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## Optical density at 650 nm in amniotic fluid, L/S ratio and foam test as indicators of fetal lung maturity

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### 1 Introduction

One of the most important findings in perinatal medicine, has been the diagnosis of fetal lung maturity (FLM), using the lecithin/sphingomyelin ratio (L/S) [9].

This technique is being used since several years ago and is today accepted as the procedure that best relates amniotic fluid phospholipids with the respiratory outcome of the neonate [1, 3, 7, 8, 10, 12, 13, 18, 19]. This method is specially indicated in cases of high risk pregnancies where complications of gestation make it necessary to know the degree of FLM. For these reasons, the L/S ratio has gained widespread popularity and is being used in the majority of the modern perinatal centers.

However the determination of the L/S ratio must be done by specialized technicians and the results cannot be obtained immediately after amniocentesis.

On the other hand, the foam test [4], which allows to obtain an immediate result and also does not require specially trained personnel, is a very good screening test although its value is limited due to the high percentage of false negatives reported [2, 3, 19]. Also, the presence of subjective factors that are responsible for a lack of reproductivity of the results has been reported [14].

The measurement of the optical density (OD) at 650 nm of amniotic fluid, proposed by SBARRA et al. [15, 16, 17] and later by COPELAND et al. [5, 6], was done in order to avoid the disadvantages of the L/S ratio, with a simple and sensitive method for estimating the FLM, which is readily available in many centers.

Based on the fact that turbidity of the amniotic fluid increases with gestational age [11], the mentioned authors correlated the L/S ratio with the OD at 400 nm [15], and later at 650 nm [5, 6,

16, 17], concluding that the method is useful for predicting FLM.

The objective of this study is to compare the L/S ratio, the foam test and the OD at 650 nm in their ability to predict FLM.

**2 Material and methods**

Eighty amniotic fluid samples from 60 patients were obtained by transabdominal amniocentesis due to maternal and/or fetal indication according to the course of gestation and the condition of the mother and/or the fetus.

Samples contaminated by meconium and/or blood were excluded from our study. The gestational age range was between 26 and 44 weeks, estimated from the date of the last menstrual period. The samples were obtained from pregnancies with associated pathologies, mainly diabetes, sensitized Rh negative, etc.

The L/S ratio was measured according to GLUCK's technique, as previously reported [2].

The foam test was done with a modification of the original technique described by CLEMENTS [4], also mentioned in our previous publication [2].

For the obtention of the OD at 650 nm, we proceeded first to separate the cells and debris from the rest of the amniotic fluid by centrifugation at 2230 g for 30 minutes in a conic tube; 3 ml were taken from the supernatant, and the optical density was determined in a Bausch & Lomb, Spectronic 20 spectrophotometer.

The statistical analysis was performed by regression analysis and Chi square test.

**3 Results**

We selected a limit of 0.100 of OD as indicator of FLM which means that the values equal or higher than 0.100 were considered as mature, and those below 0.100, immature. Also we adopted the classic limit of 2.0 for the L/S ratio, proposed by GLUCK [9], as a criteria of FLM.

**3.1 Optical density at 650 nm and L/S ratio**

Fig. 1 shows the L/S ratio values plotted against the OD determination at 650 nm. Both measure-

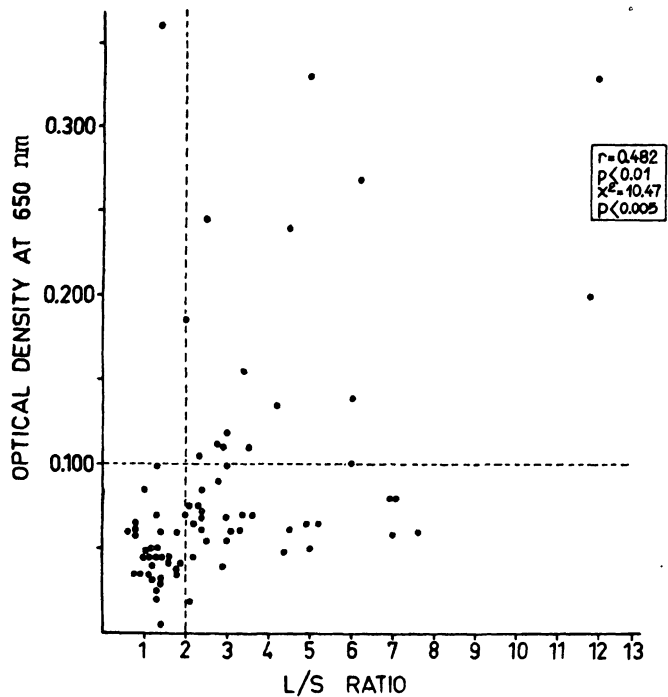


Fig. 1. The OD values have a tendency to increase when the L/S ratio becomes higher. In spite of this significant association, when the OD is lower than 0.100 there is no correlation between both indicators.

ments were made in the same amniotic fluid sample. The L/S ratio values have a tendency to increase together with the OD at 650 nm ( $r = 0.482, p < 0.01$ ).

