

## Original articles

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## Fetal pulmonary maturity as determined by particle electrophoresis

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### 1 Introduction

Assessment of fetal maturity can be important for successful clinical management of high risk obstetric patients. Different in vitro methods are available for the evaluation of fetal lung maturity [1–25]. These tests specifically consider several phospholipids, fatty acids, or all lipid components of the amniotic fluid. Up to now the analysis of cell free fraction of the amniotic fluid by particle electrophoresis has been overlooked. We are able to describe the electrophoretic differentiation of particles from the amniotic fluid. These particles under consideration are defined mainly as liposomes.

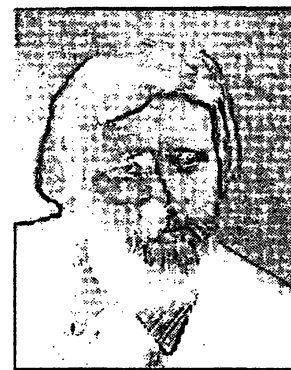
The objective of this study was to assess the results and clinical value of the electrophoretic determination as a possible index of fetal lung maturity by comparing the electrophoretic results to the total phospholipid contents as well as to the outcome of the newborn infants in 42 cases.

### 2 Material and methods

Analyses of 172 amniotic fluid samples from 132 patients are covered in this report. Amniotic fluid was mainly obtained by transabdominal amniocentesis between 26 and 42 weeks of gestation although in some cases the fluid was collected at the time of artificial rupture of the membranes. Amniotic fluid which was free of blood or

### Curriculum vitae

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meconium was obtained from normal patients (40 cases) as well as from patients with hypertension (20 cases), Rh immunization (29 cases), intrauterine fetal growth retardation (7 cases), anomalies of the duration of gestation (29 cases) and other diseases (7 cases). In ten of these cases, serial amniotic fluid samples were available for examination. Forty two patients were delivered within 48 hours of amniotic fluid sampling. RDS (respiratory distress syndrome) was diagnosed by the usual clinical and radiologic criteria. The modified method we used to determine the total phospholipid content P was based up on the method described by GUSDON [12]. The electrophoretic mobility of liposomes from the amniotic fluid samples was measured with automatic cell electrophoresis equipment Parmoquant (CARL ZEISS JENA, GDR). This technique allows a very exact and rapid determination of the electrophoretic mobility of single particles. The mean

electrophoretic mobilities of standard particles (e.g. human erythrocytes) are reproducible within  $\pm 1\%$  [20]. Fresh amniotic fluid was centrifuged at  $1000 \times g$  for 10 minutes and stored at  $-20^\circ\text{C}$ . Before measurement 0.5 ml of amniotic fluid was mixed with 2.5 ml of phosphate buffered saline (PBS) for 3 minutes. The sample was then ready for the analysis. Electrophoretic mobilities of liposomes were determined in PBS. Selection and measurement of particles were made in a subjective manner. From each sample 50 of the smallest particles ( $< 0.5 \mu\text{m}$ ) were measured. The measuring time was about 5 min/sample. Human erythrocytes with mean electrophoretic mobilities of  $1.08 \cdot 10^{-8} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$  served as standard particles. For freeze-fracture electron microscopy, the samples were centrifuged and mixed in the same manner as used for particle electrophoresis, and then the samples were incubated with 40% glycerol for 30 minutes. A small droplet on a specimen holder was frozen by immersion in liquified propan and cooled with liquid nitrogen. The specimen was fractured and shadowed in a Balzers B A 360M freeze-fracture device. Replicas, cleaned in sodium hypochloride and rinsed with distilled water, were examined in a Tesla BS 500 electron microscope. The direction of shadowing in the electron micrographs is from bottom to top.

