

L/S Ratio and Cortisol in Amniotic Fluid According to Gestational Age

RAÚL BUSTOS AND GUSTAVO GIUSI⁽²⁰⁾

Latin American Center of Perinatology and Human Development, Pan American Health Organization, World Health Organization, Montevideo, Uruguay

Summary

It is well known that corticoids act to induce fetal lung maturation when administered to the fetus or mother. However, their physiologic role has been recently questioned with regard to stimulation of surfactant production.

We investigated the process of fetal lung maturation to determine whether or not it is associated with changes in amniotic fluid cortisol.

Sixty-two amniotic fluid samples from 53 patients were obtained by transabdominal amniocentesis strictly due to maternal and/or fetal clinical indication, according to the course of pregnancy and the maternal and/or fetal status.

The L/S ratio was measured on thin-layer plates by reflectance densitometry and calculated as previously described by Gluck *et al.*

Cortisol levels in amniotic fluid were measured by radioimmunoassay using commercial reagent kits (Gamma coat I¹²⁵ cortisol; Clinical Assays, Inc.).

The level of amniotic fluid cortisol increased with gestational age from wk 30 to 41; a sharp increment was observed at 39 to 41 wk.

No significant differences were found when the mean values of cortisol were compared at wk 30 to 32, 33 to 35, and 36 to 38. When the fourth group was included (39 to 41 wk), a statistically significant difference was found ($F = 4.63$; $P < 0.01$).

There was a progressive increase in L/S ratio during gestation as has already been described, plus a value greater than 2 was found during the 33 to 35 wk period. The statistical analysis performed on L/S ratios at weekly intervals 30 to 32, 33 to 35, and 36 to 38 showed a significant difference in the mean values in the groups studied ($F = 6.84$; $P < 0.005$).

Therefore, the increment of L/S ratio over 2 was already observed during the 33 to 35 wk period of gestation, whereas the mean cortisol amniotic fluid values remained unchanged between the 30th and the 38th wk. The cortisol peak was observed only during the 39 to 41 wk period.

Speculation

It has been demonstrated that glucocorticoids have an important role in accelerating the process of the development of fetal lung maturation. The physiologic fetal lung surfactant production does not necessarily depend on the increase of fetal cortisol levels. It is speculated that cortisol has a permissive role which allows the action of other hormones.

It has been suggested, through clinical observations (10) and experimental studies (12-17) that glucocorticoids have an important role in the normal process of biochemical development of the fetal lung.

A rise in the amniotic fluid cortisol level with increasing gestational age has been shown (4, 5, 15). It is also well known that the lecithin/sphingomyelin (L/S) ratio in amniotic fluid increases

with gestational age and that its determination is one of the best tests for evaluating fetal lung maturation (7).

It has been shown that the increased L/S ratio that occurs with increased gestational age is accompanied by greater cortisol values in amniotic fluid (4).

Furthermore, maternal administration of glucocorticoids produces an increase in the L/S ratio in amniotic fluid, as well as a reduction in the incidence of respiratory distress syndrome (RDS) in the preterm newborn (1).

Although it is well known that corticoids act to induce fetal lung maturation when given to the fetus or mother, their physiologic role has recently been questioned with regard to stimulation of surfactant production. Animal experiments have shown that the increase in surfactant production is not preceded by an increase in cortisol levels, even though there is an increment of other hormones (11).

Therefore, we investigated the process of fetal lung maturation to determine whether or not it is associated with changes in amniotic fluid cortisol.

MATERIALS AND METHODS

Sixty-two amniotic fluid samples from 53 patients were obtained by transabdominal amniocentesis strictly due to maternal and/or fetal clinical indication, according to the course of pregnancy and the maternal and/or fetal status.

All patients had complicated pregnancies due mainly to diabetes and to Rh-negative sensitization (Table 1).

Samples taken from patients with polyhydramnios and contaminated by meconium or blood were excluded from our study.