In 17 samples where the value of OD was equal or greater than 0.100, 16 (94%) has a mature L/S ratio, and only one (6%) was immature. In 63 samples with an OD of less than 0.100, 32 (51%) had a mature L/S ratio, and 31 (49%) were immature (see Tab. I).

Tab. I. Comparison of OD at 650 nm with the L/S ratio ( $X^2 = 10.47, p < 0.005$ ).

L/S ratio	≥ 2.0	< 2.0	Total
OD ≥ 0.100	16	1	17
OD < 0.100	32	31	63
Total	48	32	80

The statistical analysis performed showed a significant association between the L/S ratio values higher than 2 and the OD measurement greater than 0.100 ( $X^2 = 10.47, p < 0.005$ ).

### 3.2 Optical density at 650 nm and the foam test

The foam test was performed in 64 samples, in which the OD had been previously determined. An OD equal or greater than 0.100 was present in 15 samples, in which the distribution according to the foam test result was: Negative 3 (20%), intermediate 5 (33%) and positive 7 (47%).

An OD less than 0.100 was present in 49 samples, showing the following distribution: Negative 40 (82%), intermediate 4 (8%) and positive 5 (10%) (see Fig. 2 and Tab. II).

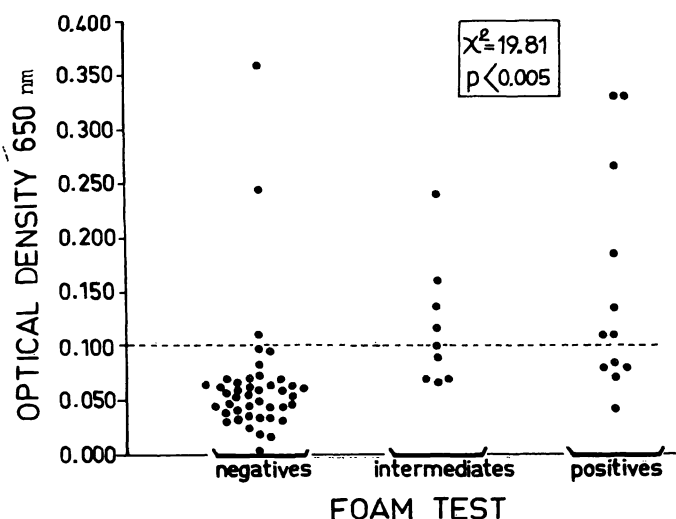


Fig. 2. A statistically significant association is shown between a positive foam test and OD values higher than 0.100. The reverse situation is found with a negative foam test (significant association between negative result and OD values lower than 0.100).

Tab. II. Comparison of OD at 650 nm with the foam test ( $X^2 = 19.81$ ,  $p < 0.005$ )

Foam test	Negatives	Intermediates	Positives	Total
OD $\geq$ 0.100	3	5	7	15
OD < 0.100	40	4	5	49
Total	43	9	12	64

The statistical analysis performed shows a significant association between a positive foam test and higher values of OD ( $X^2 = 19.81$ ,  $p < 0.005$ ).

### 4 Discussion

When the OD was 0.100 or greater, the L/S ratio was always higher than 2.0 except in one case, which represents approximately 6% ("false positives").

When the sample had an OD of less than 0.100, a wrong diagnosis of "immaturity" would be made in 50% of the cases. This percentage of "false negatives" is very important, and is higher than the 30% of "false negatives" previously reported by us for the foam test in relation to the L/S ratio [2]. The OD percentages of "false negatives" and "false positives" are similar to those obtained in the study of COPELAND et al. [5] (59.09 and 4.76% respectively), using the same OD limit that we used. However, our percentages and COPELAND's results are very different from those published by SBARRA et al. [17], who obtained a 1.5% of "false positives" and 8% of "false negatives". This difference cannot be explained by the different limit of OD used (0.150). It is of interest to note that in COPELAND's last publication [6] when the author changed the OD limit to 0.150, he obtained less false negatives (34%) and the same percentage of false positives (5%). This finding cannot be explained by the change in OD limit. When we compare the determination of OD at 650 nm with the foam test, we may conclude that a sample of 0.100 OD, or greater, will be mature by the foam test in 50% of the cases, intermediate in 30% and immature in 20% (approximate values). The last group are the "false positives" in relation to the foam test.

