# Rapid Prediction of Pulmonary Maturity by Amniotic Fluid Lipid Globule Formation

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**Utilizing the surface tension (ST) lowering properties of an amniotic fluid lipid extract, it was noted that the amniotic fluid lipid layer formed a subsurface globule when the ST reached 36.9 dynes/cm ± 0.1877 (SEM). The amount of required extract to achieve globule formation varied with the degree of fetal pulmonary maturity and the volume required could be used to differentiate pulmonary maturity (≤320 μl), transitional status (340 to 440 μl), and pulmonary immaturity (>460 μl), as related to fetal outcome (amniotic fluid obtained within 48 hours of delivery). Of the 70 patients studied, the 7 (100%) who developed respiratory distress syndrome (RDS) were predicted correctly. Nine of 70 (12.1%) gave false negative results (predicted pulmonary immaturity, no RDS developed); there were no false positives. Globule measurements of a separate series of 74 samples were compared with their L/S ratios. Twenty-two of 24 (91.7%) samples termed mature had an L/S > 2.0, while 32 of 42 with a value termed immature had an L/S ≤ 1.9. The physical events in the establishment of the monolayer and subsequent globule formation are discussed. This method now provides a rapid and reliable screening indicator of fetal pulmonary maturity.**

THE ABILITY to predict, antenatally, the risk of neonatal respiratory distress syndrome (RDS) is of extreme importance to infants in whom premature delivery is necessitated by maternal or fetal indications or to be avoided in cases of elective termination of pregnancy (induction or repeat cesarean section).

A major determinant of neonatal survival and the absence of RDS is the presence of adequate quantities of pulmonary surfactant. The role of surfactant for the maintenance of alveolar stability has been confirmed by numerous investigators and by studies of its biochemical, physical, morphologic, and clinical relations.[1-9] Gluck and his co-workers conducted an elaborate series of experiments[10-13] which culminated in the establishment of the lecithin/sphingomyelin (L/S) ratio of amniotic fluid[14] as the current standard to predict pulmonary maturity. Further clinical studies confirmed the test's reliability.[15-18] Clements et al[19] developed a rapid analysis of the foam stability of amniotic fluid as a measure of the surfactant content. The relative reliability of this procedure has also been confirmed.[20]

Enhorning[21,22] attempted to evaluate the surface tension of amniotic fluid, first in animals and then in humans, as it related to the stage of fetal lung development. Müller-Tyl et al[23] looked at surface tension of amniotic fluid in human pregnancy and noted a rise in surface activity during the last half of pregnancy.

We[24,25] have investigated the surface tension of an amniotic fluid lipid extract and found excellent correlation with the presence or absence of RDS. No false positive values have been obtained. Comparisons also have been made with the L/S ratio with excellent correlation. In our present working system, utilizing a DuNouy tensiometer, we determined pulmonary maturity by a surface tension (ST) of <56 dynes/cm after adding 120 ml of amniotic fluid lipid extract and <46 dynes/cm at 220 ml of extract. Values greater than these define immaturity. Standard curves were constructed of the surface tension values at increasing volumes of amniotic fluid lipid extract from babies with pulmonary maturity and immaturity.[25] Each is statistically significantly different from the other. (At 120 μl of extract mature mean, 43.8 dynes/cm ± SEM 0.98; immature mean, 62.8 dynes/cm ± SEM 0.59; and at 220 μl of extract mature mean, 37.4 dynes/cm ± SEM 0.46; immature mean, 54.5 dynes/cm ± SEM 0.61.) As a result of this research, it was found that when the surface tension values of both mature and immature amniotic fluids reached a plateau, a subsurface globule formed in the system (Figure 1). The volume of extract required to produce this globule seemed different depending on the degree of pulmonary development.

This present study was undertaken to confirm these observations and if true, perhaps to develop a rapid method of determining pulmonary maturity utilizing formation of the subsurface globule of an amniotic fluid lipid extract.

