The concentration of surfactant protein-A in amniotic fluid decreases in spontaneous human parturition at term

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Abstract

Objective. The fetus is thought to play a central role in the onset of labor. Pulmonary surfactant protein (SP)-A, secreted by the maturing fetal lung, has been implicated in the mechanisms initiating parturition in mice. The present study was conducted to determine whether amniotic fluid concentrations of SP-A and SP-B change during human parturition.

Study design. Amniotic fluid SP-A and SP-B concentrations were measured with a sensitive and specific ELISA in the following groups of pregnant women: (1) mid-trimester of pregnancy, between 15 and 18 weeks of gestation ($n = 29$), (2) term pregnancy not in labor ($n = 28$), and (3) term pregnancy in spontaneous labor ($n = 26$). Non-parametric statistics were used for analysis.

Results. SP-A was detected in all amniotic fluid samples. SP-B was detected in 24.1% (7/29) of mid-trimester samples and in all samples at term. The median amniotic fluid concentrations of SP-A and SP-B were significantly higher in women at term than in women in the mid-trimester (SP-A term no labor: median 5.6 μg/mL, range 2.2–15.2 μg/mL vs. mid-trimester: median 1.64 μg/mL, range 0.1–4.7 μg/mL, and SP-B term no labor: median 0.54 μg/mL, range 0.17–1.99 μg/mL vs. mid-trimester: median 0 μg/mL, range 0–0.35 μg/mL; both $p < 0.001$). The median amniotic fluid SP-A concentration in women at term in labor was significantly lower than that in women at term not in labor (term in labor: median 2.7 μg/mL, range 1.2–10.1 μg/mL vs. term no labor: median 5.6 μg/mL, range 2.2–15.2 μg/mL; $p < 0.001$). There was no significant difference in the median amniotic fluid SP-B concentrations between women in labor and those not in labor (term in labor: median 0.47 μg/mL, range 0.04–1.32 μg/mL vs. term no labor: median 0.54 μg/mL, range 0.17–1.99 μg/mL; $p = 0.2$).

Conclusion. The amniotic fluid concentration of SP-A decreases in spontaneous human parturition at term.

Keywords: *Surfactant protein, SP-A, SP-B, amniotic fluid, term, labor, parturition*

Introduction

Pulmonary surfactant, a complex molecule matrix containing lipids (90%) and specific proteins (10%), reduces surface tension at the air–liquid interface of the lung [1–3]. Lipids in surfactant are critical for pulmonary function, and attention has shifted to the role of the surfactant proteins (SPs). Currently, four types of SP have been characterized: SP-A, SP-B, SP-C, and SP-D. SP-A and SP-D are relatively

hydrophilic, C-type collagenous lectins, involved in host defense and the regulation of surfactant structure or homeostasis [4–9]. In contrast, SP-B and SP-C are relatively hydrophobic and play important roles in surfactant homeostasis and function after birth [10–12]. SP-A is the most abundant of the SPs in human lung.

Recently, SP-A was implicated in a mechanism initiating parturition in mice [13]. The experimental basis for this is that: (1) the amniotic fluid

concentration of SP-A increased with advancing gestational age in mice; (2) the administration of SP-A into the amniotic cavity on E15 of gestation caused preterm delivery; (3) intra-amniotic injection of a neutralizing antibody to SP-A was associated with prolonged gestation [13]. The proposed hypothesis is that SP-A produced by the maturing fetal lung is secreted into the amniotic cavity where it induces local macrophage activation and infiltration of these cells into the uterine wall, leading to a pro-inflammatory response, suspension of progesterone action, and parturition [13].

It is unclear whether SP-A plays a role in the initiation of parturition in humans. The present study was conducted to determine whether human spontaneous parturition at term is associated with changes in amniotic fluid concentrations of SP-A and SP-B.

