Lung liquid production rates and volumes do not decrease before labor in healthy fetal sheep

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Lines, A., S. B. Hooper, and R. Harding. Lung liquid production rates and volumes do not decrease before labor in healthy fetal sheep. J. Appl. Physiol. 82(3):927–932, 1997.—Previous studies have suggested that the volume and production rate of fetal lung liquid decrease late in gestation, before the onset of labor, in preparation for the clearance of lung liquid at birth. In contrast, our earlier studies have not shown a decrease in lung liquid volume near term, although these studies were not continued to the onset of labor. Our aim was to determine the changes in lung liquid volume and production rate in fetal sheep during the last 2 wk of gestation up to the onset of labor at term (~147 days). In eight chronically catheterized fetal sheep, the volume and production rate of fetal lung liquid were measured at 130, 135, and 140 days of gestation and then on every 2nd day until the onset of labor. Labor was detected by monitoring uterine muscle activity and intraterine pressure changes. On the day of labor onset, which occurred at 147 ± 1 days of gestation, fetuses weighed 5.0 ± 0.2 kg. The volume of fetal lung liquid was 40.4 ± 2.7 ml/kg at 19 ± 1 days before labor onset and had not significantly changed by 0.7 ± 0.2 days (44.8 ± 5.1 ml/kg) before labor. Similarly, lung liquid production rates at 19 ± 1 days before labor (5.1 ± 1.8 ml·h⁻¹·kg⁻¹) were not significantly different from those at 0.7 ± 0.2 days before labor (3.4 ± 0.7 ml·h⁻¹·kg⁻¹). We conclude that, in healthy ovine fetuses, lung liquid volumes and production rates do not decrease before the onset of labor. Our results indicate that the entire volume of fetal lung liquid (~222.5 ± 36.6 ml) must be cleared after the onset of labor.

Lung liquid volume; birth

Throughout gestation, the potential air spaces of the fetal lungs are filled with a liquid that is secreted by the pulmonary epithelium (24). It is now well established that an adequate volume of this liquid is necessary for the normal growth and development of the fetal lungs because it maintains them in an expanded state (1). However, this liquid must be cleared from the potential air spaces before the onset of air breathing at birth to establish effective gas exchange.

Reabsorption of liquid by the pulmonary epithelium is considered to play a major role in the clearance of lung liquid at birth (23). An important component of this reabsorptive process is thought to be an increase in circulating catecholamine (23, 34) and arginine vasopressin (25, 31) concentrations in the fetus during labor. It is proposed that these hormones activate luminal-surface Na⁺ channels, which lead to a reversal of the net ion flux across the lung epithelium, thereby promoting the uptake of liquid from the lung lumen (18, 23).

Previous studies have suggested that the volume and production rate of fetal lung liquid decrease up to 10 days before the onset of labor (10, 19), leading to the perception that the volume and production rate of fetal lung liquid decrease several days before the onset of labor (30). Although it has been suggested that such decreases may contribute to the clearance of lung liquid at birth, the mechanisms responsible for these decreases before labor onset are unknown and have not been investigated. In contrast to previous studies by others, our laboratory has been unable to find any evidence of a decrease in lung liquid volume or production rate in late-gestation fetal sheep (33). Indeed, collated results from numerous studies of fetal sheep under control conditions in our laboratory show that lung liquid volumes and production rates continue to increase up to 145 days of gestation (17); labor is expected at 145–147 days of gestation in this breed of sheep. However, our previous studies were not continued to the onset of labor, and, therefore, we were unable to determine whether a decrease in lung liquid volume would have occurred before labor onset. In this study, we wished to resolve the apparent discrepancies between our previous studies and those of others. Our aim was to determine whether the volume and production rate of fetal lung liquid decrease before the onset of spontaneous labor at term in healthy fetal sheep.

METHODS

Surgical preparation. Aseptic surgery was performed on eight pregnant Merino × Border Leicester ewes 121–125 days after they mated; term is 145–147 days. Anesthesia was induced with thiopentone sodium (1 g iv) and maintained with halothane (0.5–2.0% in O2 and N2O; 50:50 vol/vol). Two large-diameter saline-filled silicone rubber cannulas were inserted into the midcervical fetal trachea; one was directed toward the lungs, and the other was directed toward, but did not enter, the larynx (15). These cannulas were joined externally to form an exteriorized tracheal loop that allowed the normal movement of tracheal fluid. Polyvinyl catheters were inserted into a fetal jugular vein and carotid artery and into the amniotic sac; stainless steel wire electrodes (model AS632, Cooner) were also sewn into the uterine muscle for the detection of uterine electromyographic (EMG) activity (14). Ewes and fetuses were allowed to recover from surgery for at least 5 days before the start of experiments.

