Amniotic fluid prolactin and fetal lung maturation

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Concentrations of prolactin in amniotic fluid, fetal plasma, and maternal plasma were determined in 34 rhesus monkeys delivered by hysterotomy under general anesthesia at gestational ages of 110 to 160 days (term, 165 days). Included were 15 cases (gestational ages 110 to 143 days) in which the mothers received 2 mg of betamethasone intramuscularly daily for 3 days prior to delivery. Fetal lung maximum volumes were determined in addition to the following indices of fetal lung surfactant: lung alveolar stability, lung phosphatidylcholine concentrations, lung extract surface tensions, and amniotic fluid lecithin to sphingomyelin ratios. Amniotic fluid prolactin was found to correlate significantly with lung alveolar stability ($r = 0.51$; $p < 0.01$), lung phosphatidylcholine ($r = 0.51$; $p < 0.01$), lung extract surface tension ($r = -0.39$, $p < 0.05$) and amniotic fluid lecithin/sphingomyelin ratio ($r = 0.50$; $p < 0.01$). These correlations remained statistically significant even when the effects of gestational age were taken into account. These findings suggest that amniotic fluid may modulate fetal production of surfactant via its prolactin content. (Am J Obstet Gynecol 1985;153:372-80.)

Key words: Rhesus monkey, prolactin, fetal lung development, surfactant, alveolar stability, betamethasone

Maturation of the fetal lung is thought to be influenced by a number of hormones. Glucocorticoids, prolactin, thyroxin, and insulin[1-3] have all been implicated in the processes of fetal lung growth and development. An important event in lung maturation is the onset of the production of alveolar surfactant by type II pneumocytes at about 24 to 26 weeks of human gestation. The neonatal respiratory distress syndrome (RDS) is thought to be due to a relative deficiency in alveolar surfactant.[1] The exact stimuli for the initiation and continuation of the production of surfactant are unknown, but one stimulus might be prolactin.[2] The metabolic similarities between maturing fetal lung and lactating mammary tissue have been emphasized by Hauth et al.[4] Both tissues have a high rate of fatty acid synthesis, with an almost unique ability to incorporate palmitic acid into the *sn*-2 position of phospholipids. Several groups have observed that the addition of prolactin to fetal lung cell cultures is associated with an increased production of saturated lecithin.[3, 5]

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Hamosh and Hamosh[2] reported that treatment of fetal rabbits with intramuscular prolactin increased lung content of lecithin, but their findings were not confirmed by others.[6] Several clinical studies have shown that low levels of fetal plasma prolactin were associated with a high incidence of RDS, whereas other studies have not.[1, 6, 7]

These conflicting findings might be due to failure to consider another source of prolactin, the amniotic fluid. The decidua is thought to produce large quantities of amniotic fluid prolactin.[8] In the human and in the rhesus monkey, concentrations of amniotic fluid prolactin tend to be tenfold to 100-fold those of maternal or fetal plasma.[9] Peak concentrations of amniotic fluid prolactin occur around 24 weeks of human gestation,[6, 8] at approximately the same time that the production of lung surfactant is thought to begin. Fetal breathing movements could facilitate the movement of amniotic fluid prolactin to alveolar cells. A significant tidal flow of amniotic fluid into the fetal lung occurs during the last two thirds of pregnancy, secondary to fetal breathing activity.[10]

Glucocorticoids are thought to accelerate lung maturation, and the possibilities of interactions with prolactin merit consideration.[5] Glucocorticoids are reported to increase the number of prolactin receptors within mammary cells[11] and might have the same effect upon prolactin receptors in fetal lung cells. Such an effect could result in an earlier or increased influence of prolactin on surfactant.

The purpose of the present study was to determine

Table I. Comparison of findings in animals in groups 1 and 2

	*Group 1: Betamethasone (15)**	*Group 2: Saline solution (14)**
Gestational age (days)	128.9 ± 2.3†	129.3 ± 2.4
Fetal body weight (gm)	277 ± 13.7	296 ± 18.2
Placental weight (gm)	68 ± 2.3	81 ± 4.5‡
Adrenal weight (mg) / Body weight (gm)	0.53 ± 0.04(14)	0.81 ± 0.03§
Lung weight (gm)	5.8 ± 0.36	5.7 ± 0.37
Maximum lung volume (ml at 40 cm transpulmonary pressure)	8.06 ± 0.6	5.69 ± 0.8‡
Residual deflation lung volume (%)	47.6 ± 4.4	42.0 ± 3.4
Amniotic fluid L/S	0.61 ± 0.08	0.49 ± 0.05
Lung phosphatidylcholine/deoxyribonucleic acid (μg/μg)	0.4 ± 0.03	0.3 ± 0.03
Minimum extract surface tension (dynes/cm) = min	27.2 ± 1.9(8)	30.0 ± 0.5(7)
Amniotic fluid prolactin (ng/ml)	1458‖ [712-5510]¶	1583‖ [375-3350]
Fetal plasma prolactin (ng/ml)	12.8‖ [4-75]	15.1‖ [5-110]
Maternal plasma prolactin (ng/ml)	5.1‖ [2-19]	5.4‖ [2-44]

*Number of animals in each group (unless otherwise indicated).
†Arithmetic mean ± SE (unless otherwise indicated).
‡$p < 0.025$.
§$p < 0.005$.
‖Geometric mean.
¶Range in values.

the relationship of amniotic fluid prolactin values to indices of fetal lung maturation in the *Macaca mulatta*. In addition, the effects of administration of glucocorticoids to the mother were studied in regard to (1) concentrations of prolactin in maternal plasma, fetal plasma, and amniotic fluid and (2) the associations between concentrations of prolactin and characteristics of fetal lung development.