Materials and methods

Study design

A cross-sectional study was conducted to measure SP-A, SP-B, and total protein concentration in amniotic fluid samples obtained from women during the second trimester (15–18 weeks) of pregnancy ($n = 29$), at term (≥ 37 weeks of gestation) not in labor ($n = 28$), and at term gestation in spontaneous labor ($n = 26$). Amniotic fluid was obtained by transabdominal amniocentesis. The fluid was centrifuged and the supernatant stored. Patients with meconium-stained amniotic fluid samples were excluded. Second trimester amniotic fluid samples were obtained from patients who had consented to have an amniocentesis for prenatal diagnosis of genetic disorders. All patients from this group delivered a normal neonate at term. Women at term, but not in labor, underwent amniocentesis for the assessment of fetal lung maturity prior to cesarean section, whereas those in labor underwent amniocentesis because of either uncertain gestational age or for the diagnosis of intra-amniotic infection. All women at term had no evidence of medical or obstetrical complications, had negative amniotic fluid cultures for microorganisms, and delivered a normal infant with neonatal birth weight of more than 2500 g. Amniotic fluid not required for clinical assessment was centrifuged for 10 min at 4°C and the supernatant was aliquoted and stored at -70°C until analysis.

All women provided informed consent prior to the collection of amniotic fluid. The collection and utilization of the samples was approved by the Human Investigation Committee of the Sótero del Río Hospital, Santiago, Chile (a major affiliate of the Catholic University of Santiago), the Pennsylvania Hospital, and the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child

Health and Human Development. Many of these samples were used in previous studies of cytokines, matrix metalloproteinases and chemokines in human parturition [14–17].

ELISA for determining the SP-A and SP-B concentrations in amniotic fluid

SP-A and SP-B concentrations in amniotic fluid were determined by a sandwich ELISA system, which has been validated for amniotic fluid as previously described in detail [18,19]. The inter-assay coefficient of variation (CV) for SP-A was 33.5% and for SP-B was 34.2%, and the intra-assay CV was 7.7% for SP-A and 18.7% for SP-B. The sensitivity for SP-A was 10 ng/mL and for SP-B was 2 ng/mL.

Statistical analysis

The Mann–Whitney U-test was utilized to determine differences in the median between groups. Contingency tables and Chi-square tests were employed for comparisons of proportions. The statistical package used was SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

Results

SP-A was detected in all amniotic fluid samples. SP-B was detected in 24.1% (7/29) of mid-trimester samples (as early as 15 weeks of gestation) and in all samples at term. Table I summarizes demographic and clinical characteristics of the three study groups. As expected, the median maternal age of women in the mid-trimester group was significantly higher than women at term not in labor. Among women at term, there was no significant difference in median maternal age, gestational age at amniocentesis, and neonatal birth weight.

The median amniotic fluid concentrations of SP-A and of SP-B were significantly higher in women at term not in labor than those in the mid-trimester (SP-A term no labor: median 5.6 μg/mL, range 2.2–15.2 μg/mL vs. mid-trimester: median 1.64 μg/mL, range 0.1–4.7 μg/mL; $p < 0.001$ (see Figure 1a), and SP-B term no labor: median 0.54 μg/mL, range 0.17–1.99 μg/mL vs. mid-trimester: median 0 μg/mL, range 0–0.35 μg/mL; $p < 0.001$ (see Figure 1b)).

The median amniotic fluid concentration of SP-A in women at term in spontaneous labor was approximately 50% of that found in women at term not in labor (term in labor: median 2.7 μg/mL, range 1.2–10.1 μg/mL vs. term no labor: median 5.6 μg/mL, range 2.2–15.2 μg/mL; $p < 0.001$; see Figure 1a). There was no significant difference in the median amniotic fluid SP-B concentration between the two groups of women at term (term in labor: median 0.47 μg/mL, range 0.04–1.32 μg/mL vs.

Table I. Demographic and clinical characteristics.

Characteristics	Mid-trimester ($n = 29$)	$p^{\dagger}$	Term no labor ($n = 28$)	$p^{\ddagger}$	Term in labor ($n = 26$)
Maternal age, years	36 (26–40)	<0.001★	24 (14–42)	0.2	22 (15–36)
Gestational age at amniocentesis, weeks	16 (15–18)	<0.001★	39.3 (38–42)	0.3	40 (38–42)
Gestational age at delivery, weeks	39 (37–42)	0.6	39.3 (38–42)	0.3	40 (38–42)
Birth weight, grams	3460 (2866–4043)	0.3	3330 (2810–4470)	0.2	3325 (2540–3760)
Male gender, n (%)	17 (58.6%)	0.1	11 (39.3%)	0.03★	18 (69.2%)