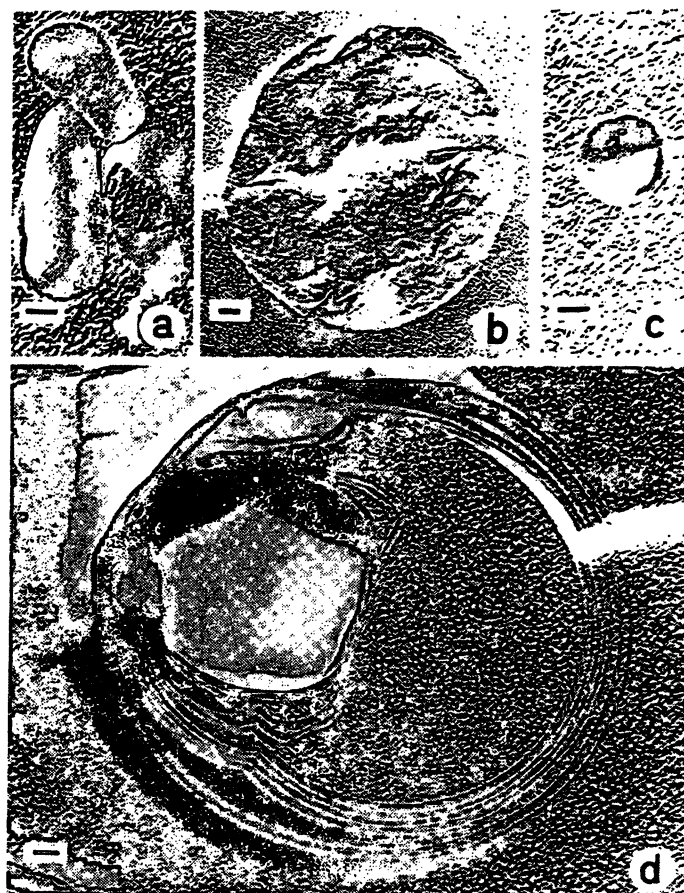


Fig. 1. Freeze-fracture demonstration of particles in the amniotic fluid. Unilamellar liposomes (a), lipid droplets (b), membrane vesicle with intramembranous particles on the fracture face (c) and multilamellar liposome (d); the bar represents  $0,1 \mu\text{m}$

### 3 Results

As seen with freeze-fracture electron microscopy, the great majority of particles in amniotic fluids are liposomes, unilamellar (Fig. 1a) or multilamellar (Fig. 1d), and characterized by smooth fracture faces. In addition lipid droplets (Fig. 1b) and occasionally small membrane vesicles with intramembranous particles on the fracture faces (Fig. 1c) are present.

Cytopherograms of the measured particles from the amniotic fluid samples showed at least two populations with mean electrophoretic mobilities (EPM) at  $0.90$  resp.  $1.30 \cdot 10^{-8} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$  (Fig. 2). In the instance of fetal lung maturity, an electrophoretically faster population is observed. On the other hand an immature lung is mainly characterized by the occurrence of an electrophoretically slower population. The quotient

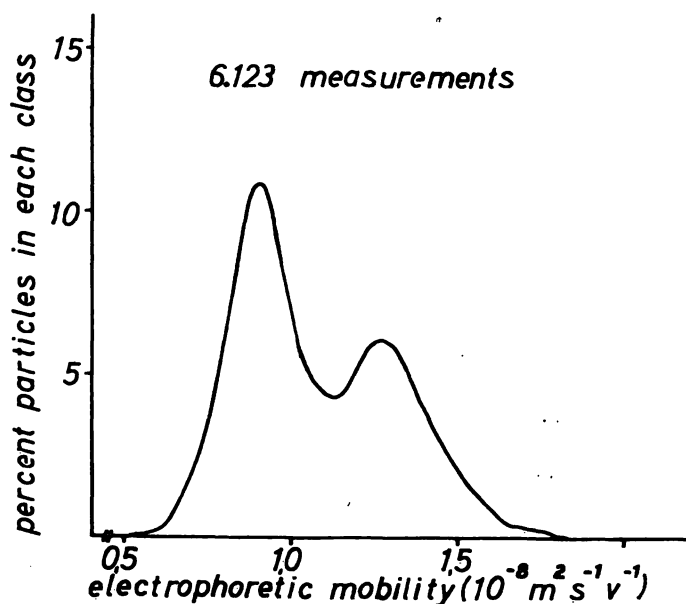


Fig. 2. Cytopherogram of 6.123 particles from amniotic fluids

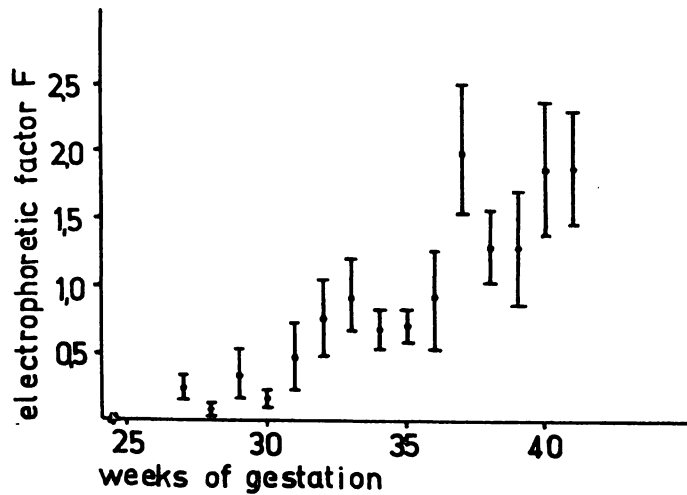


Fig. 3. Ratio F of the numbers of electrophoretically fast to slow particles from amniotic fluids during pregnancy (132 samples) with the standard error of the mean values