Methods

Thirty-four pregnant rhesus monkeys with known gestational ages were used for these studies. Group 1 consisted of 15 pregnant rhesus monkeys treated with 1 mg of betamethasone phosphate and 1 mg of betamethasone acetate (0.3 ml) intramuscularly daily for 3 days prior to delivery at gestational ages of 110, 115, 120, 124, 125, 126, 128, 130, 131, 133, 135, 136, 138, 140, and 143 days. This daily dosage of betamethasone (Celestone Soluspan, Schering Corp., Kenilworth, New Jersey) is one sixth the dosage recommended for clinical use[12] and was selected because the body weight of the rhesus monkey fetus is about one sixth that of the human fetus. The other 19 pregnant rhesus monkeys

Table II. Comparison of findings in male and female fetuses

	Groups 1, 2, and 3	
	*Female (15)**	*Male (19)**
Gestational age (days)	134 ± 3.3†	132 ± 2.9
Fetal body weight (gm)	297 ± 21	315 ± 17
Maximum lung volume	1.5 ± 0.14	1.3 ± 0.11
Lung alveolar stability	44.3 ± 3	50.6 ± 4.8
Amniotic fluid L/S	0.5 ± 0.04	0.6 ± 0.08
Lung phosphatidylcholine/deoxyribonucleic acid (μg/μg)	0.336 ± 0.02	0.409 ± 0.03
Lung extract minimum surface tension	27.5 ± 1.0 (9)	25 ± 2.8 (11)
Amniotic fluid prolactin (ng/ml)	1597 ± 288	1897 ± 264
Fetal plasma prolactin (ng/ml)	27.5 ± 7.7	21.2 ± 6.8
Maternal plasma prolactin (ng/ml)	7.7 ± 1.6	9.1 ± 2.4

*Number of animals in each group (unless otherwise indicated).
†Mean ± SE.

were treated with isotonic saline solution (Med Tech, Inc., Elwood, Texas) (0.3 ml) intramuscularly daily for 3 days prior to delivery at gestational ages of 110 to 160 days. The fourteen saline solution–injected animals which delivered at gestational ages of 110, 115, 120, 125, 126, 128, 130, 131, 133, 135, 136, 138, 140, and 143 days, were designated as group 2. The other five saline solution–injected animals which delivered at 150 to 160 days were designated as group 3.

All animals were delivered by cesarean section between 9 AM and 11 AM, at which time samples of maternal plasma, amniotic fluid, and cord plasma were obtained for analyses of prolactin. The determination of the duration of pregnancy, the care of animals, and the cesarean section techniques have been described previously.[12] The residual deflation volume at 15 cm of water transpulmonary pressure was used as the functional index of alveolar stability.

As soon as a fetus was delivered, its trachea was clamped to prevent spontaneous lung inflation, and death occurred soon afterward. Then the following studies were performed on all fetuses to assess fetal lung maturation: lung pressure-volume studies; lung phosphatidylcholine analyses; and amniotic fluid lecithin/sphingomyelin (L/S) ratios. Lung extract surface tension studies were also performed on 20 fetuses. Pressure-volume studies provided maximum lung volume, expressed as the absolute volume at 40 cm of water transpulmonary pressure. Partial volumes during inflation and deflation were ascertained as a proportion of the maximum lung volume.[12]

After the pressure-volume studies, the lungs were weighed, homogenized, and lyophilized. Lung phos-