Values are presented as median (range) or number (%). ★$p < 0.05$. †Comparison between mid-trimester and term no labor. ‡Comparison between term no labor and term in labor.
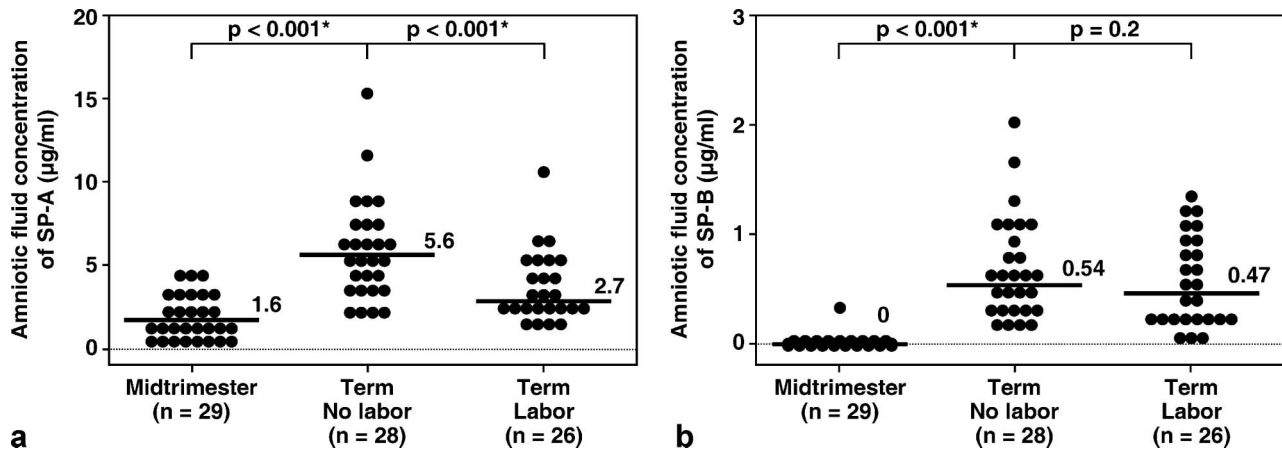

Figure 1. The median concentrations of SP-A and of SP-B in amniotic fluid were significantly higher at term than in the mid-trimester of pregnancy (SP-A term no labor: median 5.6 μg/mL, range 2.2–15.2 μg/mL vs. mid-trimester: median 1.64 μg/mL, range 0.1–4.7 μg/mL; $p < 0.001$, and SP-B term no labor: median 0.54 μg/mL, range 0.17–1.99 μg/mL vs. mid-trimester: median, 0 μg/mL, range 0–0.35 μg/mL; $p < 0.001$). The median amniotic fluid concentration of SP-A in women at term in spontaneous labor was significantly lower than in those not in labor (term in labor: median 2.7 μg/mL, range 1.2–10.1 μg/mL vs. term no labor: median 5.6 μg/mL, range 2.2–15.2 μg/mL; $p < 0.001$). There was no significant difference in the median amniotic fluid concentration of SP-B between women with and without labor (term in labor: median 0.47 μg/mL, range 0.04–1.32 μg/mL vs. term no labor: median 0.54 μg/mL, range 0.17–1.99 μg/mL; $p = 0.2$). ★$p < 0.05$.

term no labor: median 0.54 μg/mL, range 0.17–1.99 μg/mL; $p = 0.2$; see Figure 1b). The results remained similar after adjusting for total amniotic fluid protein content ($p = 0.001$ and $p = 0.2$, respectively; see Figure 2).

Although more male fetuses were included in the group of women at term in labor than in those not in labor (in labor: 69.2% (18/26) vs. not in labor: 39.3% (11/28); $p = 0.03$) (Table I), there was no significant difference in the median amniotic fluid concentration of SP-A and SP-B stratified by fetal gender in both the term in labor and term not in labor groups (all $p > 0.05$; see Figures 3 and 4).

Discussion

Principal findings of this study

(1) The median amniotic fluid SP-A concentration, but not SP-B, is lower in women with spontaneous labor at term than in women not in labor. (2) SP-A

and SP-B amniotic fluid concentrations are higher in term gestation than in the mid-trimester of pregnancy.

