Current perspectives of Assessment of Fetal Lung Maturity

Ali El Shabrawy Ali, Hala E. Mohamed Mowafy, Ahmed Mostafa Ibrahim Mostafa Hadhoud, Mohamed El huseiny Radwan

Department of Obstetrics and Gynecology, Faculty of Medicine, Zagazig University, Egypt

***Corresponding author:** Ahmed Mostafa Ibrahim Mostafa Hadhoud,

Mobile: (+20)01002947404, **E-mail:**dr.ahmed.had88@gmail.com

Abstract:

Respiratory distress syndrome of the newborn infant caused by immaturity of the fetal lung continues to be a clinical problem. Measurement of pulmonary surfactant production is the most effective way to evaluate pulmonary maturity. Since the first fetal lung maturity test was described more than two decades ago, advances in methodology have produced diagnostically sensitive tests that are both rapid and precise. Unfortunately, currently available tests continue to demonstrate low diagnostic specificity and remain poor predictors of fetal lung immaturity. We review the background, methodology, pre-analytical and analytical concerns, and clinical performance of various fetal lung maturity assays, and discuss the appropriate use and interpretation of these tests.

DOI Number: 10.48047/NQ.2022.20.20.NQ109006 **NeuroQuantology2022;20(20): 33-45**

Introduction:

The pulmonary system is among the last of the fetal organ systems to mature, both functionally and structurally. Race may affect the timing of fetal lung maturation: fetal lung maturity appears to be achieved earlier in gestation in blacks and South Asians **(1).**

Fetal pulmonary maturity is sometimes assessed before iatrogenic preterm delivery and can be a factor in determining the timing of delivery, because the immature pulmonary system may not oxygenate the neonate adequately, so preterm birth can lead to significant neonatal morbidity or mortality **(2).**

Several tests are available for this purpose. The gold-standard methods to assess fetal lung maturity are the chemical, biological and physical properties of amniotic fluid obtained by amniocentesis, which is an indirect assessment of the likelihood of lung maturity, as the direct assessment of fetal pulmonary function are not possible **(3)**.

Fetal pulmonary maturity can be expected if the review of the patient's prenatal record confirms a gestational age of ≥39 weeks of gestation, or tests on lung maturity suggest a mature lung **(4).**

When to perform lung maturity tests?

Testing is only indicated in women of uncertain gestational age. Testing to confirm pulmonary maturity in order to proceed with an elective delivery before 39 weeks should generally be accepted. Even when fetal lung maturity testing results are mature before 39 weeks of gestation, neonates delivered

before 39 weeks are at higher risk of adverse outcome than those delivered at 39 to 40 weeks of gestation without fetal lung maturity testing **(4).**

Fetal pulmonary maturity is sometimes evaluated before semi-elective but medically indicated preterm birth, as this information significantly impacts assessment of the balance between the maternal-fetal risks of continuing the pregnancy and the maternal-fetal risks of preterm birth, however assessment of fetal lung maturity should not be performed if delaying delivery because of pulmonary immaturity will place the mother or fetus at significant risk **(3)**.

Additionally, fetal lung maturity is generally not indicated prior to 32weeks of gestation, given the high prevalence of fetal pulmonary immaturity and the lower predictive value of a mature test result at this gestational age.

Fetal lung maturity tests:

The gold-standard methods to assess fetal lung maturity are the chemical, biological and physical properties of amniotic fluid obtained by amniocentesis, which is an indirect assessment of the likelihood of lung maturity, as the direct assessment of fetal pulmonary function are not possible **(3).**

Tests to assess fetal lung maturity include:

a) Amniotic fluid tests
b) Lung-to-liver signal intensity ratio at MRI.
c) Placental grading.
d) Fetal pulmonary artery flow velocity waveform
e) Direct estimation of fetal lung volume.
f) Thalamic Echogenicity.

A)Amniotic fluid tests:

Two types of tests are used to determine pulmonary maturity using the amniotic fluid obtained by amniocentesis:

1. Biochemical tests measure the concentration of particular components of pulmonary surfactant, and
2. Biophysical tests evaluate the surface-active effects of these phospholipids.