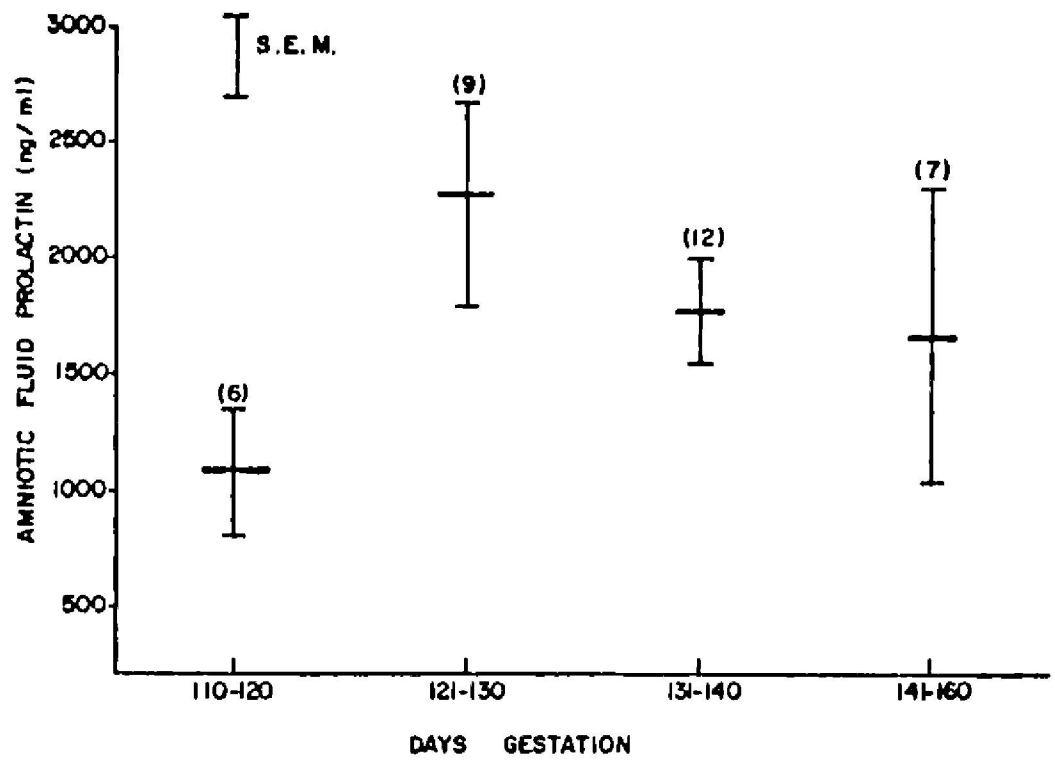

Fig. 1. Graph demonstrating changes in rhesus amniotic fluid prolactin concentrations with respect to gestational age for groups 1, 2, and 3. The mean values at 121 to 130 days and 131 to 140 days are significantly higher than those at 110 to 120 days.

pholipids were extracted and quantified as described previously.[12] A modification of the fluorometric method of Kissane and Robbins was used to perform the analyses of deoxyribonucleic acid.[12]

Saline extracts of lung tissue were prepared for surface tension studies by mincing 3 gm of lung sample in 50 ml of isotonic saline solution. This preparation was then filtered onto the trough of a modified Wilhelmy surface tension balance. After 2 hours of continuous cyclic compression and expansion of the surface film, the maximum and minimum surface tensions recorded for the final cycle were designated as the surface tension values for each lung sample.

As soon as the amniotic fluid was obtained, it was centrifuged at 1500 rpm, at 4° C, for 15 minutes. The supernatant was removed and frozen for subsequent analyses of lecithin/sphingomyelin ratio. These were performed with the fetal lung maturity test (Helena Laboratories) based on the method of Gluck et al.[13] After extraction with a chloroform/methanol mixture, the sample was concentrated and spotted on a thin-layer chromatographic plate. Quantitation was accomplished with a densitometer.

Samples of plasma and amniotic fluid obtained at delivery were stored at −20° C for 6 to 18 months until analyses of prolactin could be performed. The methodology used for analysis of prolactin was previously described by Tyson et al.[11] The intra-assay variation has been found to be 4%, with an interassay variation of 12%. No significant alteration in sample prolactin values have been noted after 3 years of storage of samples at −20° C (Tyson JE. unpublished data).

All of the above-mentioned studies were done without prior knowledge of gestational age or maternal glucocorticoid treatment. Differences in prolactin values and fetal lung studies were sought between groups 1 and 2 by unpaired *t* test analyses. Determinations in female and male fetuses were analyzed similarly. In the case of prolactin observations, because of the possibilities of outlying values, differences were also sought with the nonparametric Kolmogorov-Smirnov two-tailed test. Correlation coefficients were sought between prolactin values and fetal lung characteristics. Analyses of partial correlation coefficients were performed to test for concurrent effects of gestational age.

Results

A significant correlation was present between gestational age and fetal body weight ($r = 0.81$; $p < 0.001$), thus attesting to the adequacy of assessment of gestational age. When group 1, or the 15 betamethasone-treated animals (gestational age of 110 to 143 days), was compared to group 2, or the 14 saline-treated animals (gestational age of 110 to 143 days), no significant differences in mean gestational age, fetal body weights, or fetal lung weights were noted (Table I). The mean adrenal weight–to–body weight ratio and placental weight were significantly reduced in the betamethasone-treated animals, as reported in prior studies.[12]

Prolactin studies. Among all animals, there were marked variations in amniotic fluid prolactin, maternal plasma prolactin, and fetal plasma prolactin. The amniotic fluid prolactin values were consistently the highest ($p < 0.005$), and fetal plasma prolactin values were higher than maternal plasma prolactin values in each maternal-fetal pair except two ($p < 0.005$). Fetal plasma prolactin and maternal plasma prolactin values did not

