

Ontogeny of Surfactant Proteins A and B in Human Amniotic Fluid as Indices of Fetal Lung Maturity

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ABSTRACT. Surfactant proteins A and B (SP-A and SP-B) were measured in human amniotic fluid by ELISA and correlated with lecithin to sphingomyelin ratio (L/S), phosphatidylglycerol (PG), and perinatal outcome. Amniotic fluid SP-A, SP-B, and L/S increased with advancing gestation. SP-A was detected at 19 wk gestation and increased dramatically in the 3rd trimester of pregnancy. SP-B was first detectable at 31 wk gestation and increased significantly to term. SP-A was a more specific predictor of non-respiratory distress syndrome (RDS) than L/S or SP-B; however, the sensitivity of SP-A in predicting RDS was less than $L/S < 2.0$ (26.3 versus 82.3%, respectively). In 209 pregnancies assessed within 48 h of delivery, the sensitivity of SP-B in predicting RDS (nondetectable SP-B) was comparable to the L/S, however, SP-B = 0 was frequently observed in mature infants, limiting its specificity for prediction of RDS. The greatest sensitivity and specificity were achieved with the measurement of $L/S < 2.0$ and negative PG, which correctly predicted 100% of the infants with RDS and 94% of those who did not develop the disorder. Measurement of SP-A or SP-B did not improve the prediction of RDS. SP-A, SP-B, and L/S were not affected by infant sex, Apgar score, rupture of membranes, size for gestational age, maternal diabetes, hypertension, or exposure to medications. SP-A, SP-B, and L/S were significantly elevated in amniotic fluid from black mothers. SP-A was significantly elevated in amniotic fluid from mothers who smoked during pregnancy. Although amniotic fluid SP-A and SP-B concentrations increased with advancing gestation, the predictive value of the L/S and PG for RDS was not enhanced by the additional measurement of SP-A and/or SP-B. (*Pediatr Res* 30: 597-605, 1991)

Abbreviations

RDS, respiratory distress syndrome
SP-A, surfactant-associated protein, $M_r = 28\,000-36\,000$
SP-B, hydrophobic surfactant-associated protein, $M_r = 8700$
L/S, lecithin to sphingomyelin ratio
PG, phosphatidylglycerol

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[SP-A], SP-A concentration
[SP-B], SP-B concentration

In 1959, Avery and Mead described a deficiency of pulmonary surfactant in lungs of low birth weight infants dying of RDS (1). Recent investigations indicate that pulmonary surfactant is a complex mixture of phospholipids and associated proteins. Although surfactant phospholipids reduce surface tension *in vitro*, the phospholipid-associated proteins confer unique surface properties to the lipids (2). SP-A, an $M_r = 28\,000-36\,000$ polypeptide, is the most abundant surfactant-associated glycoprotein. SP-A enhances the surface properties of phospholipid mixtures containing the hydrophobic proteins SP-B and SP-C. SP-A also inhibits phospholipid secretion, enhances reuptake of phospholipids by type II epithelial cells *in vitro*, and confers calcium-dependent organization to the phospholipids (for review, see Ref. 3). SP-B is an $M_r = 8700$ hydrophobic polypeptide that imparts biophysical properties of rapid spreading and adsorption to surfactant phospholipids (for review, see Ref. 4). The surfactant phospholipids and associated proteins are developmentally regulated, appearing in the fetal rat lung between 18 and 19 d of gestation and increasing until birth (5-7). Lung fluid, including surfactant, contributes to the composition of amniotic fluid. Amniotic fluid content of saturated phosphatidylcholine (lecithin), PG, and phosphatidylinositol parallel the expression of these phospholipids in the fetal lung and have been used to estimate fetal lung maturity (8-13). Human amniotic fluid content of SP-A also increases with advancing gestation and is predictive of fetal lung maturity and development of RDS (14-18).

RDS continues to be a major cause of morbidity and mortality in premature infants. Accurate estimates of fetal lung maturity are required for optimal perinatal-neonatal management of high-risk pregnancies. The L/S and the presence of PG are routinely used to predict the development of RDS (9, 11, 14). The accuracy of the combined L/S and PG tests is approximately 90-95%. A high incidence of false-negative results occurs particularly with the L/S, such that fetal lung immaturity is predicted in many cases where neonatal pulmonary function is mature. In the present study, concentrations of SP-A and SP-B in human amniotic fluid were determined by capture ELISA. The predictive value of the amniotic fluid concentration of SP-A and SP-B for neonatal pulmonary disease was compared with that of the L/S and PG.

MATERIALS AND METHODS

Amniotic fluid samples ($n = 473$) were obtained from 408 high-risk pregnancies at the University of Cincinnati between

June 1986 and December 1987. Amniotic fluid was obtained by amniocentesis ($n = 334$) or vaginal collection after rupture of membranes ($n = 139$). Indications for amniocentesis were either elective delivery, premature labor, or potential blood group incompatibility. Twenty-five samples were analyzed separately because of oligohydramnios ($n = 23$) or polyhydramnios ($n = 2$) as determined by ultrasonography. Amniotic fluid was sampled within 48 h of delivery in 209 cases. Maternal and neonatal histories were determined by retrospective chart review. The prenatal use of cigarettes, alcohol, street drugs, or medications including tocolytics, theophylline, β -adrenergics, and corticosteroids was reported if it occurred during the pregnancy in question. Substance use was not further quantitated. Assignment of gestational age at the time of delivery was based on the last menstrual period, confirmed by fetal sonography and/or clinical assessment using the Ballard scale for newborn maturity (19). If the last menstrual period varied by more than 2 wk from the Ballard rating, gestational age was assigned by physical examination. Gestational age at the time of amniotic fluid sampling was extrapolated from the gestational age at birth. Administration of exogenous surfactant was noted ($n = 1$). Appropriateness of birth weight for gestational age was determined by reference to the method of Battaglia and Lubchenco (20).