The surfactant/albumin ratio (TDx-FLM ll), lecithin/ sphingomyelin (L/S) ratio, lamellar body count, and detection of phosphatidylglycerol (PG) are the tests that have been most commonly used to assess fetal lung maturity, the foam stability index and optical density at 650 nm are not widely used, the choice of test should be based upon availability, presence or absence of contaminants and physician preference **(5).**

Although randomized trials comparing all of these tests have not been performed, controlled studies found that none of the tests performed significantly better than the others **(6)**.

All are better at predicting the absence, rather than the presence, of respiratory distress. All performed less well at earlier gestational ages, which should be taken into account when interpreting results **(6).**

1- **Lamellar body count**: This is probably the most common test currently in use for assessing fetal lung maturity in the United States **(7).**

Lamellar body count is a direct measurement of surfactant production by type II pneumocytes.

A standard Coulter counter can be used for quantification because of the similarity in size of lamellar bodies and platelets. Values less than 15,000 per microliter are almost always associated with pulmonary immaturity; values ≥50,000 per microliter strongly suggest pulmonary maturity **(7).**

However, there is no consensus on the optimal threshold for predicting pulmonary maturity; values greater than 30,000 to 40,000 per microliter, as well as higher levels, have been suggested **(6).**

Therefore, some clinicians use the lamellar body count as an initial screening test and perform the lecithin/sphingomyelin (L/S) ratio if the lamellar body count is neither clearly mature (≥50,000) nor immature (<30,000). Compared to thin-layer chromatography techniques, the lamellar body count is faster, more objective, less labor intensive, less technique dependent, and less expensive. Blood contamination can lead to false elevation of the lamellar body count because platelets are counted as lamellar body; the effect of meconium is minimal **(7).**

2- **Optical density at 650 nm:** An indirect measurement of lamellar bodies can be performed by measuring optical density of amniotic fluid at a wavelength of 650 nm. It is based upon the concept that increasing opalescence is due to increasing numbers of lamellar bodies. An optical density reading of ≥0.15 is used as an indicator of pulmonary maturity **(8).**

3- **Lecithin/sphingomyelin ratio:** The lecithin/sphingomyelin (L/S) ratio for assessment of fetal pulmonary maturity.

It is based upon the observation that there is outward flow of pulmonary secretions from the lungs into the amniotic fluid. This process changes the phospholipid composition of amniotic fluid, thereby enabling indirect assessment of fetal lung maturity through evaluation of this fluid.

The concentrations of lecithin and sphingomyelin in amniotic fluid are approximately equal until 32 to 33 weeks of gestation, after that time, the concentration of lecithin begins to increase significantly while the sphingomyelin concentration remains about the same **(9).**

The measurement of sphingomyelin serves as a constant comparison for control

of the relative increases in lecithin because the volume of amniotic fluid cannot be accurately measured clinically. Determination of the lecithin/sphingomyelin ratio involves thin-layer chromatography after organic solvent extraction. It is a technically difficult test to perform and interpret; care at each step of sample handling is critical for consistent results**.** The sample should be kept on ice or refrigerated if transport to a laboratory is required. Improper storage conditions can change the lecithin/sphingomyelin ratio since amniotic fluid contains enzymes that can be affected by temperature **(10)**.

The amniotic fluid sample must be well mixed before testing. It takes several hours to perform the test, which is another disadvantage of this method. A threshold value for prediction of lung maturity should be calculated in individual centers by correlation with clinical outcome, as the variation within, and between laboratories can be considerable. Empirically, the lungs are considered mature and the risk of respiratory distress is exceedingly low when the lecithin/sphingomyelin ratio is greater than 2.0 **(11).**

4- **Phosphatidyl-glycerol:** Phosphatidyl-glycerol (PG) is a minor constituent of surfactant. It begins to increase appreciably in amniotic fluid after 35 weeks, several weeks after the rise in lecithin **(10).**

Because PG enhances the spread of phospholipids on the alveoli, its presence indicates an advanced state of fetal lung development and function. Phosphatidyl-glycerol testing can be performed by thin-layer chromatography, so it can be determined alone or in conjunction with testing for the lecithin/sphingomyelin ratio. It may be reported qualitatively as positive or negative, where positive represents an exceedingly low risk of respiratory distress, or in a quantitative fashion, in which a value ≥0.5 is associated with a minimal rate of respiratory distress **(12).**

Because thin-layer chromatography is a complicated and time-consuming technique, a rapid semi-quantitative immunologic slide agglutination test (AmnioStat-FLM) and several enzymatic assays were developed and have been validated as acceptable alternative techniques **(13).**

The slide agglutination test is the most common method for testing for phosphatidylglycerol; however, it appears to be less sensitive for detecting fetal lung maturity than thin-layer chromatography **(14)**.