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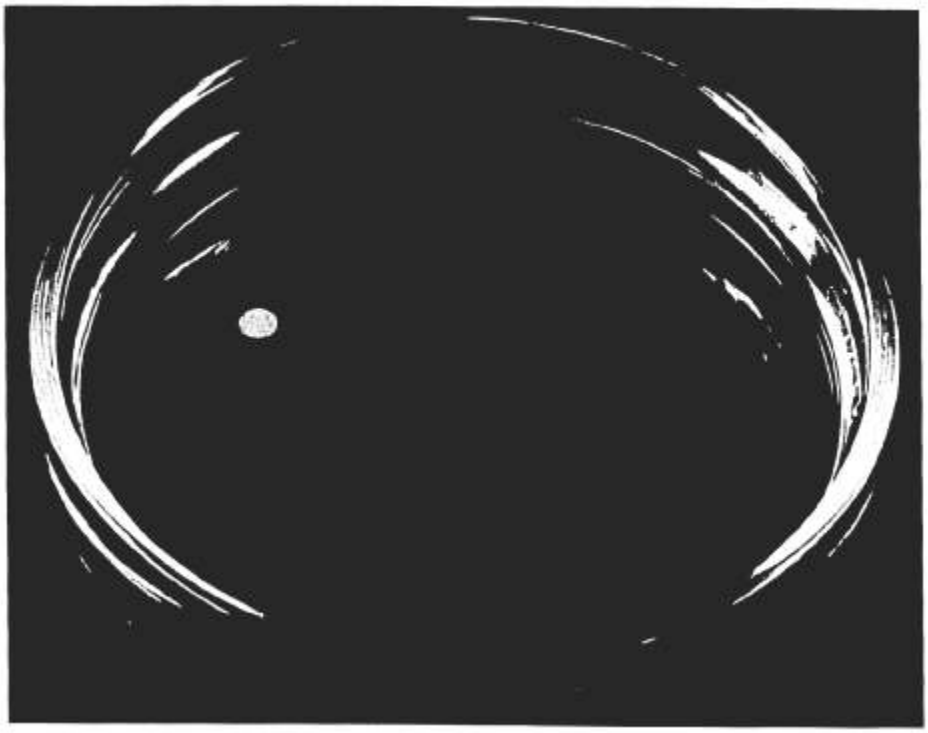

Fig 1. Subsurface globule of an amniotic fluid lipid extract. Photograph as it would appear in the petri dish during surface tension determination.

## MATERIALS AND METHODS

### *Patient Population*

Patients were selected from the obstetric service of the University of Nebraska Medical Center (Figure 2). No amniotic fluid samples were taken from women whose pregnancies were less than 28 weeks as determined by any or all of the following: last menstrual period, biparietal diameter, ultrasonography, fetal weight, and neonatal gestational age assessment.

The amniotic fluid was collected by 1) transabdominal amniocentesis, 2) transcervically at the time of amniotomy, and 3) via transcervical intrauterine pressure catheter. Amniocentesis was performed for accepted obstetric indications irrespective of the study. The conduct of labor, amniotomy, intrauterine monitoring, and mode of delivery were performed under accepted obstetric practice and sampling was incidental to the foregoing procedures.

### *Laboratory Procedure*

Amniotic fluid samples were divided into two aliquots: 1) fresh fluid for L/S ratio analysis utilizing a modified Gluck method;[14] 2) fresh fluid for surface tension and subsurface globule analysis.

The fresh raw amniotic fluid was centrifuged at 3000*g* for 15 minutes.* The supernatant was either analyzed immediately or stored at 4 C in clean glass screw cap vials. A 1–2 ml aliquot of the amniotic fluid was transferred to a 15-ml glass stoppered centrifuge tube. An equal volume of methanol† was added and the tube mixed on a vortex mixer for 1 minute. A volume of chloroform† equal to the total volume in the tube was added and mixed on a vortex mixer for 2 minutes. The emulsion thus formed was centrifuged at 1000*g* for 15 minutes.‡ This resulted in the appearance of three layers: upper aqueous, interfacial protein, and lower chloroform containing the lipids. A Pasteur pipet was introduced into the lower layer, and this layer was removed. Caution was required not to remove any of the other layers as these may affect the surface tension analysis.

The amniotic fluid lipid extracts were analyzed for surface tension utilizing a DuNouy Tensiometer§ equipped with a platinum ring of known circumference, 5.992 cm. The tensiometer was calibrated and checked before each sample was analyzed by measuring the surface tension of twice distilled water (72.0 dynes/cm).