F of the number of electrophoretically faster and slower particles seems likely to be an index of fetal lung maturity. The ratio  $F < 1$  indicates fetal lung immaturity and the ratio  $F > 1$  fetal lung maturity. The limiting EPM to define particles as electrophoretically fast and slow ones is  $1.10 \cdot 10^{-8} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$  (referred to the mean EPM of human red blood cells at  $1.08 \cdot 10^{-8} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$ ). There is a correlation between F and P ( $r = 0.38$ ;  $p < 0.01$ , s.). 132 of 172 F values (77 per cent) correspond to P

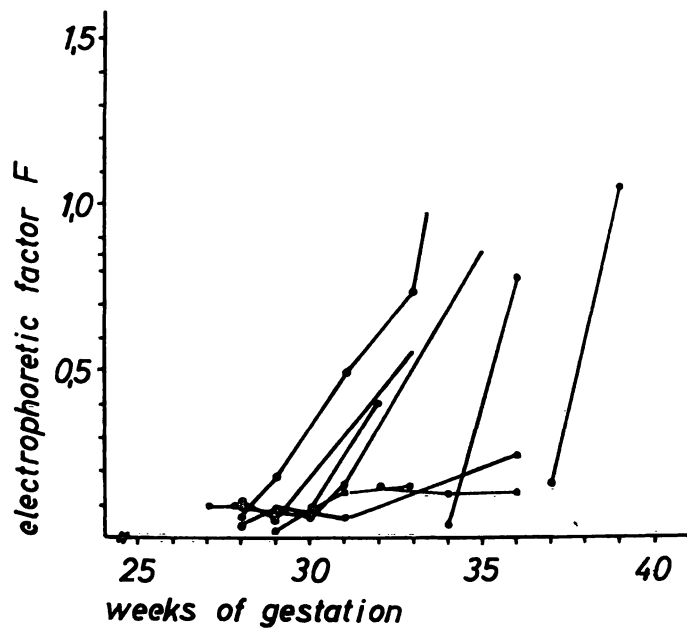


Fig. 4. Ratio F of the numbers of electrophoretically fast to slow particles from amniotic fluids in 10 cases of Rh sensitization with serial amniocentesis (in 3 cases Rh sensitization not confirmed)

values with regard to the prediction of fetal lung maturity resp. immaturity. P is also related to the mean EPM values ( $r = 0.37$ ;  $p < 0.01$ , s.). There is a adequate correlation of immature P values ( $< 65 \mu\text{Mol}$ ) to immature F values ( $F < 1$ ). Only 15 per cent of samples with immature F values show mature F values; however, 32 per cent of mature P values are followed by immature F values, especially at  $P > 160 \mu\text{Mol}$ . The electrophoretic results of 132 amniotic fluid samples between 26 and 42 weeks of gestation are shown in Fig. 3. In ten cases in which serial amniocentesis were done (Fig. 4), the F value increased as pregnancy progressed. The comparison between F and P with regard to a correct prediction of RDS in 42 patients is illustrated in Tabs. I and II.

Tab. I. Prediction of RDS by P and F value in 42 patients delivered within 48 hours of the amniotic fluid sampling.

	No. of cases	RDS cases	% correct prediction
P > 65 $\mu\text{Mol}$	32	4	88
P < 65 $\mu\text{Mol}$	10	4	40
F > 1	28	0	100
F < 1	14	8	57

Tab. II. Prediction of fetal lung maturity by means of parameters P and F

F \ P	P		$\Sigma$
	Correct	False	
Correct	29	3	36
False		8	8
$\Sigma$	32	10	42

There is a significant difference between F values of the mature resp. immature fetal lung. In 28 cases the F value was greater than 1.0 and no RDS was found. In 14 cases the F value was lower than 1.0 and in eight of them RDS was diagnosed. All RDS cases were followed by  $F < 1.0$  and in 82 per cent of cases with a mature lung the F value was  $> 1.0$ . The electrophoretic results in patients delivered within 24 hours of the amniotic fluid sampling correspond to those obtained 48 hours before delivery. Compared to F the prediction rate

of P seems to be lower, particularly in the immature zone. However, the assessment of prediction rates is difficult without further investigations. Just as GUSDON [12] we found by means of phospholipid determination in 102 cases a prediction rate of 96 per cent with regard to the mature fetal lung. In 21 cases, the P value was lower than 65  $\mu$ Mol and in 6 of them RDS was diagnosed.