Physiology of SP-A and SP-B

SP-A is a large, oligomeric protein from the collectin family of C-type lectins, encoded by two genes (*SP-A1* and *SP-A2*) located on chromosome 10. SP-A plays important roles in host defense against infection and is required for the formation of tubular myelin, the major form of alveolar surfactant. SP-A binds bacteria, viruses, allergens, and apoptotic cells and functions as an opsonin, enhancing uptake by alveolar macrophages, monocytes, neutrophils, and dendritic cells [4,7,11,12]. SP-A may also induce or inhibit the production of cytokines and inflammatory mediators (e.g., tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β), reactive oxygen species, and nitric oxide metabolites depending on the types of pathogens or stimuli,
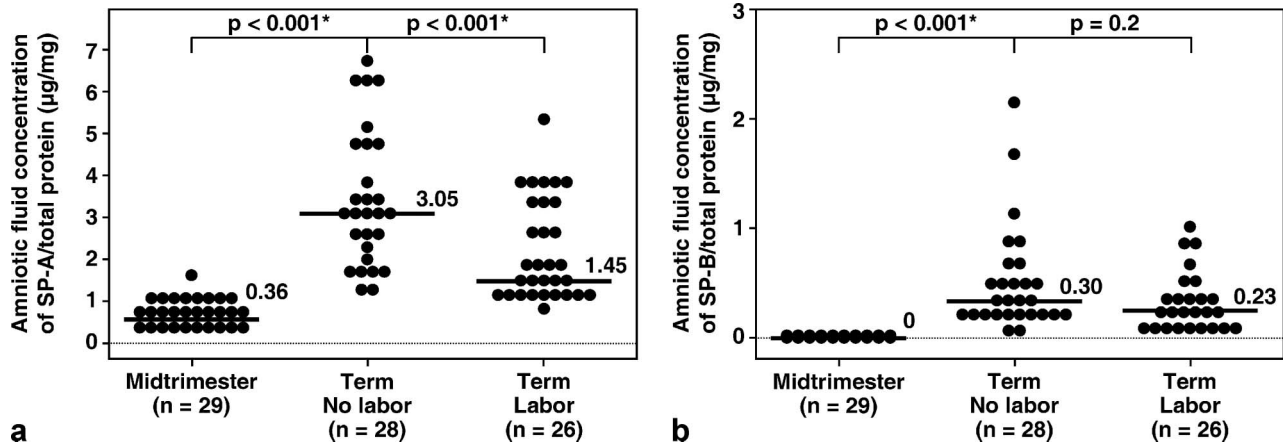
Figure 2. After adjusting for total protein concentration, the median amniotic fluid concentrations of SP-A and of SP-B were significantly higher at term than during the mid-trimester of pregnancy (SP-A/total protein, term no labor: median 3.05 μg/mg, range 1.15–6.87 μg/mg vs. mid-trimester: median 0.36 μg/mg, range 0.09–1.59 μg/mg; $p < 0.001$, and SP-B/total protein, term no labor: median 0.30 μg/mg, range 0.03–2.11 μg/mg vs. mid-trimester: median 0 μg/mg, range 0–0.08 μg/mg; $p < 0.001$). Amniotic fluid SP-A concentration after adjusting for total protein concentration in women at term in labor was lower than that for those not in labor (term labor: median 1.45 μg/mg, range 0.8–25.18 μg/mg vs. term not in labor: median 3.05 μg/mg, range 1.15–6.87 μg/mg; $p < 0.001$). There was no significant difference in the median amniotic fluid concentration of SP-B/total protein in women with and without labor (term labor: median 0.23 μg/mg, range 0.04–5.58 μg/mg vs. term not in labor: median 0.30 μg/mg, range 0.03–2.11 μg/mg; $p = 0.2$). One data point in the term labor group was an outlier (SP-B/total protein: 5.58 μg/mg) and is not shown in the figure. *$p < 0.05$.
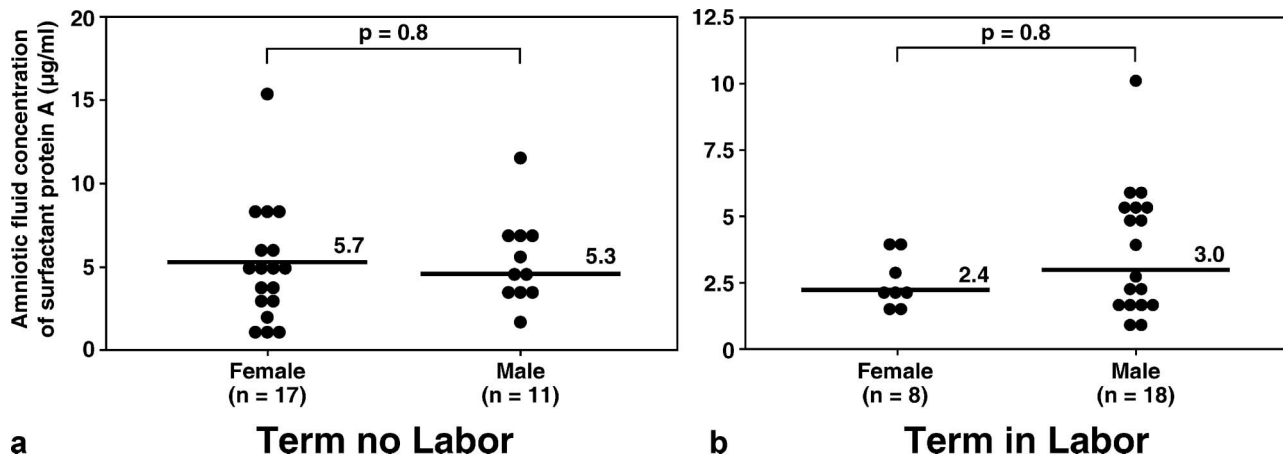