* Sorvall, RC-2B
† Certified, ACS
‡ IEC, PR-2 Centrifuge
§ Cenco, Model #70535

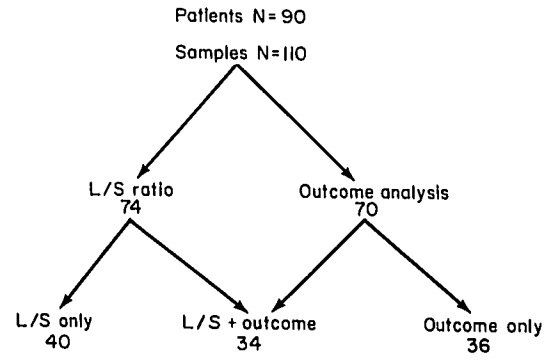


Fig 2. Patients and sampling distribution.

The cover of a standard 50 × 15 mm glass petri dish was filled with 15 ml of twice distilled water. Twenty-microliter aliquots of amniotic fluid extract were layered on the water surface with a glass micro pipet.* The droplets were allowed to disperse and a surface tension measurement was made and recorded. This was continued until the addition of more extract did not disperse on the surface, but rather the added extract remained as a globule on the surface and then sank below the water. The volume of extract and the surface tension were recorded. It was imperative that all glassware and distilled water were absolutely clean.

*Neonatal Evaluation*

Outcome analysis was utilized when the baby was delivered within 48 hours (usually 24 hours) after obtaining the amniotic fluid. The pediatricians evaluated the presence of respiratory distress syndrome (RDS) without knowledge of which babies were being studied. Only classic RDS Type I which leads to hyaline membrane disease was included as abnormal. RDS II, transient tachypnea of the newborn, because of its rapid resolution and absent sequelae was grouped with normal.

*Statistical Analysis*

Statistical analysis between groups was performed by Student *t* test, $\chi^2$, and compared with 97.5% confidence limits.

## RESULTS

The 70 samples of amniotic fluid from which a direct fetal outcome was predicted were utilized to establish the ranges of volumes at which the globule appeared. The surface tension values for these fluids were first determined and classified mature or immature by the previously mentioned criteria: mature—<56 dynes/cm at 120 μl extract added and <46 dynes/cm at 200 μl extract; immature—values greater than the mature. The Student *t* test (Table 1) demonstrated that the extract volume at which the globule appeared was different between the groups and good discrimination could be accomplished. It also reaffirmed our belief in the lack of difference between normal and RDS Type II. Using 97.5% confidence limits, ranges were then set as follows: mature—globule formation at ≤320 μl extract added; transitional—340-440 μl; and immature—≥460 μl.

TABLE 1. STUDENT *t* TEST ANALYSIS OF THE AMNIOTIC FLUID LIPID EXTRACT GLOBULE FORMATION AND NEONATAL OUTCOME (PRESENCE OR ABSENCE OF RDS)

| | | | *t* | *P* |
|---|---|---|---|---|
| Normal | vs | RDS II | 0.543 | Not significant |
| Normal | vs | RDS I | 12.69 | <0.001 |
| RDS I | vs | RDS II | 7.51 | <0.001 |
| RDS I | vs | RDS II & normal | 7.81 | <0.001 |

With these ranges established, the direct outcome analysis was performed (Table 2). All 7 (100%) of the patients who developed RDS I had globule formation at ≥460 μl extract. In only 9 of the 70 (12.1%) did the test predict immaturity but no RDS developed (false negative). There were no false positive results, and $\chi^2$ analysis yielded $P < 0.001$.

Similar analyses were performed on 74 samples comparing the globule formation and L/S ratio. The Student *t* test (Table 3) demonstrated significant differences between the groups of values.

The ranges of extract volumes required to produce globule formation were compared to the standard determinations of the L/S ratio (mature, ≥2.0; transitional, 1.0–1.9; and immature, <1.0). As shown in Table 4, 22 of 24 samples (91.7%) with a mature volume of extract at which the globule appeared had a mature L/S ratio. Significantly, in the other 2 samples established as mature, both had an L/S ratio <2.0, but neither infant developed RDS. Thirty-two of 42 (76.2%) samples with an extract volume defined as immature had an L/S ratio

TABLE 2. OUTCOME ANALYSIS: AMNIOTIC FLUID LIPID EXTRACT GLOBULE AND THE PRESENCE OR ABSENCE OF RESPIRATORY DISTRESS SYNDROME

| | *Mature <320μl* | *Transitional 340-440 μl* | *Immature >460μl* |
|---|---|---|---|
| RDS present | 0 | 0 | 7 |
| RDS absent | 46 | 8 | 9 |

$\chi^2$ Yields $P<0.001$

* Drummond Scientific Co.