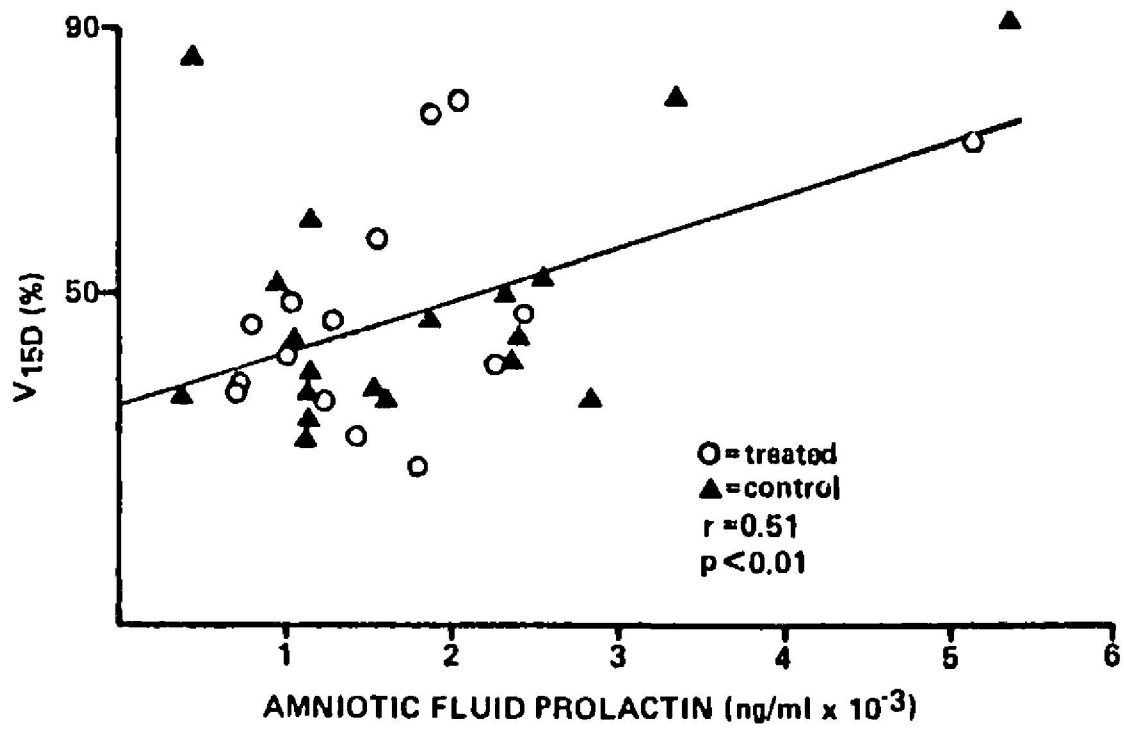

Fig. 2. Graph demonstrating correlation between amniotic fluid prolactin values and alveolar stability ($V_{15D\%}$) (expressed as a residual volume during deflation). The latter is a functional measurement of alveolar surfactant ($r = 0.51$; $p < 0.01$).

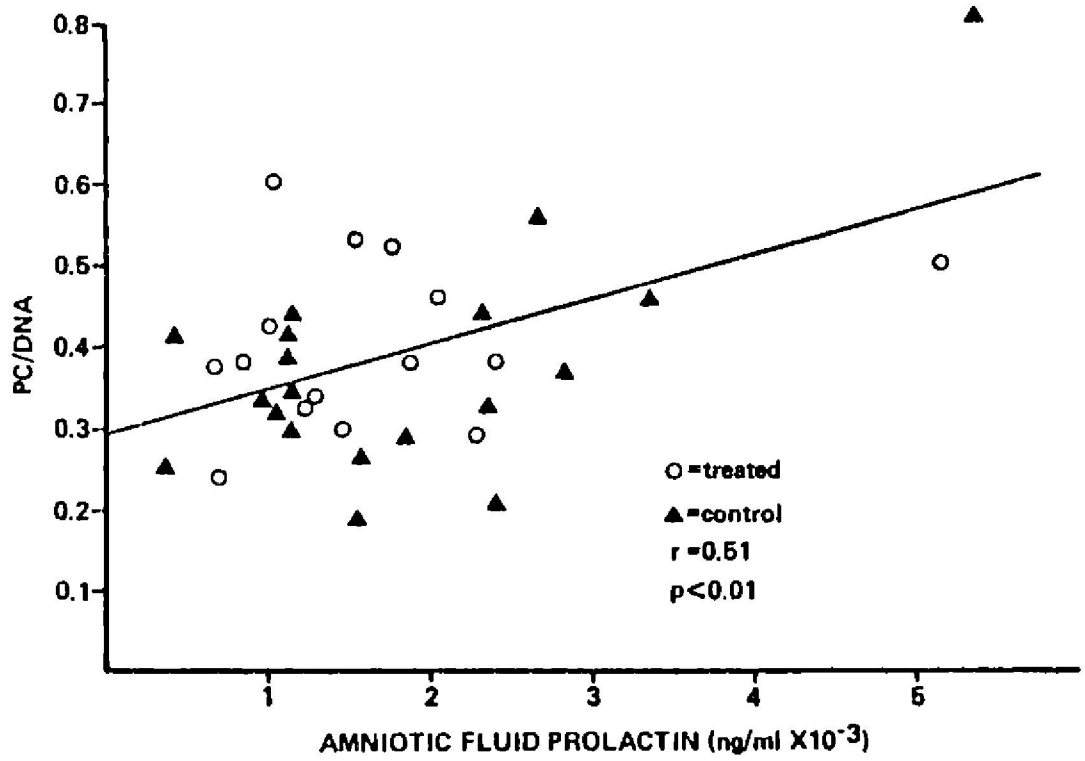

Fig. 3. Graph depicting correlation between amniotic fluid prolactin values and lung phosphatidylcholine concentrations (*PC/DNA*) ($r = 0.51$; $p < 0.01$). The difference between betamethasone-treated and saline solution–treated fetuses is not significant.