Figure 3. Amniotic fluid concentrations of SP-A in women at term with and without labor stratified by gender of the fetus. There was no significant difference in the median amniotic fluid concentration of SP-A stratified by fetal gender in both the term in labor and term not in labor groups (term no labor SP-A, female: median 5.7 μg/mL, range 2.2–15.2 μg/mL vs. male: median 5.3 μg/mL, range 2.6–11.7 μg/mL; $p = 0.8$, and term in labor SP-A, female: median 2.4 μg/mL, range 1.7–4.1 μg/mL vs. male: median 3.0 μg/mL, range 1.2–10.1 μg/mL; $p = 0.2$).

activation state of cells, and cellular receptors. The CD91–calreticulin complex, signal-inhibitory regulatory protein-α (SIRP-alpha), or Toll-like receptors (TLRs) 2 and 4 have been proposed as mediators of cellular responses to SP-A [7,12,20]. In the context of the host response of the lung, the immunomodulatory properties of SP-A might benefit the host [7,12]. For example, in the resting state, clearance of apoptotic cells or pathogens without invoking a robust inflammatory response may serve as a protective role in the lung. However, in the context of infection, the recruitment of immune cells or inflammatory mediators might be warranted [7,12]. While SP-A-deficient mice are susceptible to infection and lack tubular myelin, surfactant function does not significantly alter by the lack of SP-A [21,22]. SP-A has been detected in amniotic fluid and maternal and umbilical cord blood [23,24].