An advantage of this test is that usually it is not affected by the presence of blood or meconium; however a disadvantage is that the absence of phosphatidylglycerol, especially before 36 weeks of gestation, is less predictive of the occurrence of

respiratory distress than immature results from other tests **(15)**.

5- **Surfactant/albumin ratio:** The surfactant/albumin ratio is based on the principle of fluorescence polarization and uses an automated analyzer to quantitate the competitive binding of a fluorescent probe to both surfactant and albumin in a sample of amniotic fluid; thus, it is a true direct measurement of surfactant concentration **(16).**

An elevated surfactant/albumin ratio has been correlated with the presence of fetal lung maturity; the threshold for maturity is 55 mg of surfactant per gram albumin **(17).**

The performance of this test compares favorably with the well-established lecithin/sphingomyelin ratio and phosphatidylglycerol tests and is gestational age-dependent **(18).**

Advantages of this test are that it is a simple, automated, rapid test that varies minimally between laboratories, and requires only a small volume of amniotic fluid **(15)**

A disadvantage to the TDx-FLM ll method is the large quantification scale. Values greater than 55 are regarded as mature and values less than 40 are considered immature, while values of 40 to 54 are considered "indeterminate" Blood or meconium in the amniotic fluid also affects results **(19).**

6- **Foam stability index**: The foam stability index (FSI) is a rapid predictor of fetal lung maturity based upon the ability of surfactant to generate stable foam in the presence of ethanol **(15).**

Ethanol is added to a sample of amniotic fluid to eliminate the effects of nonsurfactant factors on foam formation. The mixture is then shaken and will demonstrate generation of a stable ring of foam if surfactant is present. The foam stability index (FSI) is calculated by utilizing serial dilutions of ethanol to quantitate the amount of surfactant present **(20).**

Amniotic fluid samples should not be collected in silicone tubes when this test is planned, as the silicone will produce "false foam." The discriminating value indicative of lung maturity is usually set at ≥47. A positive result virtually excludes the risk of respiratory distress; however, a negative test often occurs in the presence of mature lungs and the presence of blood or meconium interferes with results of the FSI **(15).**

Factors affecting maturation of the lung

- **Gestational age:** For each test, the ability to predict absence of respiratory distress is influenced by the prevalence of respiratory distress in the population tested; thus, the predictive value varies with gestational age **(15).**

As an example, in one analysis, the risk of respiratory distress after a TDx-FLM ll of 60 mg/g at 29 and 37 weeks of gestation was 16 and 1 percent, respectively **(15).**

The presence of fetal lung maturity is not necessarily indicative of readiness for neonatal life and absence of neonatal morbidity. This was illustrated in a retrospective cohort study of neonatal outcomes of infants with mature fetal pulmonary indices on prenatal testing who delivered at 34 to 36 weeks or 37 to 38 weeks versus those who delivered at ≥39 weeks of gestation, the rates of composite neonatal adverse outcome (defined as NICU admission, phototherapy, antibiotic treatment, intravenous fluids for hypoglycemia, or N/G feeding) for the three groups were significantly different at 21, 13, and 4 percent, respectively **(12).**

- **Blood, meconium:** Phosphatidylglycerol determination generally is not affected by blood, meconium, or other contaminants; its ability to predict pulmonary maturity is the same whether or not contamination is present, this is an advantage for assessing fetal lung maturity status since these substances are commonly found in amniotic fluid. The surfactant/albumin ratio is usually reliable if mature since contaminants tend to cause falsely immature findings, although the degree and direction of interference are not well fined **(21)**.

The presence of blood or meconium can interfere with interpretation of the lecithin/ sphingomyelin ratio; bloody samples give false information due to the presence of sphingomyelin in blood and decreased extraction of lecithin by cold acetone techniques in the presence of red blood cells. Therefore, if blood or other particulate matter is present in the amniotic fluid sample, a low speed, short centrifugation should be used to remove the cellular component however, this does not guarantee an accurate result, especially when there is a lot of blood or meconium **(22)**.