RDS was diagnosed in infants with clinical signs of respiratory distress (tachypnea greater than 60 breaths/min, grunting, or intercostal retractions) who required supplemental oxygen within 3 h of birth and for greater than 24 h and whose chest x-rays were consistent with a diffuse reticulogranular pattern with air

bronchograms ($n = 30$). Infants with x-ray signs of pneumonia or bacteriologic evidence of sepsis and respiratory distress were analyzed separately ($n = 5$). Transient tachypnea of the newborn was diagnosed in those infants with clinical signs of respiratory distress (tachypnea greater than 60 breaths/min, grunting, or retractions) who required supplemental oxygen for less than 24 h after birth or whose chest x-rays demonstrated perihilar streaking and intralobar fluid ($n = 62$). Pulmonary hypoplasia associated with congenital anomalies was diagnosed in two infants.

Phospholipid assays. The L/S and PG content were determined in the clinical laboratory immediately after fluid collection. The remainder of the sample was then stored at -20°C for future analysis of surfactant protein content. The L/S was determined by two-dimensional, thin-layer chromatography after cold acetone precipitation (8). Each sample was run in duplicate and samples with a difference in the ratio of greater than 0.2 were remeasured. L/S less than 2.0 were considered immature. PG was determined by thin-layer chromatography using dichlorofluorescein (0.02%) and UV light for detection (8). PG was reported as positive (mature) or negative (immature).

SP-A ELISA. Amniotic fluid contents of SP-A and SP-B were determined by ELISA. SP-A was purified from surfactant obtained by lung lavage of a patient with alveolar proteinosis (21, 22). Polyclonal anti-human SP-A antibodies were prepared in goats and New Zealand rabbits by repeated injection of approximately 1 mg purified SP-A in Freund's complete adjuvant. The antisera were specific for SP-A as assessed by immunoblot of alveolar proteinosis lavage and human amniotic fluid. The goat

Table 1. Characteristics of sampled pregnancies

Amniotic fluid samples:	473		
Pregnancies:	408	Sampled within 48 h of delivery: 209	
Race:	White: 231	Black: 174	Other: 3
Sex of infant:	Female: 207	Male: 201	
Source of amniotic fluid:	Amniocentesis: 334	Vaginal collection: 139	
Indications for amniocentesis:	Elective delivery: 156	Preterm labor: 98	Other: 80
Range of gestational age:	19–42 wk		
Mode of delivery:	Vaginal: 191 Cesarean section without labor: 129 Cesarean section with labor: 88		
Status of amniotic membranes:	Premature rupture (<37 wk gestation): 58 Prolonged rupture (>24 h): 7 Both (premature and prolonged): 82 Neither (premature or prolonged): 261		
Smoke:	Yes: 215 No: 193		
Alcohol:	Yes: 90 No: 318		
Marijuana:	25		
Cocaine:	4		
Complications of pregnancy:			
Diabetes mellitus:	Nondiabetic: 361	Class A: 25	Class B: 6 Class C: 5 Class D: 7 Class RF: 4
Hypertension:	No hypertension: 340	Chronic hypertension: 34 Pregnancy-induced hypertension: Mild: 26 Severe (requiring medication): 8	
Medications:	Ritodrine: 100 Theophylline: 12	Magnesium sulfate: 54 Aerosol β -adrenergics: 11	Steroids: 21
Infant diagnosis (pulmonary):	Well: 306 Pulmonary hypoplasia: 2 RDS: 30	TTN: 62	Pneumonia: 5 Aspiration: 3
Size for gestational age*:	SGA (<10%): 11	AGA (10–90%): 348	LGA (>90%): 49

* SGA, small for gestational age; AGA, average for gestational age; LGA, large for gestational age.

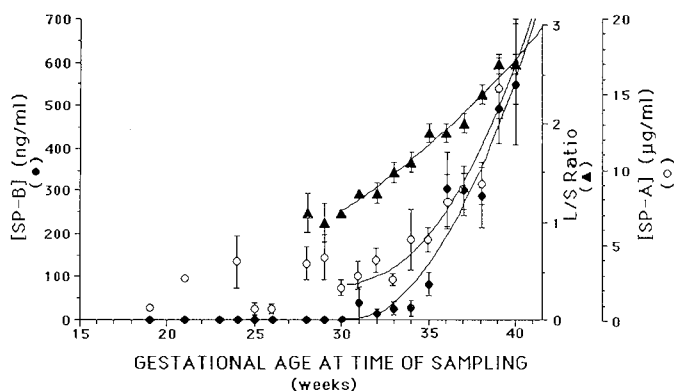


Fig. 1. Mean human amniotic fluid L/S, [SP-A], and [SP-B] at various gestational ages. The values are expressed as mean \pm SD. [SP-A] (open circle, $n = 442$) and [SP-B] (closed circle, $n = 443$) were measured by ELISA; L/S (closed triangle, $n = 278$) was determined by thin-layer chromatography as described in Materials and Methods. Values from 14 41- to 42-wk gestation pregnancies and from 33 pregnancies complicated by lung oligohydramnios ($n = 23$), polyhydramnios ($n = 2$), or congenital anomalies ($n = 8$) were excluded. Best fit regression lines, in each case a 2nd order polynomial, are given for amniotic fluid samples of 30 to 40 wk gestation.

and rabbit anti-SP-A antisera were used as first and second antibodies in a capture ELISA as previously described (18). Each assay plate included a standard curve generated with purified human SP-A (5–100 ng/mL) and four serial dilutions of each amniotic fluid sample adjusted to within the linear range of the assay. The SP-A ELISA was quantitated by determination of absorbance at 490 nm.