TABLE 3. STUDENT *t* TEST ANALYSIS OF THE AMNIOTIC FLUID LIPID EXTRACT GLOBULE FORMATION AND THE LECITHIN/SPHINGOMYELIN (L/S) RATIO

| *L/S ratio* | | | *t* | *P* |
|---|---|---|---|---|
| <2.0 | vs | >2.0 | 9.54 | <0.001 |
| <2.0 | vs | >2.9 | 10.75 | <0.001 |
| <2.0 | vs | 2.0–2.9 | 7.84 | <0.001 |
| <1.0 | vs | 1.0–1.9 | 2.048 | <0.025 |

≤1.9. Chi square analysis of these relations yielded a $P < 0.001$.

Contamination of amniotic fluid with as little as 10 mg of meconium in 6 ml of pooled immature amniotic fluid gave falsely mature values to the volume required for globule formation. On visualization, this mixture has only a light green appearance. This parallels the results of meconium contamination on the surface tension of the amniotic fluid extract.[25] An immature value in the face of this contamination should, however, remain valid.

## DISCUSSION

Alveolar expansion and contraction are explained within the Law of LaPlace ($P = 2\gamma/r$: P = pressure, $\gamma$ = surface tension, r = radius). Thus, on expiration, the surface tension increases directly with the pressure and the alveoli would tend to collapse. This is the situation in the neonate with inadequate surfactant and Type I RDS.[5,26] The addition of surfactant, however, introduces a component which tends to decrease the surface tension as pressure increases, and, thus maintains alveolar stability and provides easier re-expansion on inspiration of the now only partially collapsed alveolus.

Surfactant acts with the establishment of a surface monolayer on the alveolar surface.[8] This basically creates an interface between the air–liquid phases whereby the polar (hydrophilic) end of surfactant is adsorbed to the liquid and the nonpolar (hydrophobic) end projects into the gaseous phase. Using lecithin, a major component of surfactant, as an example, there is a phosphatidyl choline polar end and two fatty acid chains as nonpolar ends off the basic glycerol molecule[27,28] (Figures 3 and 4). The fatty acid chains vary: in

TABLE 4. COMPARISON OF THE AMNIOTIC FLUID LIPID EXTRACT GLOBULE FORMATION AND THE LECITHIN/SPHINGOMYELIN (L/S) RATIO

| | *Mature <320 µl* | *Transitional 340–440 µl* | *Immature >460 µl* |
|---|---|---|---|
| L/S mature (>2.0) | 22 | 4 | 10 |
| L/S transitional (1.0–1.9) | 1 | 2 | 17 |
| L/S immature (<1.0) | 1 | 2 | 15 |

$\chi^2$ Yields $P<0.001$

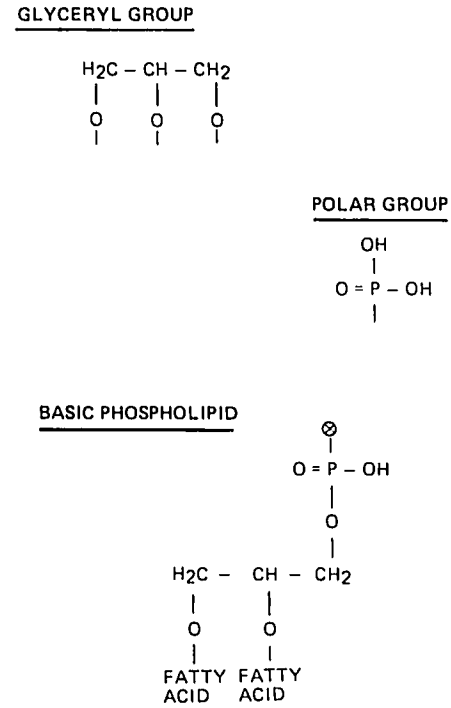


Fig 3. Basic components of the surface active phospholipids—surfactant (modified after Chapman[27]).