Amniotic fluid samples were 10 ml or more. Phospholipids and cortisol were measured after centrifugation at 3500 rpm for 10 min.

Extraction of phospholipids from amniotic fluid supernatant was performed using the procedure described by Gluck *et al.* (6).

After extraction, acetone precipitation was done, and both fractions (acetone-precipitated and acetone-soluble) were plated and run on heated, activated thin layers of silica gel H containing 5% ammonium sulfate.

The acetone-precipitated fraction was developed on one-dimensional thin-layer chromatography using chloroform-methanol-acetic acid-H₂O (390-150-48-24 v/v).

After development of the chromatograms the plates were charred at 280°C for 10 min to visualize the compounds. The L/S ratio was measured on the thin-layer plates by reflectance densitometry and calculated as previously described by Gluck *et al.* (7).

Cortisol levels in amniotic fluid were measured by radioimmunoassay (4) using commercial reagents kits (Gamma coat I¹²⁵ cortisol; Clinical Assays, Inc.).

RESULTS

The relationship among amniotic fluid cortisol levels, L/S ratio, and gestational age is shown in Figure 1.

The level of amniotic fluid cortisol increased with gestational age from wk 30 to 41; a sharp increment is observed at 39 to 41 wk.

Samples were divided into four groups according to gestational age for statistical purposes. Mean values, standard deviations, and standard errors were calculated for each group.

No significant differences were found when the mean values of cortisol were compared at wk 30 to 32, 33 to 35, and 36 to 38 (Fig. 2). When the fourth group was included (39 to 41 wk), a statistically significant difference was found ($F = 4.63$; $P < 0.01$) (Fig. 1).

A similar tendency was observed when the L/S ratio was analyzed in relation to gestational age (Fig. 1).

There was a progressive increase during gestation as has already been described. The L/S ratio was already found to be greater than 2 during the 33 to 35 wk period. A statistical analysis of the L/S ratio at weekly intervals 30 to 32, 33 to 35, and 36 to 38 showed a significant difference in the mean values in the groups studied. ($F = 6.84$; $P < 0.005$) (Fig. 3).

Therefore, a L/S ratio greater than 2 had already been observed during the 33 to 35 wk period of gestation, whereas the mean

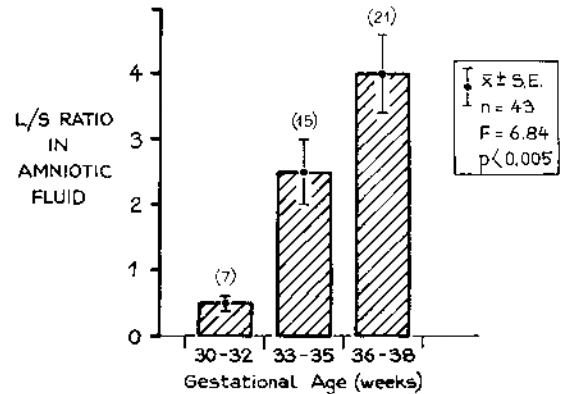


Fig. 3. The statistical analysis showed a significant difference between L/S ratio mean values of the studied groups.

cortisol amniotic fluid values remained unchanged between wk 30 and 38. The cortisol peak was observed only during the 39 to 41 wk period.

Table 1. Distribution of associated pathologies

Chronic hypertension	7
Diabetes B and C	6
Diabetes D, F, R	6
Rh sensitization	10
Rh sensitization + hypertension	1
Rh sensitization + diabetes	2
Other pathologies	21
Total	53

DISCUSSION

Liggins (9) proposed that glucocorticoids have an important role in the maturation of the sheep fetal lung.

Corticosteroids have been used in human patients, in cases of threatened premature labor, to hasten the maturing process of the lung of the fetus (10).

Cortisol levels in umbilical cord blood of newborns in whom RDS subsequently developed were found lower than that in newborns of comparable gestational age without the syndrome (14).