At low ionic concentration the expected effects are evident. In saline solution at pH 7.2 the increase of the EPM of electrophoretically slow particles is 35 per cent at 3.6 mS and 50 per cent at 0.9 mS compared with isotonic samples. Similar values were found in the case of electrophoretically fast particles (25 resp. 40 per cent). On the other hand the populations are different as influenced by pH. Compared with measurements at pH 7.2 there is only a small change of the EPM of electrophoretically slow particles at pH 3.4 and pH 9.9, whereas the EPM of electrophoretically fast particles decreases at pH 3.4 (40 per cent). Using the described conditions, reproducible results were obtained.

#### 4 Discussion

Several in vitro tests are used in clinical practice in order to ascertain lung maturity before delivery in high risk pregnancies. The measurement most commonly used in clinical laboratory is the ratio of lecithin to sphingomyelin in the amniotic fluid [8, 9, 23, 25]. Other in vitro tests specifically consider several phospholipids [2, 5, 7, 13, 14, 15, 18] or fatty acids [1, 4, 24]. Alternative methods reflecting all lipid components of the amniotic fluid are surface tension measurements [3, 11, 21], spectrophotometry [10], fluorescent polarization [6, 22] and determination of the total phospholipid content [12, 16, 17]. On the other hand the analysis of the cell free fraction of the amniotic

#### Summary

Particle electrophoresis has been used to investigate particles obtained from amniotic fluid. These particles are defined mainly as unilamellar and multilamellar liposomes by electron microscopy. A total of 172 samples of amniotic fluids from 132 pregnant were included in this

fluid by particle electrophoresis has been overlooked. Our preliminary results demonstrate that particles from the amniotic fluid are differentiated by their electrophoretic mobilities. As revealed by freeze-fracture electron microscopy these particles are mostly unilamellar and multilamellar liposomes, and only some lipid droplets and a few small membrane vesicles are present. Formation of some liposomes from the lipid droplets during sample mixing cannot be excluded; therefore, this procedure must be routinely performed in the same manner each time. Without further investigation an exact chemical characterization of the measured particles is not possible. The occurrence of mixed liposomes cannot be excluded. The sources of the electrophoretically measured amniotic fluid particles are vague. SCHMIDT et al. [19] assume that liposomes from amniotic fluids are derived from the amnion. Further investigation will be necessary to clarify the origin of amniotic fluid particles which were differentiated by particle electrophoresis. The different behavior of electrophoretically slow and fast particles at low pH supports the assumption that several lipids are evaluated by cell electrophoretic measurements. The preliminary results show that the electrophoretic factor F seems to be related to the fetal lung maturity. Seventy-seven per cent of F values correspond to P values which are used for the prediction of fetal lung maturity resp. immaturity. Moreover, in 100 per cent of cases with  $F > 1.0$  no RDS was found, whereas 57 per cent of cases with  $F < 1.0$  correlated to clinical RDS.

A final assessment of the clinical value of electrophoretic measurements for the prediction of fetal lung maturity depends on further investigation of the relationship between F and the lung maturity of the newborn infant. Preliminary investigation suggests that the present results correlate to those obtained by objective cell electrophoretic measurements.

study. The electrophoretic results were compared to the total phospholipids and to neonatal outcomes in 42 cases.

At least two kinds of particles from amniotic fluids with different electrophoretic mobilities (EPM) are

evident. With a ratio of  $F > 1.0$ , the presence of electrophoretically faster particles was found to be associated with apparent fetal lung maturity. On the other hand when the ratio was  $F < 1.0$ , this seems to indicate an immature fetal lung. Seventy-seven per cent of  $F$  values correspond to the total phospholipid content  $P$  regarding the prediction of fetal pulmonary maturity resp. immaturity.

The correct prediction of fetal lung maturity was demonstrated in 42 patients delivered within 48 hours of amniotic fluid sampling. In 28 cases with a mature  $F$  value

( $> 1.0$ ) the correct prediction of respiratory distress syndrome (RDS) was 100 per cent. Eight out of 14 patients with immature  $F$  values ( $< 1.0$ ) developed RDS. The  $F$  values were found to increase as pregnancy progressed.

The electrophoretically differentiated amniotic fluid particles behave differently at low pH. Selection and electrophoretic measurement of particles were made in a subjective manner. This preliminary investigation suggests that the results correspond to those obtained by objective cell electrophoretic measurements.