SP-B ELISA. SP-B was purified from bovine lung lavage as previously described (23). The purity and concentration of the bovine SP-B standard were confirmed by amino acid composition using reverse-phase HPLC on a Waters Pico-Tag column (Waters Associates, Millipore Corp., Milford, MA) to quantitate phenylthiocarbamyl-amino acid derivatives and by sequence analysis by Edman degradation and gas phase sequencer (24–27). The homology between bovine and human SP-B is approximately 80% (28). Polyclonal anti-human SP-B antibodies were generated in New Zealand rabbits by repeated injection of a human-based synthetic SP-B polypeptide (AA 1-60) in Freund's complete adjuvant and were used to coat the ELISA plate (29). Monoclonal anti-human SP-B antiserum was prepared from ascitic fluid of nude mice injected intraperitoneally with mouse spleen-myeloma hybridomas selectively producing anti-human SP-B antibodies. The murine spleen cells were isolated by dilutional cloning from mice repeatedly immunized with human cadaver lung lavage in Freund's adjuvant. The murine anti-SP-B antiserum was used as second antibody in the capture ELISA. The specificity of the anti-SP-B antisera was demonstrated by immunoblot of bovine serum, whole bovine surfactant, purified bovine SP-B, and human surfactant (23). Nonspecific binding was blocked with 5 mg/mL BSA and 5% human serum. Peroxidase-conjugated anti-mouse IgG was used to quantify the bound antibody SP-B complexes as previously described (18). Each assay plate included a standard curve generated with purified bovine SP-B (5–100 ng/mL). Four serial dilutions of each amniotic fluid sample were made in 50 mM phosphate buffered solution with 0.5% Nonidet P-40 (Sigma Chemical Co., St. Louis, MO) and were adjusted to within the linear range of assay. The SP-B ELISA was quantitated by determination of absorbance at 490 nm. Analysis of the ELISA was performed on an Apple IIE computer in line with a Dynatech (Chantilly, VA) microtiter plate reader.

Data were expressed as mean \pm SD unless otherwise indicated. Associations of the [SP-A], [SP-B], L/S, and PG content with the occurrence of neonatal RDS were determined by contingency

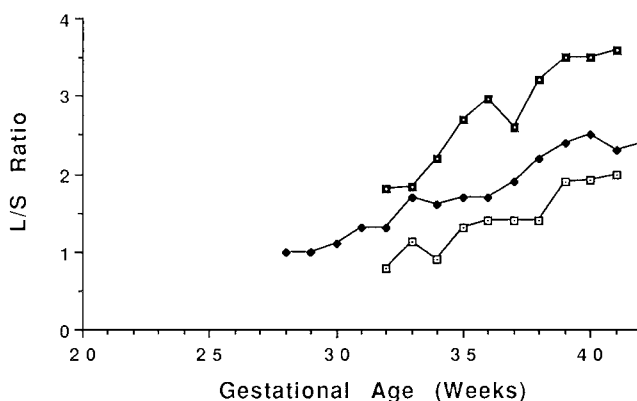
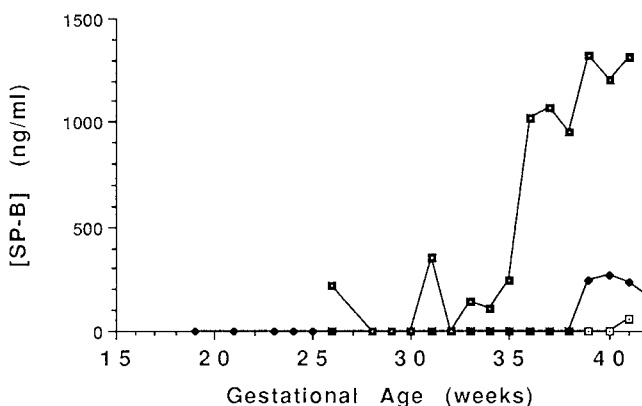
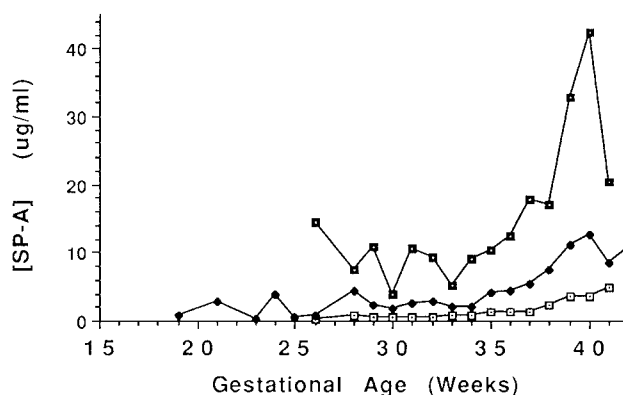


Fig. 2. Tenth (open square), 50th (black diamond), and 90th (closed square) percentile values of human amniotic fluid [SP-A] ($n = 442$), [SP-B] ($n = 443$), and L/S ($n = 278$) at various gestational ages. Values from 33 pregnancies complicated by oligohydramnios ($n = 23$), polyhydramnios ($n = 2$), or congenital anomalies ($n = 8$) were excluded from the analysis. [SP-A] and [SPA-B] were measured by ELISA; L/S was determined by thin-layer chromatography as described in Materials and Methods.

table analysis. [SP-A] $< 1 \mu\text{g/mL}$, [SP-B] = 0 ng/mL, L/S < 2.0 , and negative PG were selected as cutoff values indicating fetal pulmonary immaturity (positive test). The effects of potentially confounding variables including infant sex, size for gestational age, race, Apgar scores, prolonged or premature rupture of membranes, maternal diabetes, hypertension, smoking, use of ethanol, and medications were screened by Mann Whitney U or Kruskal-Wallis nonparametric tests. Variables significantly as-

Table 2. Aberrant surfactant indices detected in amniotic fluids sampled from pregnancies complicated by oligohydramnios, polyhydramnios or congenital abnormalities*