SP-B is a hydrophobic protein encoded by a single gene located on chromosome 2 [25]. SP-B plays a critical role in surfactant homeostasis by promoting adsorption of lipid surfactant into the expanding surface film and maintaining its stability during
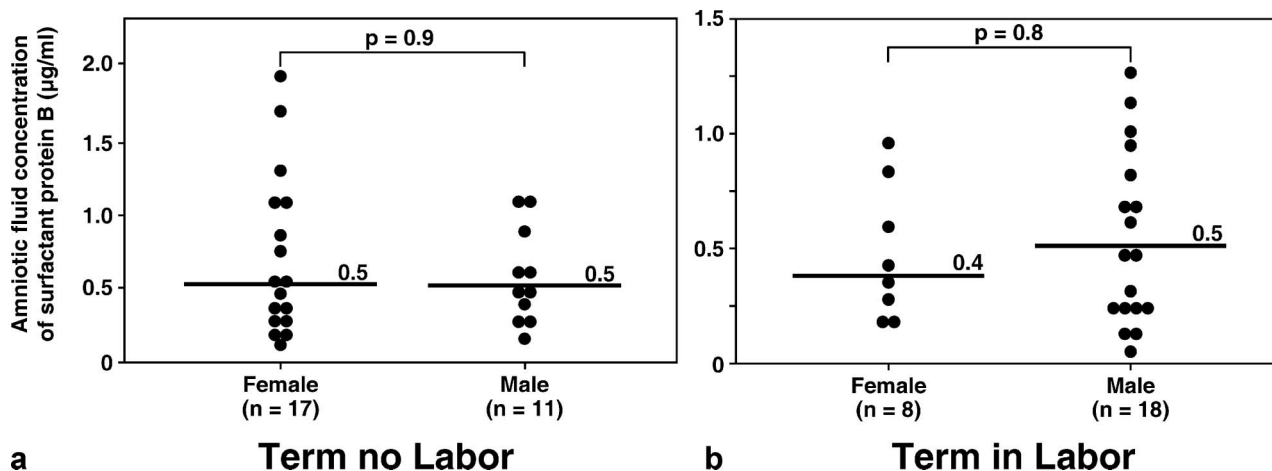
Figure 4. Amniotic fluid concentrations of SP-B in women at term with and without labor stratified by gender of the fetus. There was no significant difference in the median amniotic fluid concentration of SP-B stratified by fetal gender in both the term in labor and term not in labor groups (term no labor SP-B, female: median 0.5 μg/mL, range 0.2–1.9 μg/mL vs. male: median 0.5 μg/mL, range 0.2–1.1 μg/mL; $p = 0.9$, and term in labor SP-B, female: median 0.4 μg/mL, range 0.2–0.9 μg/mL vs. male: median 0.5 μg/mL, range 0.04–1.3 μg/mL; $p = 0.8$).

respiratory cycles to lower the surface tension within the alveolar spaces [1,26]. Infants with inherited deficiency of SP-B develop severe respiratory distress at birth [27]. SP-B has been detected in amniotic fluid and in adult serum [28].

Alveolar type II cells synthesize all four surfactant proteins and surfactant lipids. Surfactant proteins are also synthesized by airway cells, including Clara cells and submucosal cells. SP-A, but not SP-B, has been localized to non-pulmonary sites [12] including brain, trachea, testis, salivary glands, lacrimal glands, heart, prostate, kidney, pancreas, vagina [29], and human chorio-amniotic membranes [30], supporting the role of SP-A in host defense rather than reducing surface tension.

SP-A and SP-B in mid-trimester amniotic fluid

The finding that amniotic fluid concentrations of SP-A and SP-B in women at term are higher than in the mid-trimester of pregnancy is consistent with that of previous studies [18,30–33]. Pryhuber and colleagues [18] reported that amniotic fluid concentrations of SP-A and SP-B, and the lecithin–sphingomyelin (L/S) ratio increased with advancing gestational age. The mean amniotic fluid concentration of SP-A gradually increased after 19–23 weeks of gestation, followed by a quadratic increase between 30–40 weeks of gestation. In the current study, SP-A was detectable in the amniotic fluid at 15–18 weeks of gestation. SP-A expression has been detected by *in situ* hybridization in the human fetal respiratory tract by 16 weeks [18,34]. At that time SP-A protein and mRNA expression were observed only in the tracheal epithelium. However, expression by bronchiolar cells and pre-type II lining terminal airways

were not observed until 19–20 weeks of gestation [18,34]. It is possible that amniotic fluid SP-A in the early mid-trimester derives from an extrapulmonary origin, most likely chorioamniotic membranes. The presence of SP-A in the amniotic cavity at 17 weeks in a patient with fetal tracheal atresia (without tracheo-esophageal fistula) supports this view [35].

The observation that amniotic fluid SP-B was detectable in 24% of the mid-trimester samples and as early as 15 weeks of gestation was unexpected. However, all of the samples except one had low concentrations of SP-B. Contrary to the present findings, SP-B has been previously reported to be first detectable in amniotic fluid at 31 weeks of gestation [18]. SP-B mRNA and pro-protein have been detected in lung tissue as early as 13–15 weeks of gestation [36–38]. The mature SP-B protein has been detected after 19 weeks [37,38] and not consistently detectable until the 24th week when the epithelial cell lining begins to differentiate into type II cells containing lamellar bodies.