- **Oligohydramnios and polyhydramnios:** The effect of amniotic fluid volume (oligohydramnios, polyhydramnios) on test results has not been studied extensively ,Theoretically, tests that are expressed as a ratio or proportion of two solutes released into the amniotic fluid should remain accurate independent of amniotic fluid volume, while tests that reflect the concentration of a substance in the amniotic fluid (eg,

lamellar body count, phosphatidylglycerol) may be affected by amniotic fluid volume.

- **Vaginal pool samples:** In women with intact membranes, amniotic fluid is obtained by amniocentesis, while in women with ruptured membranes, a syringe can be used to aspirate amniotic fluid pooled in the posterior vaginal fornix. In a study of 16 patients who underwent both amniocentesis and vaginal collection of amniotic fluid at a mean gestational age of 33 weeks, the vaginal pool TDx-FLM ll result was lower than the amniocentesis result in every patient **(23).**
- **Antenatal corticosteroids:** corticosteroids enhance fetal lung maturity by accelerating the development of type 1 and type 2 pneumocytes, leading to structural and biochemical changes that improve both lung mechanics and gas exchange. Induction of type 2 pneumocytes increases surfactant production by inducing production of surfactant proteins and enzymes necessary for phospholipid synthesis, so standard tests for predicting fetal lung maturity may be less reliable for predicting fetal lung maturity in women who have received antenatal corticosteroids.
- **Maternal diabetes mellitus:** Assessment of the effect of maternal diabetes on fetal lung maturity has yielded controversial findings. Some studies suggest that fetal lung maturation is delayed in diabetic pregnancies, resulting in a greater risk of RDS, when compared with non-diabetic pregnancies of similar gestational age. Other studies illustrate normal fetal lung development in pregnancies with well-controlled diabetes (pregestational or gestational diabetes) **(24)**.
- **Twin pregnancy:** the twin pregnancy delay the maturity of fetuses' lungs, and the lung are less mature compared to a singleton fetus at the same gestational age. So careful evaluation of the fetal lung maturity of each fetus should be done separately.

B) Lung-to-liver signal intensity ratio at MRI (LLSIR):

The T2-weighted images of MRI are able to predict the fluid content of fetal organs. The fetal lung exhibit low signal intensity on T2-weighted images because of less fluid content in early pregnancy. A previous study using MRI found that high intensity points to a substantial amount of fetal lung fluid in the voluminous small airways and alveoli, whereas low intensity indicates the absence of fetal lung fluid **(25).**

Using this phenomenon, MRI was used to predicate fetal lung maturity through measuring the signal intensity ratio between both the fetal liver and lungs at MRI assessment, introducing a relation between

both organs as Lung-Liver Signal Intensity Ratio (LLSIR) **(26)**.

Oka et al (27) performed a study in 2014 on 120 fetuses who underwent MRI examination in various indications after 22nd week of gestation. LLSIR was measured on T2-weighted images of MRI. Changes of the ratio with the progress of gestational age were studied, then the relations between LLSIR and presence of the RDS after birth were calculated. The best cutoff value of the LLSIR to predict respiratory outcome after birth was calculated using Receiver Operating Characteristic (ROC) analysis. LLSIR correlated significantly with advancing of gestational age. The non-RDS group had higher LLSIR when compared with the RDS group. ROC curve analysis showed that fetuses with an LLSIR below 2.00 were more likely to have immature lungs and develop RDS (sensitivity: 100%, specificity: 75%). So The fetal LLSIR of MRI on T2- weighted images was found as an accurate prenatal evaluative method for fetal lung maturity.

C) Placental grading :

Many studies suggested the use of placental maturity grading systems to evaluate the fetal lung maturity **(28).**

Many grading systems has been proposed, the most accepted one is the Grannum Classification system, which classifies placental maturity into four grades 0 - III, based on the ultrasound appearances of the placenta. Grading was based on the changes occurring in three zones of placenta: chorionic plate, placental substance and basal layer, as the pregnancy advances.