Amniotic fluid status	Gestational age (wk)	Associated anomaly	Test abnormality
Oligohydramnios	30	Pulmonary hypoplasia	[SP-A] > 90%
		Hydronephrosis	
	36	Trisomy 21	[SP-A] > 90%
		Congenital heart disease	
	36	Maternal SLE	L/S > 90%
		Fetal heart block	
	40	Unknown etiology	[SP-B] > 90%
	37	Unknown etiology	[SP-B] and L/S > 90%
		Villamentous cord insertion	
	39	Unknown etiology	[SP-A], [SP-B], and L/S > 90%
	38	IUGR by US, not by clinical exam	[SP-A] and [SP-B] > 90%
	36	IUGR, unknown etiology	[SP-B] > 90%
	39	Unknown etiology	L/S > 90%
Polyhydramnios	31	Pulmonary hypoplasia	[SP-A] and [SP-B] > 90%
		Dysplastic kidneys	
		Congenital heart disease	
Normal amniotic fluid	33	Severe polyhydramnios	[SP-A] and L/S < 10%
		Hydrocephalus	
Normal amniotic fluid	36	+ Cytomegalovirus culture	[SP-A] > 90%
		Microcephaly	
	39	Hydrocephalus, <i>ex vacuo</i> Osteogenesis imperfecta	[SP-A] and L/S < 10%

* SLE, systemic lupus erythematosus; US, ultrasound; IUGR, intrauterine growth retardation.

sociated with amniotic fluid concentrations of SP-A or SP-B univariately were included in multiple regression analyses. Because of SP-A and SP-B not being normally distributed within each gestational age grouping and because of the polynomial rise in the surfactant indices with advancing gestation, the multiple regression analyses were performed using the logarithmic transformation of SP-A, SP-B, and L/S ratio and including (gestational age)² and (gestational age) as independent variables. Statistical significance was defined as $p < 0.05$ unless otherwise specified. All statistical analyses were performed using Statview 512+ software (Brainpower, Inc., Agoura Hill, CA).

RESULTS

Surfactant protein immunoassays. Characteristics of the antisera used in the SP-A ELISA have been previously reported (18). The polyclonal rabbit anti-human SP-B antiserum and the monoclonal mouse anti-human SP-B antibody were highly reactive with the mature, $M_r = 8700$ SP-B protein as determined by single-phase ELISA and immunoblot of either purified bovine SP-B or whole bovine surfactant after separation by SDS-PAGE. The reproducibility and precision of the SP-B ELISA, assessed as interassay and intraassay coefficients of variation, were within 25%. Correlation coefficients of the standard curves were generally 0.95–0.99. The limit of detection was reliably 25 ng/mL, and the sensitivity (change in absorbance per change in [SP-B]) was approximately 0.003. SP-A and SP-B were stable in aliquots of an amniotic fluid sample assayed over a 6-mo period of storage at -20°C . SP-B was 100% recoverable by the ELISA from amniotic fluid spiked with standard SP-B within the range of concentrations detected in the current study samples. Addition of 2 to 50% (vol/vol) EDTA-anticoagulated whole human blood or meconium to standard concentrations of SP-A and SP-B did not significantly alter the ELISA of SP-A or SP-B. Meconium did not react with the ELISA of either SP-A or SP-B. Addition of 2–30% blood or 10% meconium to amniotic fluid samples decreased (3–20%) the concentrations of SP-A and SP-B compared with noncontaminated samples.

Ontogeny of L/S, SP-A, and SP-B. Characteristics of the pregnancies included in this study are presented in Table 1.

Gestational age at the time of amniotic fluid sampling was 19 to 42 wk ($n = 1-67$ samples per gestational week). RDS was diagnosed in 30 infants (19 Caucasian, 11 black; 14 male, 16 female) with a mean gestational age of 30.7 ± 4.2 wk (mean \pm SD). Transient tachypnea of the newborn was diagnosed in 62 infants. Pneumonia or sepsis was detected in five infants. Amniotic fluid aspiration and meconium aspiration were diagnosed in two infants and one infant, respectively. Exogenous surfactant was administered only for treatment of established RDS (*i.e.* rescue protocol) and did not alter the assessment of respiratory disease. No pulmonary disease was detected in 306 neonates. Mean gestational age of those without RDS was 36.3 ± 3.0 wk (mean \pm SD).

Amniotic fluid concentration of SP-A and SP-B and the L/S increased with advancing gestation (Fig. 1). SP-A was detected in the single amniotic fluid samples obtained at 19, 21, and 23 wk. A gradual increase in mean [SP-A] occurred over the next 7 wk, followed by a quadratic increase between 30 and 40 wk gestation ($R^2 = 0.96$). The increase in L/S after approximately 30 wk gestation was also best described by a quadratic equation ($R^2 = 0.98$). SP-B was first detectable in amniotic fluid at 31 wk gestation. Thereafter, mean [SP-B] increased in a quadratic fashion to term ($R^2 = 0.94$). When considered by gestational week, the mean [SP-A] and [SP-B] values correlated with each other and with the mean L/S ($R^2 = 0.83$, [SP-A] versus [SP-B]; $R^2 = 0.74$, [SP-A] versus L/S; $R^2 = 0.64$, [SP-B] versus L/S, 28–42 wk gestation). Correlation of the raw data for [SP-A] and [SP-B] with L/S ($R^2 = 0.26$ and 0.23 , respectively) reflected the wide variability in amniotic fluid content of SP-A, SP-B, and L/S within each week of gestation. The concentrations of SP-A and SP-B were not normally distributed within each week of gestation. The median, 10th, and 90th percentiles of [SP-A] and [SP-B] are represented in Fig. 2.

Amniotic fluid samples obtained from 33 pregnancies complicated by an abnormal quantity of amniotic fluid and/or congenital anomalies are not included in Figure 2. Twenty-five of these pregnancies were complicated by oligohydramnios ($n = 23$) or polyhydramnios ($n = 2$) as determined by ultrasonography. Congenital anomalies or intrauterine growth retardation were associated with oligohydramnios or polyhydramnios in 11 cases.