If we consider a sample with a value below 0.100 OD, it will be immature by the foam test in 82% of the cases, intermediate in 8% and mature in 10%. The last value is considered as the "false negatives".

The discussed method is simple, rapid and easily performed. The laboratory equipment required is the same as that which is used for the reading at 450 nm for the determination of bilirubin in amniotic fluid. Therefore, any laboratory that performs this last technique, can determine the OD at 650 nm.

In spite of these advantages, the OD method does not replace the L/S ratio. This is due to the presence of false positives of the method, and mainly

to its very high percentage of false negatives (50%).

Also, it cannot replace the shake test, mostly due to the fact that the OD method presents a higher percentage of false negatives.

Whenever the shake test cannot be performed, we recommend the OD as a screening test, keeping in

mind the small, but real percentage of false positives involved.

Further studies should be made by correlating the OD at 650 nm with the neonatal outcome, before an extensive use of this method may be recommended.

### Summary

In order to develop a sensitive, simple and rapid technique for assessing the FLM, we have compared OD at 650 nm in amniotic fluid as previously reported, against L/S ratio, and the foam test, two methods which have been repeatedly studied for their ability to predict FLM.

Eighty samples of amniotic fluid (obtained by transabdominal amniocentesis) were studied. These samples were obtained from 60 pregnancies with associated pathologies, mainly Rh negative sensitization and diabetes.

In all these samples L/S ratio was determined according to GLUCK's method, as well as OD at 650 nm. In 64 of these samples we also performed the foam test with a modification of the original technique described by CLEMENTS.

An OD limit of 0.100 was considered as immature, as described by COPELAND. Also, we adopted the classic limit of 2.0 for the L/S ratio as criteria of FLM.

The L/S ratio values showed a tendency to increase together with the OD although the correlation was poor (Fig. 1).

The measurement of OD showed 6% of false positives in relation to the L/S ratio and 50% of false negatives (Tab. I). These results are similar to those obtained by

COPELAND, using the same OD limit of 0.100, but very different from those reported by SBARRA. This difference cannot be explained by the different OD limit of 0.150 that was used.

In 64 samples the foam test and the OD reading were performed. The OD showed 20% false positives in relation to the foam test and 10% of false negatives (Fig. 2, Tab. II).

This method is quick, simple and easily performed by using the same laboratory equipment necessary for the reading of OD at 450 nm for bilirubin determination in amniotic fluid.

In spite of these advantages it does not replace the L/S ratio due to its higher percentage of false positives and false negatives. Also it cannot replace the foam test due to its higher percentage of false negatives.

In case that the foam test cannot be done, we recommend the OD determination at 650 nm as a screening test, keeping in mind the small but real percentage of false positives involved.

Further studies should be made by correlating the OD at 650 nm with the neonatal outcome, before an extensive use of this method may be recommended.

**Keywords:** Amniotic fluid, fetal lung maturity, L/S ratio, "shake test", phospholipids, optical density.

### Zusammenfassung

#### Extinktionsmessung bei 650 nm, L/S-Quotient und Schaumtest als Indikatoren der fetalen Lungenreife

Die Messung der optischen Dichte des Fruchtwassers scheint eine empfindliche, einfache und schnelle Methode zur Feststellung der fetalen Lungenreife zu sein. In dieser Untersuchung wurde ein Vergleich zwischen OD<sub>650nm</sub>, L/S-Ratio und Schaumtest angestellt. Die Eignung von L/S-Ratio-Bestimmungen und Schaumtest zur Feststellung der fetalen Lungenreife ist wiederholt untersucht worden.

Das untersuchte Kollektiv bestand aus 60 pathologischen Schwangerschaften, wobei in der Hauptsache Rh-Sensibilisierung bzw. Diabetes die Komplikationen darstellten. Insgesamt wurden 80 Fruchtwasserproben mittels der transabdominale Amniozentese gewonnen.

Bei allen Proben wurde sowohl die L/S-Ratio nach GLUCK bestimmt wie auch die OD<sub>650nm</sub> gemessen. An 64 Proben wurde zusätzlich der Schaumtest nach CLEMENTS in einer modifizierten Form durchgeführt.

Hinsichtlich der OD sahen wir nach COPELAND 0.100 als Limit für eine unreife Lunge an. Bezogen auf die L/S-Ratio übernahmen wir als Grenzwert 2.0, oberhalb dessen mit einer reifen Lunge zu rechnen ist.