The grading system is as follows **(29):**

- **Grade 0:** <18 weeks
  - Uniform echogenicity
  - Smooth chorionic plate
- **Grade I:** 18-29 weeks
  - Occasional parenchymal calcification/hyperechoic areas
  - Subtle indentations of chorionic plate
- **Grade II:** >30 weeks
  - Occasional basal calcification/hyperechoic areas
  - Deeper indentations of chorionic plate (does not reach up to basal plate)
  - Seen as comma type densities at the chorionic plate
- **Grade III:** >39 weeks
  - Significant basal calcification
  - Chorionic plate interrupted by indentations (frequently calcified) that reach up to basal plate: cotyledons
  - An early progression to a grade III placenta is concerning and is sometimes associated with placental insufficiency
  - Associated with smoking, chronic hypertension, SLE, diabetes

D) Fetal pulmonary artery flow velocity waveforms (PATET):

The amniotic fluid biomarkers were studied against the fetal pulmonary artery Doppler waveforms acceleration/ejection time ratio to evaluate its ability to predict fetal lung maturity. **Schenone et al (30)** studied 43 pregnant women for fetal lung maturity testing. They studied the amniotic fluid biomarkers against the fetal pulmonary artery Doppler waveforms acceleration/ejection time ratio (PATET). An ultrasound examination that included measurement of the PATET was performed before the results of the amniocentesis were reported. The results of the PATET and the surfactant/albumin ratio were compared, and a receiver operating characteristic curve was used to determine the PATET cutoff with the optimal sensitivity and specificity for predicting surfactant/albumin ratio results.

The results demonstrated that a PATET cutoff of 0.3149 provided a specificity of 93%, a sensitivity of 73, a negative predictive value of 87%, and a positive predictive value of 85% for predicting immature surfactant/albumin ratio results. An inverse correlation between the acceleration-tome/ejection-time ratio in the fetal pulmonary artery and the amniotic fluid lecithin/sphingomyelin ration was found. This suggests that ultrasound evaluation of fetal pulmonary artery blood flow may be a promising new non-invasive technique to evaluate fetal lung maturity **(31).**

E) Direct estimation of fetal lung volume:

Direct estimation of fetal lung volume was investigated as a marker for fetal lung maturity, the total volume of normal fetal lungs can be expressed by the second-degree regression equation: $0.08 \times (\text{gestational week} - 30.1)^2 + 3.28 \times \text{gestational week} - 67.2$**.** Values below the 25^{th} percentile of this curve were considered abnormal. This method was found to be affected by liquor volume, fetal congenital malformation & any abnormalities in the utero-placental circulation, and it do not provide any additional physiological information relating to potential function of the fetal lung **(32).**

F) Ultrasound evaluation of the Thalamus echogenicity :

Rasheed et al (2) evaluated fetal thalamic echogenicity by ultrasound as a possible marker of fetal lung maturity in comparison with other ultrasound makers. A prospective longitudinal study performed in Al- Elwiya Maternity Teaching Hospital in Baghdad, Iraq during the period from April 2010 to March 2011. On 142 pregnant women (36 to 42 weeks of gestation) who were admitted for elective cesarean section and referred for an obstetric ultrasound scan at the same day of their elective cesarean

section were included. Scanning with linear ultrasound with convex transducer frequency of 3.5 MHz was utilized to measure the biparietal diameter and the state of echogenicity was recorded as echogenic or echolucent, in addition to amniotic fluid vernix and the placental changes. Rasheed et al found that the presence of echogenic thalamus as a sign of fetal lung maturity had a specificity of 86.53% which is higher than the other signs of lung maturity; the positive predictive value was (89.6%) which is also higher than the other signs, but the sensitivity was 63.33% and negative predictive value was 57.69% which is lower than the presence of vernix in the amniotic fluid, 86.66 and 67.56 respectively. Rasheed et al concluded that evaluation of echogenic thalamus is beneficial, and can be considered as a new marker of fetal lung maturity; however, further studies are required to strengthen such idea **(32).**

Conclusion:

When an elective delivery is planned before 39 weeks' gestation, documentation of fetal lung maturity is recommended. Fetal lung maturity in pregnancies complicated by adequately controlled maternal diabetes is achieved at a gestational age in similar pregnancies of non-diabetic women. Lamellar body count is a rapid and reliable test of fetal lung maturity. An algorithm for fetal lung maturity testing is included.