Experimental protocol. In each fetus, the volume and production rate of lung liquid were measured at 130, 135, and 140 days of gestation and then on every 2nd day until the onset of labor. The number of lung liquid volume and production rate measurements performed over the study period was considered to be the minimum required to detect changes during late gestation. Two blood samples were collected on the day of each lung liquid volume and production rate measurement to monitor arterial PO₂ (PaO₂), arterial PÇO₂, PaO₃, and arterial pH (pHₐ) and for the measurement of fetal plasma cortisol concentrations. The gestational age-related increase in fetal plasma cortisol concentrations provides confirmation of the normal timing of labor, whereas a premature increase in cortisol concentrations is indicative of...
a compromised fetus. Amniotic fluid and fetal tracheal pressures, from which amniotic fluid pressure was subtracted, were recorded on a polygraph throughout the study period. Myometrial EMG activity was also displayed on the polygraph.

Detection of labor. The onset of labor was determined from recordings of both uterine EMG activity and amniotic fluid pressure. Nonlabor uterine contractions (–0.5 to 1 contractions/h) were characterized by a 5- to 15-min burst of uterine EMG activity coinciding with a small rise (<2 mmHg) in amniotic fluid pressure (14). In contrast, uterine contractions associated with labor were more frequent (–6 contractions/h) and were characterized by discrete, brief (0.5- to 1-min) bursts of uterine EMG activity that coincided with increases in amniotic fluid pressure (<4–5 mmHg); the rises in amniotic fluid pressure increased in amplitude and frequency as labor progressed. Recordings of amniotic fluid pressure and uterine EMG were continued for at least 6 h after the first signs of labor were detected to ensure that the ewe was in labor. The ewe and fetus were then killed by an overdose of pentobarbital sodium (iv), and a postmortem examination was performed. An estimate of the time of labor onset was obtained from the polygraph recording.

Analytic methods. Fetal PaO2, PaCO2, and pH, corrected to core fetal body temperature (39°C), and percent oxygen saturation of hemoglobin (SaO2) were measured by using a blood-gas acid-base analyzer (model ABL30, Radiometer). Fetal lung liquid volumes and production rates were measured by using an established method of indicator dilution (15). Briefly, the fetal lung was isolated from the upper airway by connecting the descending tracheal catheter to a sterile 100-ml reservoir (open to the atmosphere via a bacterial filter) throughout the measurement period. Lung liquid was drained into the reservoir, and an impermeant indicator (200 mg; Dextran blue 2000, Pharmacia) was added; this dye does not enter the fetal circulation (15). The indicator was thoroughly mixed by repeatedly draining the lung liquid into the reservoir and then returning it to the lungs for 45 min. After the initial mixing period, lung liquid samples (1–6 ml) were collected at 15-min intervals for the next 90 min. The concentration of indicator in these samples was measured by using a multichannel absorbance meter (Titertek Multiskan, Flow Laboratories) set at a wavelength of 620 nm. We have calculated the error involved in this measurement to be 5–7% at the 95% confidence limit.

Combined amniotic and allantoic fluid volumes were measured at postmortem examination by draining and collecting all fluid surrounding the fetus. Fetal plasma cortisol concentrations were assayed by using an established method (6).

Data analysis. Although measurements of lung liquid production and volume were made at specific gestational ages in each fetus, all data were analyzed in relation to the time before the onset of labor because the age at labor onset differed among fetuses. Data from individual animals were grouped into specific time periods before the onset of labor, ensuring that the number of observations in each period were approximately equal (n = 4 or 5). The mean (± SE) time interval before the onset of labor was then calculated for each specific period, and this was used as a factor in the subsequent analysis of variance (ANOVA). The physiological data grouped into these time periods were PaO2, PaCO2, SaO2, pH, lung liquid volumes and production rates, and fetal plasma cortisol concentrations. These data were analyzed by a one-way ANOVA for repeated measures; the mean numbers of days before labor and animals used were as additional factors in the analysis. Where significant differences existed, the differences between values were identified by using a Student-Newman-Keuls test. The accepted level of significance for all statistical analyses was P < 0.05. All data in Figs. 1-4 have been presented with respect to the onset of labor.