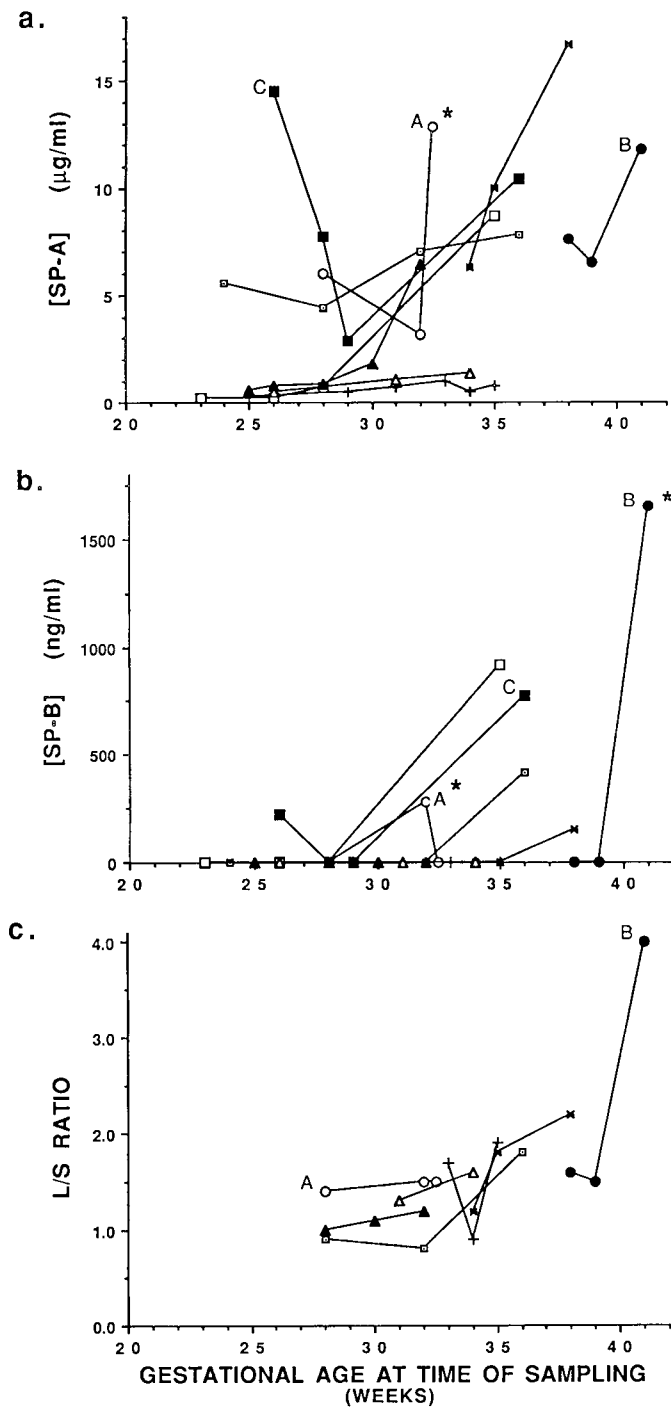


Fig. 3. Representative data of amniotic fluid [SP-A], [SP-B], and L/S from pregnancies sampled on more than one occasion. Each of the nine symbols (consistent between panels a, b, and c) represents an individual pregnancy sampled on three or more occasions. Open circles, closed circles, and closed squares represent patients A, B, and C, respectively, as referred to in the text. The surfactant proteins and phospholipids were measured as described in Materials and Methods. Asterisks highlight three indices that were greater than the 90th percentile for gestational age as defined in Figure 2.

An additional eight neonates had congenital anomalies but normal amniotic fluid volume. [SP-A], [SP-B], or L/S were outside of the 10th or 90th percentiles for gestational age (Fig. 2) in 13 of the 33 amniotic fluid samples obtained from pregnancies complicated by the abnormal quantity of amniotic fluid or by congenital anomalies, as shown in Table 2. The SP-A and SP-B content and L/S of the remaining 20 pregnancies associated with

congenital anomalies or abnormal amniotic fluid volume were within the 10th to 90th percentiles defined in Figure 2, including three infants with mild to severe hydronephrosis (one with oligohydramnios, two with normal fluid), three diagnosed to have intrauterine growth retardation and oligohydramnios, seven with oligohydramnios of unknown etiology, and one infant each with 1) oligohydramnios and Conradii Syndrome, 2) polyhydramnios, hydrocephalus, imperforate anus, and hydronephrosis, 3) Trisomy 21, 4) Arnold-Chiari malformation, 5) congenital heart disease, 6) maternal hyperthyroidism, and 7) nephroblastoma. Four infants with intrauterine growth retardation and normal amniotic fluid volumes had amniotic fluid indices within expected limits.

Amniotic fluid was sampled from 46 pregnancies by amniocentesis on more than one occasion, generally because of blood group antigen incompatibility. Representative data, composed of those patients who were sampled three or more times, are presented in Figure 3. For each patient, the L/S, the detectability of PG, and the concentrations of SP-A and SP-B generally increased with advancing gestation. Patient A (open circles) received betamethasone, ritodrine, and magnesium sulfate 4 d before her second sample, which showed a transient decrease in [SP-A] and a rise in SP-B. Patient B (closed circles) received brethine and ritodrine 5 wk before the first amniocentesis. She was treated with tocolytics and two doses of betamethasone 8 d before the second amniotic fluid sampling. Patient C (closed squares) received several antibodies between 30 and 36 wk gestation. No medications were reported in the other pregnancies represented in Figure 3. There was considerable variability with regard to peak [SP-A] and [SP-B] detected at term gestation.

Prediction of fetal lung maturity. Amniotic fluid samples were obtained from 209 pregnancies within 48 h of delivery. The values of [SP-A], [SP-B], L/S, and PG obtained from these 209 samples were tested by contingency table analysis to determine the correlation with the development of neonatal RDS. Specificity, sensitivity, accuracy, and positive and negative predictive values calculated on the 209 samples are represented in Table 3.