**Keywords:** Amniotic fluids, fetal pulmonary maturity, liposomes, particle electrophoresis.

### Zusammenfassung

#### Fetale Lungenreifbestimmung mit Hilfe der Partikelelektrophorese

Mit Hilfe der Partikelelektrophorese wurden elektronenmikroskopisch hauptsächlich als Liposomen charakterisierte Teilchen aus dem Fruchtwasser untersucht. Die elektrophoretischen Untersuchungen umfassen 172 Fruchtwasserproben von 132 Patientinnen. Die Ergebnisse wurden mit dem Gesamtphospholipidgehalt und in 42 Fällen mit dem Zustand der Neugeborenen verglichen.

Es treten mindestens zwei Teilchenarten mit unterschiedlicher elektrophoretischer Mobilität (EPM) auf. Das Verhältnis  $F > 1,0$  von elektrophoretisch schnellen zu langsamen Teilchen scheint mit einer reifen fetalen Lunge in Beziehung zu stehen. Andererseits weist das Verhältnis  $F < 1,0$  auf eine unreife fetale Lunge hin. 77% der  $F$ -Werte stimmen mit dem Gesamtphospholipidgehalt  $P$  hinsichtlich der Voraussage der Lungenreife überein.

**Schlüsselwörter:** Fetale Lungenreife, Fruchtwasser, Liposomen, Partikelelektrophorese.

### Résumé

#### Maturité pulmonaire foetale déterminée par électrophorèse des particules

Les auteurs se sont servis de l'électrophorèse pour explorer les particules obtenues à partir du liquide amniotique. Ces particules sont représentées principalement par des liposomes uni ou multilamellaires en microscopie électronique. Au total, 172 échantillons de liquide amniotique provenant de 132 femmes enceintes ont été inclus dans cette étude. Les résultats de l'électrophorèse ont été comparés aux phospholipides totaux et dans 42 cas au devenir néonatal.

A partir du liquide amniotique au moins deux sortes de particules avec des mobilités électrophorétiques différentes sont évidentes. Un rapport  $F > 1$ , particules les plus rapides en électrophorèses sur particules les moins rapides, est associé à une apparente maturité foetale pulmonaire. A l'inverse, un rapport  $F < 1$  semble indiquer une immaturité pulmonaire foetale. 77% des valeurs de  $F$  correspondent aux phospholipides, totaux,

Die richtige Voraussage der fetalen Lungenreife wurde bei 42 Patientinnen, die innerhalb von 48 Stunden nach der Fruchtwasserentnahme entbunden wurden, überprüft. In 28 Fällen mit einem  $F$ -Wert  $> 1,0$  liegt die richtige Voraussage eines Respiratory Distress Syndroms (RDS) bei 100%. In 8 von 14 Fällen mit einem  $F$ -Wert  $< 1,0$  wurde ein RDS nachgewiesen. Die  $F$ -Werte steigen mit zunehmender Schwangerschaftsdauer an.

Die elektrophoretisch nachweisbaren Teilchenpopulationen aus dem Fruchtwasser werden bei niedrigem pH-Wert unterschiedlich beeinflusst.

Die Selektion und elektrophoretische Messung der Teilchen erfolgte in subjektiver Weise. Wie erste Untersuchungen jedoch zeigen, scheinen die subjektiv gewonnenen Ergebnisse mit den Resultaten objektiver elektrophoretischer Messungen übereinzustimmen.

correspondant à la prédiction de la maturité ou de l'immaturité pulmonaire foetale.

Chez 42 patientes ayant accouché dans les 48 heures après le recueil de liquide amniotique, on a pu établir une prévision correcte de la maturité pulmonaire foetale. Dans 28 cas avec une valeur de  $F$  mature ( $> 1$ ), le pourcentage de prédiction correcte du syndrome de détresse respiratoire (SDR) a été de 100%. 8 des 14 patientes avec des valeurs de  $F$  immatures ( $F < 1$ ) ont eu un enfant ayant présenté un SDR. Les valeurs de  $F$  augmentent au cours de l'évolution de la grossesse. Les particules du liquide amniotique avec des migrations différentes en électrophorèse, migrent de façon variable à pH bas.

La sélection et la détermination électrophorétique des particules ont été réalisées de manière subjective. Néanmoins, les investigations préliminaires suggèrent que les résultats actuels correspondent à ceux que l'on obtient à l'aide de déterminations électrophorétiques objectives.

**Mots-clés:** Electrophorèse des particules, liposomes, liquide amniotique, maturité pulmonaire foetale.

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