correlate significantly with each other or with amniotic fluid prolactin values. Significant correlations were not found between gestational age and amniotic fluid prolactin, maternal plasma prolactin, or fetal plasma prolactin concentrations. When the amniotic fluid prolactin values were analyzed by various gestational ages, they appeared to rise to a plateau at 121 to 140 days. The values were lower at 110 to 120 days, rose to a maximum value at 121 to 130 days, and declined slightly at 131 to 140 days and 141 to 160 days (Fig. 1). The mean values at 121 to 130 days and 131 to 140 days were significantly higher than the mean value at 110 to 120 days ($p < 0.025$).

Maternal plasma prolactin, fetal plasma prolactin, and amniotic fluid prolactin concentrations for groups 1 and 2 were not significantly different by the unpaired *t* test and the Kolmogorov-Smirnov test. The distribution of these values was not thought to be parametric and therefore they are described by their geometric means and the ranges of their values in Table I. Among all three groups, no significant differences in maternal plasma prolactin, fetal plasma prolactin, or amniotic fluid prolactin values were noted for female fetuses compared to male fetuses (Table II).

Lung studies. The measures of surfactant used in this study were found to correlate with gestational age for all three groups as follows: lung alveolar stability: $r = 0.46$; $p < 0.005$; lung extract minimum surface

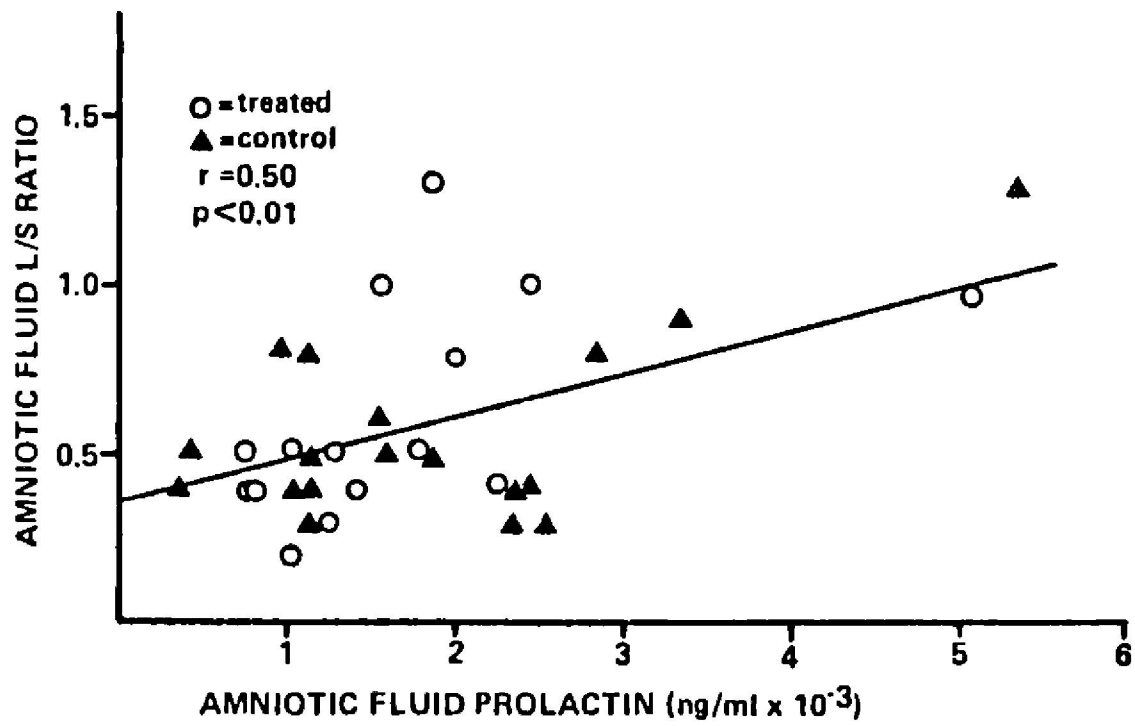

Fig. 4. Graph demonstrating correlation between amniotic fluid prolactin values and L/S ratios ($r = 0.50$; $p < 0.01$). No significant differences are noted between betamethasone-treated fetuses and their controls.