As shown in Table 3, SP-A was a more specific test for RDS than was the L/S; 93.0% of the infants who did not develop RDS had mature [SP-A] ($\geq 1 \mu\text{g/mL}$) compared with 86.7% with L/S ≥ 2.0 . However, only 26.3% of the infants who developed RDS would have been detected by [SP-A] $< 1 \mu\text{g/mL}$ compared with 82.3% correctly detected by L/S ratio < 2.0 , i.e. the sensitivity of the amniotic fluid [SP-A] < 1 was poor. The sensitivity of the SP-A concentration for RDS was improved by increasing the cutoff value (i.e. [SP-A] < 3) but the specificity and accuracy decreased. The sensitivity of [SP-B] = 0 to predict RDS was comparable to that for L/S (82.4 versus 82.3%); however, [SP-B] = 0 was less specific, incorrectly predicting 47.3% of healthy neonates to develop RDS versus 13.3% by L/S. The greatest sensitivity and specificity were attained with the commonly used combination of L/S < 2.0 and negative PG, which correctly predicted 100% of the infants who developed RDS and 94.2% of those who did not develop RDS. Other combinations of the four amniotic fluid indices did not improve both the sensitivity and specificity or overall accuracy of the test. The positive predictive value reflects the likelihood that a positive test, suggesting pulmonary immaturity, will correctly identify an infant who will develop RDS. The positive predictive value of L/S < 2.0 and negative PG was only 45.5%, yet it was not improved by consideration of the [SP-A] and [SP-B]. When only the 106 samples for which all four amniotic fluid indices were available were considered, the contingency table analysis was somewhat altered (data not shown). However, the relative probability values of the tests, relative to each other, were unchanged. The accuracy and positive predictive value of the amniotic fluid indices were greatly improved when all four values reflected immaturity, i.e. L/S < 2 , negative PG, [SP-A] < 1 , and [SP-B] = 0. However, the sensitivity for RDS, when all four tests reflected immaturity,

Table 3. Association of [SP-A], [SP-B], L/S, and PG content with the development of neonatal RDS*

Positive test	Sensitivity (%)	Specificity (%)	Accuracy (%)	+PV (%)	-PV (%)
[SP-A] < 1	26.3	93.0	86.8	27.8	92.5
[SP-A] < 3	64.7	77.1	76.0	20.4	96.0
[SP-B] = 0	82.4	52.7	55.3	14.0	97.0
L/S < 2	82.3	86.7	86.5	26.3	98.9
[SP-A] < 1 and/or [SP-B] = 0	82.4	51.7	54.3	13.1	96.9
L/S < 2, SP-A < 1, and/or [SP-B] = 0	60.0	90.4	89.0	23.1	97.9
[SP-A] < 1 or L/S < 2	83.3	84.8	84.7	23.8	98.9
[SP-B] = 0 or L/S < 2	100	57.7	59.6	10.2	100
PG negative	73.3	85.9	84.9	30.6	97.4
L/S < 2 and PG negative	100	94.2	94.4	45.5	100
L/S < 2, PG negative, [SP-B] = 0, and [SP-A] < 1	25	100	97.2	100	97.1

* Sensitivity, frequency of positive test results in those with RDS [true-positive ÷ (true-positive + false-negative results)]; specificity, frequency of negative test results in those without RDS [true-negative ÷ (true-negative + false-positive results)]; accuracy, proportion of test results that correctly predict outcome [(true-positive + true-negative) ÷ total number of tests]; +PV (+predictive value), frequency of RDS in those with positive test results; and -PV (-predictive value), frequency of no RDS in those with negative test results.

was greatly reduced; only 25% of the infants who would develop RDS were identified.

[SP-A], [SP-B], and L/S were not significantly affected by infant sex, Apgar score, prolonged or premature rupture of the membranes, size for gestational age, maternal diabetes, hypertension, use of ethanol, theophylline, ritodrine, magnesium sulfate, corticosteroids, or insulin when analyzed by multiple regression analyses with gestational age as an independent variable. Overall, [SP-A], [SP-B], and L/S values obtained by amniocentesis were statistically greater than those obtained by vaginal collection. However, when source of amniotic fluid and gestational age were included as independent variables in multiple regression analyses, the sample source was not significant and did not contribute to the variability in the data. When amniocentesis and vaginal pool samples were spiked with known quantities of SP-B, the recoverability was comparable. Amniotic fluid samples were obtained in six cases within 7 d after prenatal corticosteroid treatment and from three cases receiving chronic corticosteroids. The amniotic fluid indices of these nine steroid-exposed cases were not significantly different from those of the nonsteroid treated pregnancies. [SP-A], [SP-B], and L/S were significantly greater in amniotic fluid of black infants than of Caucasian infants despite statistically considering the gestational age at sampling, maternal hypertension, medications, or size of the infant (Fig. 4A). Maternal nutrition and prepregnancy weight were not considered in the current study. Amniotic fluid [SP-A] was significantly higher in amniotic fluid obtained from mothers who smoked cigarettes compared with that obtained from mothers who did not smoke (Fig. 4B). SP-B and L/S were not significantly affected by maternal smoking habits.

DISCUSSION

Human amniotic fluid concentration of SP-A and the L/S increased with advancing gestational age as previously reported (11, 13-18). The correlation of the L/S, PG, and [SP-A] with gestational age and fetal lung maturity reported in the present study was comparable to that described in the literature (8, 9, 14, 30). In addition, SP-B was detected in human amniotic fluid by ELISA. Like [SP-A] and L/S, amniotic fluid concentration of SP-B increased during the 3rd trimester of pregnancy. Amniotic fluid concentrations of SP-A and SP-B correlated with fetal lung maturity as assessed by the presence of RDS in the neonate. The predictive value of the amniotic fluid L/S and PG content for RDS was not improved by consideration of the surfactant-specific proteins SP-A and SP-B.

King *et al.* (17), in 1975, determined amniotic fluid content of surfactant apoproteins by latex agglutination assay, likely primarily detecting SP-A. The apoproteins were detectable as early as 29 wk gestation and increased with advancing gestation,

in association with increasing content of surfactant phospholipids. Similar ontogenic changes in SP-A and L/S were reported in amniotic fluid and in fetal rat lung (5-16). The results of the current study, presented in Figure 1, are consistent with detection of human fetal SP-A mRNA. Levels of SP-A mRNA were low or undetectable in 13- to 25-wk gestation human fetal lung explants before culture (31, 32). SP-A mRNA was first detected by *in situ* hybridization in human fetal lung at 16 wk gestation (33). The normative percentiles for [SP-A] and L/S presented in Figure 2 of the current study agree closely with the results of Hallman *et al.* (14) and Gluck *et al.* (8), respectively.