RESULTS

Fetal outcome. After postmortem examination, two of the eight fetuses studied were excluded from further analysis for reasons stated below. However, the lung liquid volume and production rate data from these fetuses have been presented separately in the text and in Fig. 3. Of the six remaining fetuses, the mean gestational age at the onset of labor was 147 ± 1 days. Fetal PaO2, PaCO2, SaO2, and pH did not change during the 20 days before the onset of labor; the values of pH (7.365 ± 0.004), PaCO2 (43.9 ± 0.3 Torr), PaO2 (22.8 ± 0.4 Torr), and SaO2 (60.3 ± 2.7%) are typical of healthy fetuses in our laboratory. At postmortem, the mean body weight of fetuses in the study group was 5.0 ± 0.2 kg and the mean volume of amniotic and allantoic fluid was 1,228 ± 250 ml.

Two fetuses were excluded from the study group because, at the time of fetal death, they had been subjected to intrauterine conditions known to cause a decrease in lung liquid volume. One fetus was growth restricted (body weight was 3.3 kg, which is >3 SDs below mean weight of study group) and appeared to be confined to one uterine horn by a larger twin (weighing 5.0 kg), which occupied the other uterine horn and the majority of the uterine body. The trunk of the fetus was markedly flexed ventrally, and the amniotic and allantoic sacs contained only a small amount of viscous fluid, which is indicative of oligohydramnios. In addition, this fetus showed an asymmetric pattern of growth, having a smaller lung (26.6 vs. 31.4 ± 1.3 g/kg) and liver (14.6 vs. 24.0 ± 1.2 g/kg) than did fetuses from the study group. In the other fetus excluded from the study, only a small volume (430 ml) of viscous fluid could be collected from both sacs surrounding the fetus, which is indicative of oligohydramnios. Although we have no knowledge as to how long these conditions had existed, both trunk flexion (13) and oligohydramnios (9, 13, 27) are known to reduce fetal lung liquid volumes within 48 h (13).

Fetal lung liquid production rates. The mean rate of lung liquid production was 12.9 ± 4.5 ml/h (5.1 ± 1.8 ml·h⁻¹·kg⁻¹) at 19 ± 1 days before the onset of labor and gradually increased to 17.0 ± 3.7 ml/h at 0.7 ± 0.5 days before labor (Fig. 1A). Although the production rate, adjusted for fetal body weight, tended to be reduced just before labor onset (0.7 ± 0.5 days), the mean value measured at this time (3.4 ± 0.7 ml·h⁻¹·kg⁻¹) was not significantly different from any of the other measurements (Fig. 1B). The mean rate of lung liquid production during the 20 days before labor was 4.2 ± 0.1 ml·h⁻¹·kg⁻¹. Lung liquid production rates measured in one fetus excluded from the study group did not decrease before the onset of labor; the value measured on the day of labor (5.1 ml·h⁻¹·kg⁻¹) was similar to that measured 4 days before labor onset (5.0 ml·h⁻¹·kg⁻¹). In the other excluded fetus, however, the lung liquid production rate decreased from 3.0...
Fetal lung liquid volumes. The volume of lung liquid increased from 102.8 ± 10.9 ml at 19 ± 1 days before labor to 222.5 ± 36.6 ml at 0.7 ± 0.5 days before labor (Fig. 2A). Adjusted for fetal body weight, lung liquid volumes tended to increase from 40.4 ± 2.7 ml/kg at 19 ± 1 days to 44.8 ± 5.1 ml/kg at 0.7 ± 0.5 days before labor, although these changes were not significant (Fig. 2B). The maximum mean lung liquid volume during the 20-day study period was 49.8 ± 4.4 ml/kg at 3.8 ± 0.4 days before labor. Fetal lung liquid volumes measured just before labor (0.7 ± 0.5 days) were not significantly different from any values measured during the experimental period.

Lung liquid volumes measured in the two fetuses excluded from the study were substantially lower (>3 SDs) than volumes measured in the remaining fetuses just before labor onset. Although lung liquid volumes measured in the two excluded fetuses were similar to the volumes in the remaining fetuses for most of the study period (i.e., 41 ml/kg at 5 days before labor in one fetus and 47 ml/kg at 4 days before labor in the other), they markedly decreased just before labor onset (Fig. 3). Within 12–14 h before labor, lung liquid volumes were 19.0 ml/kg in one fetus and 29.8 ml/kg in the other.