Table III. Correlation coefficients for groups 1, 2 and 3

	Amniotic fluid prolactin	*Fetal plasma prolactin*	*Maternal plasma prolactin*
Gestational age (days)	r = 0.16	r = 0.12	r = 0.24
Significance	NS	NS	NS
Lung extract minimum surface tension	r = −0.39	r = −0.45	r = 0.06
Significance	p < 0.05	p < 0.025	NS
Amniotic fluid L/S	r = 0.50	r = 0.15	r = −0.15
Significance	p < 0.01	NS	NS
Phosphatidylcholine/dexoyribonucleic acid	r = 0.51	r = 0.21	r = 0.22
Significance	p < 0.01	NS	NS
Lung alveolar stability	r = 0.51	r = 0.05	r = 0.19
Significance	p < 0.01	NS	NS
Maximum lung volume	r = 0.24	r = 0.28	r = 0.14
Significance	NS	NS	NS
Amniotic fluid prolactin	—	r = 0.14	r = −0.04
Significance		NS	NS
Fetal plasma prolactin	r = 0.14	—	r = 0.04
Significance	NS		NS
Maternal plasma prolactin	r = −0.04	r = 0.03	—
Significance	NS	NS	

tension: $r = -0.50$; $p < 0.025$; and lung microgram phosphatidylcholine/microgram deoxyribonucleic acid concentration: $r = 0.40$; $p < 0.025$. Gestational age correlated with amniotic fluid L/S ratio for groups 2 and 3 ($r = 0.47$; $p < 0.05$) but not for all three groups. The amniotic fluid L/S values for this study appear to be in reasonable agreement with those reported by Gluck et al.[13] in rhesus monkeys.

In comparing lung characteristics of group 1 and group 2, maximum lung volumes were significantly increased in group 1, but no significant differences in lung alveolar stability, phosphatidylcholine/deoxyribonucleic acid, lung extract minimum surface tension, or amniotic fluid L/S values were noted between the betamethasone-treated fetuses and their saline solution–treated controls (Table I). Among all cases, there were no differences between female and male fetuses (Table II).

For groups 1, 2, and 3, significant correlations were noted between lung alveolar stability and each of the following: lung extract minimum surface tension ($r = -0.83$, $p < 0.005$); amniotic fluid L/S ($r = 0.59$; $p < 0.005$); and phosphatidylcholine/deoxyribonucleic acid ($r = 0.57$; $p < 0.005$). These correlations, in addition to previous observations,[12] strongly support the hypothesis that these measurements are interrelated and reflect lung surfactant properties.

Prolactin concentrations and lung characteristics. Amniotic fluid prolactin values for all three groups were found to correlate significantly with lung alveolar

Table IV. Partial correlations

	*Amniotic fluid prolactin**	*Gestational age†*
Groups 1, 2, and 3		
Lung alveolar stability	r = 0.45	r = 0.45
Significance	p < 0.01	p < 0.01
Phosphotidylcholine/ deoxyribonucleic acid	r = 0.49	r = 0.38
Significance	p < 0.01	p < 0.025
Lung extract minimum surface tension	r = −0.51	r = −0.49
Significance	p < 0.025	p < 0.025
Groups 2 and 3		
Amniotic fluid L/S	r = 0.55	r = 0.41
Significance	p < 0.05	p < 0.05

*With gestational age held constant.
†With amniotic fluid prolactin values held constant.

stability (r = 0.51; b = 7.9×10^{-3}; p < 0.01) (Fig. 2); phosphatidylcholine/deoxyribonucleic acid (r = 0.51; b = 0.056×10^{-3}; p < 0.01) (Fig. 3); amniotic fluid L/S ratios (r = 0.50; b = 0.122×10^{-3}; p < 0.01) (Fig. 4); and lung extract minimum surface tension (r = −0.39; p < 0.05) (Table III). These correlations with amniotic fluid prolactin were significant even when the 160-day value was excluded for lung alveolar stability (r = 0.36, p < 0.025) and amniotic fluid L/S ratios (r = 0.327, p < 0.05), but not for phosphatidylcholine/ deoxyribonucleic acid (r = 0.25, p < 0.10 and lung extract minimum surface tension (r = 0.01; NS). For groups 2 and 3 combined, amniotic fluid prolactin also correlated significantly with lung alveolar stability (r = 0.48; p < 0.025); phosphatidylcholine/deoxyribonucleic acid (r = 0.66; p < 0.005) and amniotic fluid L/S (r = 0.61; p < 0.005), but not lung extract minimum surface tension (r = −0.29).

Fetal plasma prolactin values were found to correlate with lung extract minimum surface tension (r = −0.45; p < 0.05), but not when corrected for effects of gestational age. No other significant correlations were noted between amniotic fluid prolactin, fetal plasma prolactin, or maternal plasma prolactin values and these indices of lung maturation (Table III).

Although there were no significant correlations between amniotic fluid prolactin and gestational age, both amniotic fluid prolactin and gestational age correlated significantly with lung alveolar stability, phosphatidylcholine/deoxyribonucleic acid, and lung extract minimum surface tension for all three groups. Gestational age correlated with amniotic fluid L/S for groups 2 and 3, but not for all three groups. These relationships were examined in an analysis of partial correlations (Table IV). The partial correlations for both amniotic fluid prolactin and for gestational age were statistically significant, athough those for amniotic fluid prolactin tended to be stronger.