The ontogeny of SP-B in human pregnancy has not been previously reported. In the present study, ontogeny of SP-B in human amniotic fluid was similar but not identical to that previously demonstrated for amniotic fluid SP-A and L/S. SP-B was first detected and increased sharply after 32 wk gestation, several weeks after SP-A. In the present study, the surge in amniotic fluid SP-A appeared slightly later than the increase in L/S. Thus, the developmental appearance of SP-A and lecithin could be readily distinguished. Slavkin *et al.* (34) observed the appearance of lamellar bodies before detection of SP-A expression, suggesting that the surfactant apoproteins are regulated independently of the surfactant phospholipids and are not necessary for early lamellar body formation. Differences in timing of expression of SP-A and SP-B support the concept that the proteins are also independently regulated.

The L/S and the presence of PG in amniotic fluid are used routinely to estimate fetal lung maturity to optimize the management of high-risk pregnancies. A significant false-positive and false-negative rate occurs in L/S and PG testing as applied to the prediction of fetal lung maturity. Considerable variation exists in the timing of the L/S surge, especially between 33 and 37 wk of gestation. Many fetuses with L/S in the "transitional zone" (1.5-1.9) are falsely predicted to be at risk for RDS. In the current study, the frequency of RDS in neonates whose amniotic fluid L/S and PG content predicted fetal lung immaturity was only 45.5%, a positive predictive value consistent with previously published data (9, 11). The delivery of infants incorrectly predicted to be immature may be delayed, with potential detriment to mother and fetus. Infants with falsely immature L/S and PG assays may be needlessly subjected to surfactant replacement, a therapy that is showing promise for preventing or reducing the severity of RDS in prematurely born infants. Hallman *et al.* (14) reported enhanced accuracy of prediction of RDS when amniotic fluid [SP-A] was considered along with transitional zone values of L/S. However, the assessment of amniotic SP-A and SP-B in the current study did not improve the positive predictive value of the amniotic fluid tests.

The L/S may falsely predict fetal lung maturity, particularly in diabetic patients (35-37). Evaluation of PG in conjunction

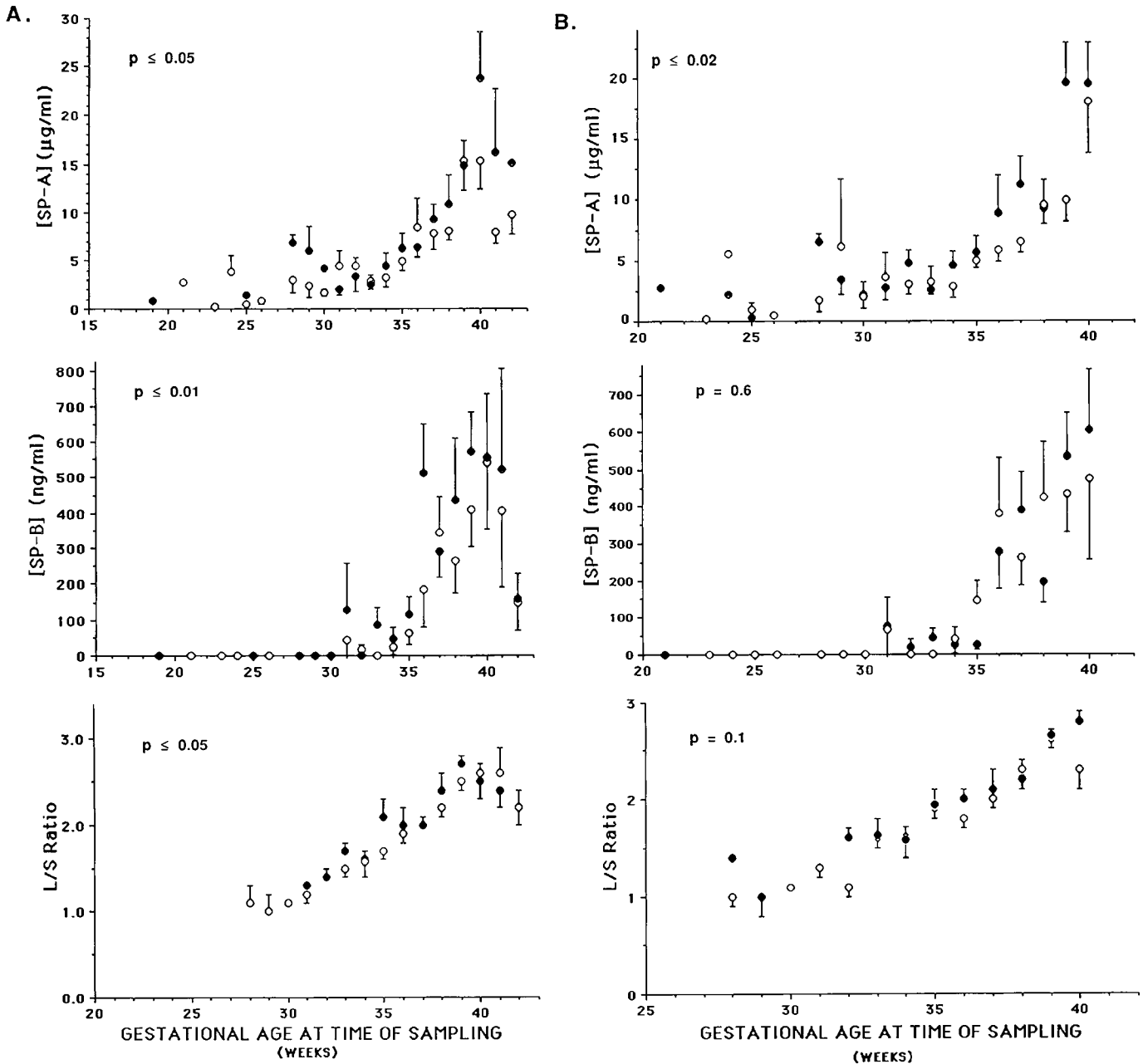


Fig. 4. Amniotic fluid indices stratified by race (A) (black, $n = 174$, closed circles; Caucasian, $n = 231$, open circles) and maternal smoking habit (B) (positive smoking history, $n = 215$, closed circles; negative smoking history, $n = 193$, open circles). The values are expressed as mean \pm SEM. Where standard error bars are not present, $n = 1$. Values from 33 pregnancies complicated by hydramnios or congenital anomalies were excluded. The p values represent the result of multiple regression analysis including (gestational age)², gestational age, and either race or maternal smoking habit as independent variables and using the logarithmic transformation of the surfactant indices.