Die L/S-Ratio verhielt sich in der Tendenz so wie die OD; die statistisch berechnete Korrelation war jedoch gering (Fig. 1). In Relation zur L/S-Ratio ergab die OD-Messung zu 6% falsch positive und zu 50% falsch negative Ergebnisse (Tab. I). COPELAND erhielt vergleichbare Zahlen, wobei derselbe Grenzwert von 0.100 zugrunde lag, während SBARRA ganz andere Ergebnisse liefert. SBARRA benutzte zwar 0.150 als Limit, was aber den Unterschied allein nicht erklären kann.

Der Vergleich OD-Messung mit Schaumtest bei 64 Proben ergab, daß die Messung der OD in Relation zum Schaumtest zu 20% falsch positive und zu 10% falsch negative Werte liefert (Fig. 2, Tab. II).

Die OD-Bestimmung ist schnell und einfach durchzuführen, wobei die gleiche Laborausstattung, wie sie zur Bili-

rubinmessung im Fruchtwasser (OD<sub>450nm</sub>) gebraucht wird, ausreicht.

Trotz dieser Vorteile kann die Methode wegen ihres hohen Prozentsatzes an falsch positiven und falsch negativen Ergebnissen die L/S-Ratio nicht ersetzen. Auch gegenüber dem Schaumtest fällt die OD-Messung wegen des höheren Prozentsatzes an falsch negativen Ergebnissen ab.

In den Fällen, wo der Schaumtest nicht durchgeführt wer-

den kann, können wir die OD-Messung als Screening empfehlen, wobei man an die möglichen falsch positiven Ergebnisse denken muß.

Bevor die OD-Messung in einem größeren Umfang eingesetzt und empfohlen werden kann, müssen in jedem Fall weitere Untersuchungen zur Korrelation von OD<sub>650nm</sub>-Werten im Fruchtwasser und tatsächlicher neonataler Lungenreife stattfinden.

**Schlüsselwörter:** Fetale Lungenreife, Fruchtwasser, L/S-Ratio, optische Dichte, Phospholipide, Schaumtest.

## Résumé

**Densité optique à 650 nm du liquide amniotique, rapport L/s et test foam en tant qu'indicateurs de la maturité pulmonaire foetale (MPF).**

Dans le but de développer une technique sensible, simple et rapide de détermination de la MPF, nous avons comparé la densité optique (DO) à 650 nm dans le liquide amniotique comme précédemment rapporté, au rapport L/S, et le test foam, deux méthodes qui ont souvent été étudiées quant à leur capacité de prédire la MPF.

Quatre-vingt échantillons de liquide amniotique (obtenus par amniocentèse transpariétale) ont été étudiés. Ces échantillons ont été obtenus chez 60 grossesses à pathologies associées, principalement des sensibilisations Rhésus et des diabètes.

Dans tous ces échantillons le rapport L/S a été déterminé selon le procédé de GLUCK, de même que la DO à 650 nm. Dans 64 de ces échantillons il a été également pratiqué le test foam avec une modification de la technique originale décrite par CLEMENTS.

La limite de DO de 0.100 été considérée comme immature, comme décrit par COPELAND. De même nous adoptâmes la classique limite de 2 pour le rapport L/S en tant que critère de MPF.

Les valeurs du rapport L/S montraient une tendance en augmentation ensemble avec la DO quoique la corrélation était faible (fig. 1).

La détermination de la DO montrait 6% de faux positifs en relation avec le rapport L/S et 50% de faux positifs (tab. I.). Ces résultats rappellent ceux de COPELAND qui utilisait la même limite de DO à 0,100, mais sont très différents de ceux de SBARRA. Cette différence ne peut être expliquée par la limite de DO différente de 0,150 qui a été employée.

Sur 64 échantillons le test foam et la lecture de la DO ont été réalisées. La DO montrait 20% de faux positifs par rapport au test foam et 10% de faux négatifs (fig. 2., tab. II).

C'est une méthode rapide, simple et de réalisation facile en utilisant le même équipement de laboratoire nécessaire pour la lecture de la DO à 450 nm pour la détermination de la bilirubinaemie. Malgré ces avantages il ne peut remplacer le rapport L/S du fait de son haut pourcentage de faux positifs et négatifs. De même, ne peut-il remplacer le test foam du fait de son haut pourcentage de faux négatifs.

Dans les cas où le test foam ne peut être pratiqué, nous recommandons la détermination de la DO à 650 nm en tant que test de dépistage en tenant compte du nombre petit mais réel des faux positifs.

D'autres investigations doivent suivre pour rechercher la corrélation entre la DO à 650 nm et l'état foetal à la naissance, avant de pouvoir conseiller l'utilisation extensive de cette méthode.

**Mots-clés:** Densité optique, liquide amniotique, maturité pulmonaire foetale, phospholipides, "shake test".

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