Comment

The primary objective of this study was to assess the possible roles of maternal plasma prolactin, fetal plasma prolactin, and amniotic fluid prolactin in stimulating the production of lung surfactant. The plasma concentrations of prolactin noted from 110 to 160 days' gestation in the rhesus monkey and her fetus appeared to be in agreement with the values reported by others.[6, 9, 15] At peak levels, values for amniotic fluid prolactin were 10 to 100 times those of fetal plasma prolactin and maternal plasma prolactin. Increases in fetal and maternal plasma levels of prolactin in the rhesus monkey have been noted to occur after 150 days' gestation,[15] but were not detected in the present study, probably because only four of 34 (12%) of the cases were analyzed during this time. There were no findings in this study to indicate that maternal plasma prolactin or fetal plasma prolactin influenced fetal lung maturation.

There have been few previous studies of amniotic fluid prolactin in the rhesus monkey. Friesen et al.[9] noted a tendency to an increase in amniotic fluid prolactin with gestational age in three monkeys. The present study provided sufficient numbers to determine amniotic fluid prolactin concentrations in the rhesus monkey for 67% to 94% of gestational age. A significant elevation occurred at about 70% of gestation. Maternal treatment with betamethasone did not appear to affect maternal plasma prolactin, fetal plasma prolactin, or amniotic fluid prolactin values.

The present study permitted analyses of the associations between amniotic fluid prolactin values and four different indices of fetal lung surfactant. The general belief is that the principal effect of lung surfactant is to lower surface tension at the alveolar lining, thereby increasing alveolar stability.[1] The most reliable measure of this functional effect of surfactant on alveolar stability is provided by lung pressure-volume studies. Of-

ten such direct measurements of the surfactant system are not possible and inferential markers must be used, such as lung tissue or lavage concentrations of disaturated phosphatidylcholine, phosphatidylinositol, or phosphatidylglycerol; lung extract or lavage surface tension studies; type II cell lamellar inclusion body densities, etc.[1] These biochemical and morphologic measures are very helpful, but on occasion may not portray accurately either the location of surfactant or its physiologic properties.[16] They cannot determine functional effects of surfactant as accurately as lung deflation characteristics.

The residual deflation volume at 15 cm of water transpulmonary pressure (lung alveolar stability) has been found by the authors to be one of the most sensitive measures of functional surfactant.[12] In the present study, lung alveolar stability was found to correlate significantly with phosphatidylcholine/deoxyribonucleic acid, lung extract minimum surface tension, and amniotic fluid L/S, thus indicating that these last three characteristics were acceptable markers for functional surfactant under the conditions of this study. Although it would have been of additional interest, other markers, such as lung tissue or lavage concentrations of disaturated phosphatidylcholine, phosphatidylglycerol, or phosphatidylinositol were not assessed.

In this study, the analyses of partial correlations suggest that associations of amniotic fluid prolactin with lung surfactant are separate and perhaps stronger than the influences of gestational age. Substantial changes in surfactant characteristics occurred over the range of values of amniotic fluid prolactin observed. The slopes of the regressions (Figs. 2, 3, and 4) suggest that when concentrations of amniotic fluid prolactin reach 4000 to 5000 ng/ml, the extrapolated lung stability values (lung alveolar stability), lung lipid concentrations (phosphatidylcholine/deoxyribonucleic acid), and amniotic fluid L/S ratios, are consistent with mature fetal lung characteristics in the rhesus monkey. [Mature values: lung alveolar stability, 71.5% ± 7.6% SEM; phosphatidylcholine/deoxyribonucleic acid, 0.46 ± 0.11 SEM; L/S, 0.9 ± 0.2 SEM (Johnson JWC, Mitzner W, Beck JC. In preparation).] These associations do not prove that amniotic fluid prolactin is the stimulus for the production of fetal lung surfactant, but there are circumstantial and in vitro data to support this hypothesis, as reviewed partly in the introduction. That the inspiration of amniotic fluid prolactin does occur is supported by recent studies in rhesus monkeys that demonstrated that concentrations of fetal tracheobronchial fluid prolactin are closely correlated with matched concentrations of amniotic fluid prolactin.[17] If prolactin is important in initiating the production of surfactant, amniotic fluid may be a more satisfactory vehicle than fetal plasma, given the relatively meager lung perfusion that exists in the fetus. In the absence of an amniotic fluid source, higher concentrations in plasma would be required to achieve comparable concentrations and distributions of prolactin in lung tissue. High plasma concentrations of prolactin beginning at midgestation might have detrimental effects on fetal osmoregulation, lactation, and intermediary metabolism.