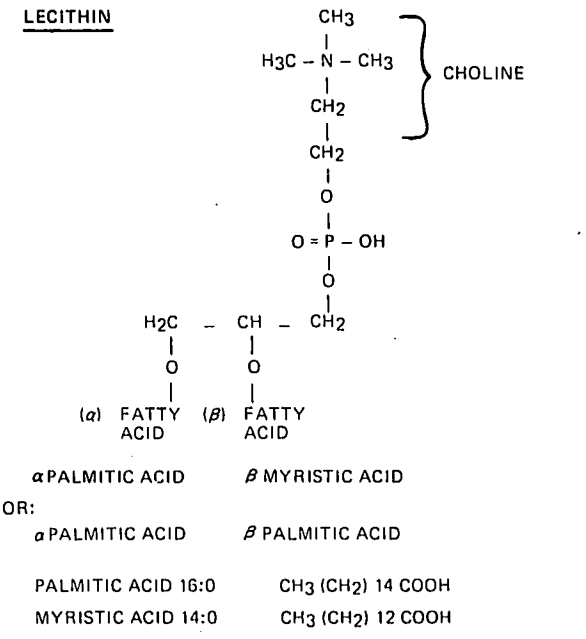


Fig 4. Structure of lecithin—a phospholipid. A major component of surfactant.

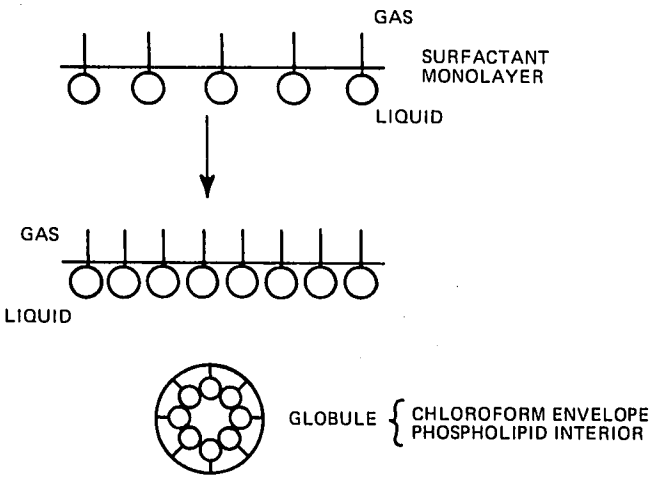


**Fig 5.** Diagram of the formation of the subsurface globule from an amniotic fluid lipid extract.

the mature baby as $\alpha$ palmitic/$\beta$ palmitic and while the premature will have, predominantly, less active and less stable $\alpha$ palmitic/$\beta$ myristic acid chains.[10-13]

Our system of measuring the surface tension lowering properties of surfactant is dependent on establishment of a monolayer of amniotic fluid lipid extract on the water surface. One sees the surface tension decrease as the monolayer is complete. However, the surface tension does not decrease indefinitely, but reaches a plateau in fluids from mature and immature babies at about the same surface tension, although the volume of extract required is obviously different. After plateau formation occurs, when the monolayer is packed, we suddenly see the appearance of the subsurface globule. This globule represents an outer layer of chloroform (a nonpolar solvent) with the surfactant within (Figure 5). The globule forms because addition of more phospholipid in chloroform onto a tightly packed surface favors the chloroform maintaining itself as a globule[29] vs the spreading of the phospholipid–chloroform solution on the surface. The chloroform, being more dense than water, and now in the formation of a globule, instead of spreading out over the surface, obeys the law of gravity and falls into the liquid phase.

The appearance of the subsurface globule can be related to the amount of surfactant in the amniotic fluid extract. Mature fluids have more surfactant in each volume of extract and thus pack the surface monolayer at lower volumes and allow globule formation to occur at lower volumes than with immature amniotic fluids. This provides the rationale for the efficacy of this type of determination.

The results presented confirm clinically, by direct outcome studies, that the volume of extract required to produce a globule can be accurately related to the degree of fetal pulmonary maturity. In no case did this test predict maturity in which the baby developed Type I RDS. Whatever error occurred was protective, in that the test predicted immaturity and no RDS developed. The results also compared favorably with the L/S ratio. This procedure, however, is to be construed as more of a screening test than the full surface tension analysis. The next phase of this work is to attempt to abbreviate the procedure by fewer additions of larger aliquots of extract so that an equally reliable but more rapid (less than 1 hour) determination may be accomplished.

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