It has been reported that the activity of cholinephosphotransferase in the fetal lung increased during normal gestation in the rat (2) and mouse (16). In the fetal rabbit, this activity was stimulated by the administration of glucocorticosteroids (3), although this has not been observed by other authors (18, 19).

However, there is no direct evidence that fetal cortisol is the primary stimulus of augmented surfactant synthesis in the human fetus. Indeed, no relation was found between the level of cord plasma cortisol and the development of RDS (8).

The results of Mescher *et al.* (11) showed that the rise in fetal plasma cortisol production during gestation occurs after the rise in surfactant in tracheal effluent in fetal sheep.

It has been reported that a sharp increase in total amniotic fluid cortisol occurs after the 34th wk of gestation (4). However, our results show that this rise occurs at wk 38 after an increase in the L/S ratio has been produced.

The difference between the results of Fencel and Tulchinsky (4) and ours could possibly be explained by the wide interval of gestational age used by these authors (20 to 34 and 35 to 40 wk), as compared with the short period used in our study (2-wk intervals).

The increase in umbilical artery blood cortisol level around the 36th wk of gestation reported by Murphy (13) may be interpreted as preceding the rise in amniotic fluid cortisol.

Because there are conflicting reports about the effects of corticosteroids on the process of fetal lung maturation, their role has not yet been clearly established. Cortisol may have a permissive role in fetal lung maturation which allows the action of other hormones.

The results of our present study lead us to conclude that the elevation of L/S ratio observed between the 30th and 38th wk of gestation was not accompanied by a previous or simultaneous increase in amniotic fluid cortisol level.

These results support findings made in animal experiments (11) and suggest that fetal lung surfactant production does not necessarily depend on the increase of fetal cortisol levels.

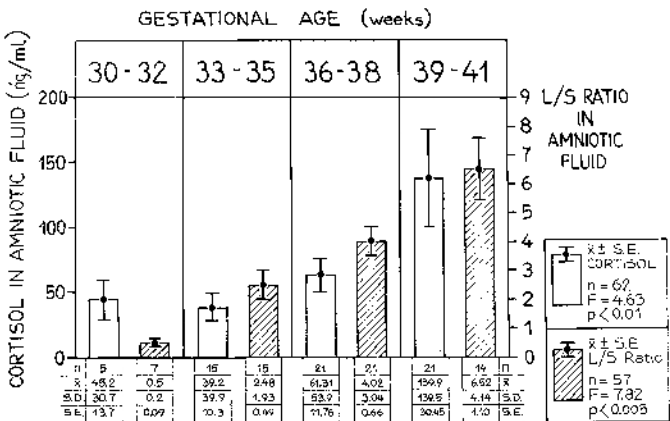


Fig. 1. The level of amniotic fluid L/S ratio increases along gestation. The mean value of amniotic fluid cortisol remains unchanged until the 36 to 38 wk period, showing a sharp increment at the end of pregnancy.

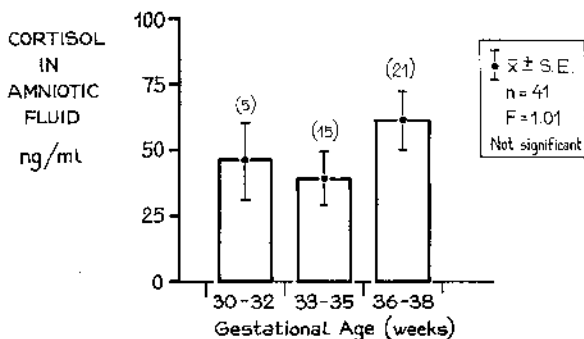


Fig. 2. No significant differences were observed in the mean values of cortisol between the different periods compared.

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20. Requests for reprints should be addressed to: Dr. Raúl Bustos, Centro Latinoamericano de Perinatología y Desarraollo Humano, Casilla de Correo 627, Montevideo, Uruguay.
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