In this study the observed correlation coefficients between amniotic fluid prolactin and indices of fetal lung surfactant were generally more significant than those for gestational age, but still they were not high. This might be explained in several ways. First of all, the study design may not have favored the optimum detection of existing amniotic fluid prolactin associations. The transfer of prolactin from the amniotic space to the alveolar cell probably requires days. Consequently, amniotic fluid prolactin–induced alterations in the characteristics of fetal lung surfactant might be expected to lag days behind changes in the concentrations of amniotic fluid prolactin. In addition, the concentrations of prolactin in amniotic fluid probably differ from corresponding concentrations of prolactin at type II alveolar cells, because of dilutional events, receptor site availability, etc. Stronger correlations were found to exist between tracheobronchial fluid prolactin and measures of lung surfactant than those reported in the present study for amniotic fluid prolactin.[17] These findings support the hypothesis that the experimental conditions of the current study contributed to the lesser correlations noted between amniotic fluid prolactin and these same lung characteristics.

Furthermore, the confounding influences of other hormone modulators of surfactant production were not assessed in the present study. For all of these reasons, stronger associations between concentrations of amniotic fluid prolactin and characteristics of lung surfactant could be artifactually reduced under the circumstances of this investigation. It is also possible that a relatively low correlation coefficient reflects coincidence and not a true association. There were few high values of amniotic fluid prolactin in the present study, although in these particular fetuses mature surfactant was present as measured by three independent assessments: lung alveolar stability, phosphatidylcholine/deoxyribonucleic acid, and L/S. On the basis of the available data, it is not possible to conclude unequivocally that the reported coefficients represent cause-and-effect associations, but it seems unlikely that the statistically significant correlations between amniotic fluid prolactin and four independently determined surfactant characteristics could all be coincidental. More studies with samples of high amniotic fluid prolactin are needed to resolve this issue.

In this study, no effort was made to identify the gestational age events associated with surfactant that ap-

pear to be independent of amniotic fluid prolactin. These could include alterations due to gestational age in the fetal lung response to thyroxin, insulin, and cortisol. To date, the effects of thyroxin and insulin on lung maturation have not been fully assessed in the rhesus monkey. Evidence exists to support the physiologic role of endogenous glucocorticoids in other species, but most studies in the rhesus monkey or human fetus have not shown a surge of plasma cortisol prior to the onset of production of surfactant.[6] Perelman et al.[18] were unable to demonstrate a relationship between rising levels of fetal blood cortisol and lung maturation in the rhesus monkey.

No significant differences in the lung characteristics of betamethasone-treated fetuses (group 1) compared to saline solution–treated controls (group 2) were found in this study, except for increased maximum lung volume values. As reported previously, the increases in maximum lung volumes associated with glucocorticoid treatment are thought to be related to changes in lung connective tissue rather than surfactant.[12] A stimulatory effect of betamethasone on the synthesis of surfactant might have been detected had lung lavage phosphatidylcholine studies been performed. Still, the functional significance of such chemical changes would be questionable, since changes in lung alveolar stability were not noted. These findings do not exclude the possibility that experiments extending into several hours of neonatal life might reveal betamethasone-induced changes in surfactant characteristics.

The relevancy of these findings in rhesus monkeys to the findings in other species remains to be determined. There are marked similarities in changes in amniotic fluid prolactin in the rhesus monkey and the human. In both species the peak values of amniotic fluid prolactin tend to be tenfold to 100-fold higher than fetal plasma prolactin values, and peak values of amniotic fluid prolactin tend to occur around 65% to 75% of gestation.[6, 8] Gradual declines in the concentration of amniotic fluid prolactin occur in late pregnancy in the rhesus monkey and human,[6, 8, 9] thus suggesting that the role of amniotic fluid prolactin may be to initiate rather than to maintain the production of surfactant. The fact that Hatjis et al.[19] could not demonstrate a correlation between human amniotic fluid prolactin and amniotic fluid L/S might be explained on the basis that most of their cases were analyzed after 85% (35 weeks) of gestation, when amniotic fluid prolactin values are decreasing[6, 8] and amniotic fluid L/S values are increasing.[1, 4] Their findings do not exclude the possibility that the observed mature amniotic fluid L/S values were induced by concentrations of amniotic fluid prolactin present prior to 35 weeks.

The increase in human fetal plasma prolactin that occurs as concentrations of amniotic fluid prolactin decline may indicate that the fetal pituitary gland is replacing the decidua as the main source of lung prolactin. A change in the source of prolactin would be appropriate in view of impending delivery and loss of the decidual source. If fetal pituitary prolactin is important to the production of lung surfactant in late pregnancy, it could also be important to the neonate, which might explain why plasma prolactin values remain high during the first 2 months of life.[6] If the hypothesis is correct that the production of surfactant is enhanced by amniotic fluid prolactin early in pregnancy and by fetal plasma prolactin later, then low values of fetal plasma prolactin might be expected to be associated with an increased risk of RDS, as has been reported by several different groups of investigators.[1, 6, 7] It has been observed that infants born to narcotic addicts are less susceptible to RDS. This apparent protective effect might be explained by the increased prolactin values noted in the newborn infants of opioid addicts.[20]

In summary, a number of investigators has suggested that several endocrine factors may be involved in the production of fetal lung surfactant.[2, 3] The present study on rhesus monkeys supports the hypothesis that amniotic fluid prolactin is one of these factors. Events that modulate decidual production of prolactin and transfer of prolactin to amniotic fluid might have important influences on neonatal lung function. The relevance of these findings to events in the human fetus remains to be determined.

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