DISCUSSION

The results of this study indicate that fetal lung liquid volumes and production rates do not significantly change before the onset of spontaneous labor at term in uncomplicated pregnancies. These findings support previous observations from our laboratory that the volume and production rate of fetal lung liquid do not decrease before 145 days of gestation (17, 33) and are in contrast to the findings of other studies (10, 19). All six fetuses included in our study group were considered to have experienced normal, uncomplicated pregnancies with no evidence of fetal compromise. This conclusion is based on fetal blood-gas and pH measurements, the pattern of fetal plasma cortisol concentration changes, gestational age at the onset of labor, fetal body and organ weights, and amniotic fluid volumes measured at postmortem. In these six fetuses, we found that the volume of lung liquid at 19 ± 1 days before labor (40.4 ± 2.7 ml/kg) was similar to that at 0.7 ± 0.2 days before labor (44.8 ± 5.1 ml/kg). Thus our findings
do not support previous studies that showed that the volume of fetal lung liquid decreases before the initiation of labor in sheep. Dickson et al. (10) reported that the volume of fetal lung liquid gradually decreases from 104.6 ml (±33.0 ml/kg) at 135 days to 70.2 ml (±17 ml/kg) at 142 days of gestation. They concluded that 80–90 ml of lung liquid remain to be cleared after the onset of labor (10). However, in that study, 5 of the 10 ewes delivered at 141–143 days of gestation and 4 delivered at 135–137 days of gestation (10). In contrast, although we used a similar breed of sheep, the mean gestational age at the onset of labor in our study was 147 ± 6 days of gestation, which is the expected full term in this breed of sheep. Thus the discrepancy between our findings and those of Dickson et al. (10) may relate to undetected fetal compromise (leading to premature labor) in the latter study or to undetected labor coinciding with the measurements of lung liquid volume and production rates; amniotic fluid volumes and indexes of uterine motility and labor were not monitored in the study of Dickson et al. (10).

It has also been reported that extravascular lung water content is reduced before the onset of labor in fetal rabbits (3). However, because the volume of lung liquid is principally controlled by fetal muscle activity, such as diaphragmatic contraction (20) and laryngeal adduction, which restricts lung liquid efflux (17), lung liquid volumes are reduced by ~50% when both sets of muscles become inactive (20), as occurs after death. Thus postmortem measurements of extravascular water content made in previous studies (4, 5) are unlikely to reflect in vivo measurements of fetal lung liquid volume.

The fetal lung liquid volumes measured in this study are in close agreement with measurements made over the last one-third of gestation in numerous previous studies in our laboratory (15–18, 20, 31–33). Our finding that fetal lung liquid volumes do not decrease before the initiation of labor is also consistent with reports of ultrastructural changes within the lung during late gestation (2). Major structural modification of the lung occurs during the final 2 wk of gestation, principally in response to the increase in circulating levels of cortisol and triiodothyronine (7). These structural changes, which include interalveolar wall thinning and reductions in connective tissue content, result in increases in the potential air space volume to 75% of total lung volume by term (2). Thus it is reasonable to assume that because lung liquid occupies the potential air spaces of the fetal lung, the volume of this liquid, relative to body weight, must also increase as the potential air space volume increases. Continued lung liquid production, combined with fetal breathing movements and the restriction of lung liquid efflux exerted by the upper airway, ensures that the lungs remain expanded late in gestation, as evidenced by the sustained pressure gradient between the lung lumen and the amniotic sac (17). Although fetal lung liquid volume may be reduced by any of a number of causes, we know of none that would be present in a healthy fetus that

Fig. 3. Mean fetal lung liquid volumes measured throughout last 20 days of gestation before onset of labor in study group of fetuses and individual data from 2 fetuses that were excluded from analysis (B108 and W347). These 2 fetuses were excluded because at postmortem examination they were both found to have been exposed to intrauterine conditions known to cause a decrease in lung liquid volume; both fetuses had been exposed to oligohydramnios, and 1 was also growth restricted. Day 0 is day that labor was first detected.

Fig. 4. Fetal plasma cortisol concentrations measured throughout last 20 days of gestation before onset of labor in study group of fetuses. Day 0 is day that labor was first detected. Values that are identified by different letters are significantly different from each other.
has an adequate volume of amniotic fluid in the absence of labor.