References:

1. **Areia A.L., Almeida M.F., Braga A.J.C., Pereira N.B., Macedo C.V. & Nogueira- Silva C.** Corticoterapia para maturação pulmonar fetal. Acta Obst. Ginecol. Port.2018. 12(4):311-313.
2. **Rasheed, Faris & Al-Sattam, Zahraa' & Hussain, Saad**. Evaluation of thalamus echogenicity by ultrasound as a marker of fetal lung maturity. Open Journal of Obstetrics and Gynecology. 2012.2. 270-275. 10.4236/ojog.2012.23056.
3. **Bonet-Carne E., Palacio M., Cobo T., Perez-Moreno A., Lopez M., Piraquive J.P., Ramirez J.C., Botet F., Marques F. & Gratacos E.** Quantitative ultrasound texture analysis of fetal lungs to predict neonatal respiratory morbidity. Ultrasound Obstet. Gynecol.2015. 45(4):427-433. <https://dx.doi. org/10.1002/uog.13441> <PMid:24919442>
4. **Avila G.L., Bovino F., Camargo D.G., Souza N.C., Santos G.G.F., Deschk M., Mendes L.C.N. & Feitosa F.L.F.** Aplicação materna de glicocorticóide nos parâmetros vitais de cordeiros nascidos a termo e prematuros. Ciência Rural.2014. 44(6):1106-1112.

5. **Bovino F.** Abordagem clínica de cordeiros prematuros: avaliação de protocolos terapêuticos emergenciais para estimulação da atividade respiratória. Doctoral Dissertation, Faculdade de Medicina Veterinária de Araçatuba, Universidade Estadual Paulista.2015. 93p.
6. **Cheong J.L.Y., Lee K.J., Boland R.A., Spittle A., Opie G.F., Burnett A.C., Hickey L.M., Roberts G., Anderson P.J. & Doyle L.W.** Changes in long-term prognosis with increasing postnatal survival and the occurrence of postnatal morbidities in extremely preterm infants offered intensive care: a prospective observational study. Lancet Child Adolesc. Health,2018. 2(12):872-879. https://dx.doi.org/10.1016/s2352-4642(18)30287-6
7. **Beamon CJ, Hardisty EE, Harris SC, Vora NL.** A single center's experience with noninvasive prenatal testing. Genet Med. 2015; 16: 681- 687.
8. **Samartha Ram H, Sandhya Ram S.** Amniotic fluid optical density (AFOD) surge coincides with the onset of spontaneous term labor. Paper presented at 55th AICOG-2014. Varanasi, Book of abstracts: page 74.
9. **Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., and Gluud, C.** Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst. Rev.2015. 3, CD007176. doi: 10.1002/14651858.CD007176.pub2
10. **Miura H., Yamazaki T., Kikichi M. & Sakaguchi M.** Plasma steroid hormone concentrations and their relationships in Suffolk ewes during gestation and parturition. Anim. Sci. J. 2019.90(11):1426-1431.
11. **Kwak, Young Hoon, Hessam Sadatsafavi, John Walewski, and Nigel L. Williams.** Evolution of Project Based Organization: A Case Study. International Journal of Project Management.2014. 33: 1652–64.
12. **Patel R.M**. Short-and-long-term outcomes for extremely preterm infants. Am. J. Perinatol. 2016.33(3):318-328.
13. **Chapman, J. F., & Herbert, W. N.** Current methods for evaluating fetal lung maturity. Laboratory Medicine, 1986, 17(10), 597-602.
14. **Yarbrough M, Grenache D, Gronowski A**. Fetal lung maturity testing: the end of an era. Biomark Med.,2014. 8(4):509-15.
15. **Jörger A., Acevedo C., Busley D., Ganter M., Schmiedl A. & Ziehank E.H.** Stereological and biophysical characteristics of the ovine surfactant system and its changes caused by ovine pulmonary adenocarcinoma. Res. Vet. Sci. 2017.114:332-340.