with the L/S has been used to improve the estimate of fetal lung maturity in diabetic pregnancies. Katyal *et al.* (37) reported that measurement of surfactant apoproteins by ELISA yielded a better estimate of fetal lung maturity in diabetic patients than did the L/S. McMahan *et al.* (18) reported no difference in SP-A concentration in amniotic fluids of diabetic patients compared with controls pair-matched for gestational age. Hallman *et al.* (38) also reported no difference in SP-A content between diabetic and control amniotic fluid. In the current study, there was no difference in SP-A and SP-B content or L/S detected at given gestational ages in diabetic *versus* nondiabetic pregnancies. Current aggressive management of maternal diabetes may result in normal fetal pulmonary maturation (39).

When the data of the current study are analyzed as a whole, the amniotic fluid [SP-A], [SP-B], and L/S were not significantly affected by maternal exposure to medications including cortico-

steroids or tocolytics. It is interesting that patients A and B in Figure 3 both demonstrated a transient decrease and then increase in [SP-A] and an increase in [SP-B] in amniotic fluid sampled after receiving betamethasone and tocolytics. The data of the present study, however, are insufficient to support an association between maternal corticosteroid therapy and human amniotic fluid or fetal lung surfactant proteins.

The concentrations of SP-A and SP-B were altered in some cases of abnormal amniotic fluid volume or congenital anomaly. Pulmonary maturation may be altered in the fetus with anomalies due to either abnormal volume or composition of the amniotic fluid or secondary to the underlying congenital anomaly itself. In the present study, increased surfactant protein and phospholipid were noted in the presence of oligohydramnios and in the presence of cytomegalovirus infection; decreased surfactant proteins and phospholipids were noted in a single case of poly-

hydramnios and in the presence of osteogenesis imperfecta. It is likely that the changes in [SP-A], [SP-B], and L/S noted in pregnancies complicated by oligohydramnios or polyhydramnios reflect dilution effects or may simply be outliers of the normal curve. The L/S, with sphingomyelin as an internal control for volume, was unexpectedly altered in five of the patients with abnormal amniotic fluid or congenital anomaly. In the current study, there is not sufficient data to support an association between abnormal amniotic fluid volume or congenital anomaly and altered surfactant indices or fetal lung development.

Amniotic fluid SP-B was not detected in a large number of infants who had no evidence of respiratory distress. Although RDS rarely occurred if SP-B was detectable, SP-B was not detected in many infants with apparently mature lungs. SP-B mRNA has been detected in human fetal lung at 13 wk gestation and rises to 50% of adult level at 24 wk gestation (40). Significant variability in SP-B mRNA was noted in fetal human lungs from 22–24 wk (41). Several factors may account for the discrepancy in expression of SP-B mRNA in the fetal lung and detection of the SP-B peptide in amniotic fluid. The mature SP-B peptide is produced by proteolysis of a larger precursor protein that is not detected by the SP-B ELISA. It is possible that SP-B is expressed at earlier stages of development but that the active SP-B peptide is either not secreted or not proteolytically processed. Alternatively, SP-B from the fetal lung may be rapidly metabolized from the amniotic fluid. It is possible that normal lung function requires levels of SP-B production below that detected by the SP-B ELISA or that SP-B secretion is enhanced in the process of delivery. It is unlikely that amniotic fluid substances interfere with the SP-B ELISA. Known quantities of SP-B added to amniotic fluid samples were recovered in the ELISA.

[SP-A] was significantly elevated in amniotic fluid from mothers who smoked during pregnancy. [SP-B] and the L/S were not affected by maternal smoking. The mechanisms and significance of this finding have not been discussed. Alterations in uteroplacental blood flow and relative fetal hypoxia due to vasoconstrictive effects of nicotine and fetal accumulation of carboxyhemoglobin may result in advanced lung maturation in fetuses whose mothers smoke (42, 43). The elevation of SP-A in the amniotic fluid of mothers who smoked during pregnancy may be a fetal stress response to chronic hypoxia.

In the current study, [SP-A], [SP-B], and L/S were significantly greater in the amniotic fluid of black women than in that of Caucasian women, despite consideration of gestational age and other complications of pregnancy. Fadel *et al.* (44) found no difference in amniotic fluid lecithin between white and black fetuses. However, black infants have a lower incidence of RDS than white infants when rate of prematurity is taken into consideration (43, 45–47). The elevated amniotic fluid surfactant proteins and phospholipids reported in the current study are consistent with the apparent advanced pulmonary maturation in black infants. Further study is needed to determine if enhanced lung maturity in black infants is due to genetic differences between races or to confounding factors such as maternal nutrition that are influenced by race.

In summary, the present study demonstrates that amniotic fluid SP-B, like L/S and SP-A, increases during the last trimester of gestation and may be used to predict pulmonary outcome at birth. Measurement of SP-A and SP-B do not further enhance the specificity or sensitivity of predicting RDS conferred by L/S and PG. Surfactant protein SP-A and SP-B determinations alone do not provide the accuracy of the commonly used indices of pulmonary maturity, namely L/S and the presence or absence of PG. Differences in amniotic fluid content of SP-A in mothers who smoked and of SP-A and SP-B in black *versus* Caucasian women suggest possible differences in fetal pulmonary development warranting further investigation.

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