Amniotic fluid concentrations of SP-A and SP-B in women at term with and without labor

Animal studies suggest that SP-A is incorporated into myelinic and vesicular forms of extracellular surfactant [39] and that labor can stimulate the release of surfactant into the pulmonary airways [40,41]. In humans, the concentration of SP-A in umbilical cord blood is higher in newborns delivered after spontaneous labor than in those delivered by elective cesarean section [24]. Cho et al. [24] suggested that this could be due to an increased transfer of pulmonary SP-A to the fetal circulation

during labor. Our finding that the median amniotic fluid SP-A concentration was lower in women at term in labor than in those not in labor was unexpected and not consistent with the view that SP-A secreted by the lung may play a role in the onset of parturition. Condon et al. [13,42] proposed that SP-A could initiate parturition by directly stimulating amniotic fluid fetal macrophages to migrate into the uterine wall, where they would produce pro-inflammatory cytokines and activate nuclear factor kappa B leading to suspension of the progesterone action and uterine quiescence. However, there is no information available about the amniotic fluid concentrations of SP-A in mice in labor compared to those not in labor. A recent study in placental bed biopsy and postpartum hysterectomy specimens from pregnant patients with male fetuses reported that macrophages in human myometrium after labor are all maternal origin [43]. This study suggested that the trafficking of fetal macrophages in labor seems to be different between humans and mice. Although *in vitro* experiments provided evidence that surfactant lipids and SP-A could stimulate prostaglandin production in human amnion [44,45], there was no significant difference in SP-A mRNA expression in the chorioamniotic membranes of women at term not in labor and those in labor [35]. Finally, the observation that mice with a genetic deletion [46] or an over-expression [47] of the SP-A gene reproduce normally contradicts the role of SP-A as an essential factor of labor.

The current study does not exclude a role for SP-A in the mechanism of parturition. Human labor is associated with an increased production of prostaglandins and pro-inflammatory cytokines by intra-uterine tissue, amniotic fluid, and invading macrophages [48–51]. The decreased amniotic fluid concentration of SP-A may be due to the uptake by macrophages and other cells including amnion. However, there is no experimental evidence at this point that this is the case. Alternatively, SP-A could potentially suppress an inflammatory response in intrauterine tissues during normal gestation [7]. A decrease in amniotic fluid SP-A could be associated with an acute intrauterine inflammatory response and parturition [7]. A recent study indicated that human fetal membranes from women in spontaneous labor, but without histologic chorioamnionitis, had a gene expression pattern consistent with inflammation [52].

Another possible explanation for the observed lower amniotic fluid concentration of SP-A in women at term in labor than in those without labor is that SP-A may be incorporated into tubular myelin [39] in preparation for neonatal exposure to the noxious elements present in room air after birth.

Incorporation of SP-A into the complex surfactant molecule matrix present in the lung leads to a decrease in amniotic fluid SP-A concentration. In an animal experiment, a time-dependent movement of exogenous SP-A from extracellular components into pneumocyte type II cells was observed after intra-tracheal instillation of SP-A [39] suggesting that alveolar clearance and recycling of SP-A into type II cells or tubular myelin could occur in humans. Finally, since there is an increased concentration of neutrophil elastase in the amniotic fluid of women at term in labor [53], and the incubation of bronchoalveolar lavage fluid from patients with cystic fibrosis (that contained high concentration of protease) with SP-A caused a time-dependent degradation of SP-A [54], it is also possible that the decreased concentrations of SP-A in the amniotic fluid are due to the action of proteases during term labor [53,54].

Strengths and limitations of the study

This is the first study examining the question whether the concentrations of SP-A and SP-B in amniotic fluid change in human parturition. Since this is a retrospective cross-sectional study, the chronological events or causal relationships would be difficult to specify. Moreover, the ELISA assay system validated for amniotic fluid [18,19] had slightly high inter- and intra-assay coefficients of variation. So far, there has been no commercially available assay for surfactant proteins (except SP-D).

Conclusion

In summary, our study demonstrates that the amniotic fluid concentrations of SP-A, but not of SP-B, are lower in normal pregnancies at term in spontaneous labor than in those not in labor.

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