Alcorn, In vitro quantification of dexamethasone-induced surfactant protein B expression in human lung cells, J. Matern. Fetal Neonatal Med. 15 (2004) 155–159.