16. **Stichtenoth G, P. Jung, G. Walter, J. Johansson, B. Robertson, T. Curstedt, E. Herting:** Polymyxin B/pulmonary surfactant mixtures have increased resistance to inactivation by meconium and reduce growth of gram-negative bacteria in vitro. Pediatr. Res., 59 (3) (2014), pp. 407-411
17. **Mimmi, M. C., Ballico, M., Amoroso, F., et al.** Phospholipid profile of amniotic fluid in ovine model of congenital diaphragmatic hernia (CDH): the effect of fetal tracheal occlusion. Journal of Proteome Research, 2015, 14(3), 1465-1471.
18. **Harrison S.M. & Goldenberg R.L**. Global burden of prematurity. Seminars Fetal Neonatal Med.2016. 21(2):74-79.
19. **Lockwood CM, Crompton JC, Riley JK, Landeros K, Dietzen DJ, Grenache DG, Gronowski AM**. Validation of lamellar body counts using three hematology analyzers. Am J Clin Pathol 2010;134(3):420-8
20. **Todorov A., Pakrashi M., Oosterhof N. N.** Evaluating faces on trustworthiness after minimal time exposure. Soc.2014. Cogn. 27, 813–833. 10.1521/soco.2009.27.6.813
21. **Srouji SS, Carr DB, Gardella CM, et al**. The effect of common clinical contaminants on amniotic fluid fluorescence polarization results. Obstet Gynecol 2004; 104:1237.
22. **Kart, B., Karsidag, A.Y.K., Buyukbayrak, E.E., Telatar, B., Turan, C. and UNAL, O:** Evaluation of fetal lung maturity by turbidity testing and tap test, 2015; J Turk Soc Obstet Gynecol. 8 (1): 25-31.
23. **Winn-McMillan T, Karon BS.** Comparison of the TDx-FLM II and lecithin to sphingomyelin ratio assays in predicting fetal lung maturity. Am J Obstet Gynecol 2005; 193:778.
24. **Melanson SE, Jarolim P, McElrath TF.** Fetal lung maturity testing in diabetic mothers. Lab Med 2007; 38:553.
25. **Moshiri M, Mannelli L, Richardson ML, et al.** Fetal lung maturity assessment with MRI fetal lung-to-liver signal-intensity ratio. AJR Am J Roentgenol 2013;201(6):1386–90. DOI: 10.2214/AJR.12.9679
26. **Mills M, Winter TC, Kennedy AM, et al.** Determination of fetal lung maturity using magnetic resonance imaging signal intensity measurements. Ultrasound Q. 2014;30:61–7.
27. **Oak P., Pritzke T., Thiel I., Koschlig M., Mous D. S., Windhorst A., Jain N., Eickelberg O., Foerster K., Schulze A. et al.** Attenuated PDGF signaling drives alveolar and microvascular defects in neonatal chronic lung disease. EMBO Mol.

Med.2014. 9, 1504-1520. 10.15252/emmm.201607308

28. **Nagwani M, Kumar PS, Singh U, Rani A, Malhotra S**. Two Dimensional Ultrasonographic Study of Placental Maturity and Its Correlation with Gestational Age and Maternal Parameters. Indian Journal of Clinical Anatomy and Physiology 2015;2(3):148-153
29. **Chen Q., Fang B., Wang Y., Li C., Li X., Wang R., Xiong Q., Zhang L., Jin Y., Zhang M. et al.** Overexpressing dominant-negative FGFR2-IIIb impedes lung branching morphogenesis in pigs. J. Genet. Genomics,2015. 45, 147-154. 10.1016/j.jgg.2018.02.002
30. **Schenone MH, Samson JE, Jenkins L, Suhag A, Mari G.** Predicting fetal lung maturity using the fetal pulmonary artery Doppler wave acceleration/ejection time ratio, Fetal Diagnosis and Therapy :2014; 36(3):208-214.
31. **Laban M, Mansour G, Elsafty M et al.** Prediction of neonatal respiratory distress syndrome in term pregnancies by assessment of fetal lung volume and pulmonary artery resistance index. Int J Gynaecol Obstet. 2015; 128: 246–50.
32. **Feitosa F.L.F., Braga G.I., Mendes L.C.N., Alcindo J.F., Souza N.C., Bovino F., Trein T.A., Trevizan J.T. & Baptista R.S**. Avaliação da maturidade pulmonar de cabritos nascidos a termo e prematuros. Arq. Bras. Med. Vet. Zootec.2020. 72(4):1313-1320.