After postmortem examination, two fetuses were excluded from our study group because they appeared, at the time of fetal death, to have experienced intrauterine conditions known to reduce fetal lung liquid volume. Both fetuses were surrounded by reduced amounts of amniotic and allantoic fluids, and their trunks were markedly flexed; one fetus was growth restricted (3.3 kg), and most of the uterus was occupied by its very much larger twin (5.0 kg). Previous studies have demonstrated that oligohydramnios results in a reduction in fetal lung liquid volume (9, 13, 27). This reduction is thought to be caused by a reduction in intrauterine space, due to the loss of amniotic fluid, and the resulting fetal compression causes increased flexion of the fetal trunk (13). This imposed flexion of the fetus causes an increase in abdominal pressure, and hence intrathoracic pressure, which results in increased efflux of lung liquid via the trachea, thereby reducing the volume of lung liquid (13). The resultant decrease in lung liquid volume is the proposed mechanism by which oligohydramnios causes fetal lung hypoplasia (13).

The findings of our study also do not support reports that the production rate of fetal lung liquid decreases before the initiation of labor (10, 19). Kitterman et al. (19) found that the rate of production of lung liquid, as measured by its collection into an intrauterine bag, decreased 2 days before delivery. The authors suggested that this may have been due to a rise in fetal plasma cortisol concentrations, leading to increased conversion of norepinephrine to epinephrine; increased circulating concentrations of epinephrine are known to inhibit fetal lung liquid production (8, 16). However, we have recently demonstrated that prolonged cortisol infusions increase fetal lung liquid production rates (32), whereas removal of the fetal adrenal glands, which removes the primary source of endogenous cortisol in the fetus, reduces fetal lung liquid production rates (33). In the present study, a relationship was not evident between the exponential increase in fetal plasma cortisol concentrations (Fig. 4) and changes in either lung liquid volume or production rate late in gestation. Although the findings of our previous studies (32, 33) indicated that such a relationship may exist, the findings of the present study have not verified this relationship; we have no explanation for this discrepancy.

In the study of Kitterman et al. (19), three of the six control fetuses appeared to deliver prematurely (129–142 days), possibly as a result of undetected infections as suggested by the authors. Thus it is possible that some of the lung liquid production rate measurements made by Kitterman et al. (19) and Dickson et al. (10) were affected by the presence of undetected labor during their measurements; it is well established that labor causes an inhibition of lung liquid production and induces its reabsorption (8). Although it is known that lung liquid production rates can be affected by endocrine and metabolic factors during late gestation, ordinarily the influences of these factors are insufficient to inhibit lung liquid production rates in healthy uncompromised fetuses. It is interesting that, in the study of Kitterman et al. (19), lung liquid production rates decreased in all three fetuses that delivered prematurely but in only one of three fetuses that delivered at the expected gestational age.

The results of our study indicate that the clearance of lung liquid does not begin before the initiation of labor. Thus the total volume (~222.5 ± 36.6 ml) of lung liquid must be cleared after the onset of labor. During the latter stages of labor, the very high circulating concentrations of epinephrine (12, 22) and arginine vasopressin (25, 29) in the fetus are thought to play an important role in the reabsorption of lung liquid (8, 31). However, we consider that other mechanisms may also be involved. For instance, we observed in one fetus that 325 ml of liquid were present within the lung ~5 h before labor onset. Lung liquid reabsorption rates of up to 40 ml/h have been reported during labor and during epinephrine infusions (8, 34), indicating that it could take 8–9 h for this volume of liquid to be cleared by reabsorption. It is interesting that, in all fetuses, we could drain very little lung liquid (46 ± 31 ml) immediately before postmortem, which occurred ~8 ± 2 h after the onset of labor. Because none of our ewes was in the second stage of labor at the time of autopsy, and because large increases in circulating epinephrine concentrations occur only late in the second stage just before delivery of the head (8), we suggest that much of the liquid may be removed early in labor by mechanisms other than lung liquid reabsorption. One possibility is increased flexion of the fetal trunk, caused by uterine contractions (28) and shortening of uterine muscle, resulting in increased intra-abdominal pressure, increased intrathoracic pressure, and increased lung liquid efflux via the trachea (13). In addition to the reabsorption of lung liquid via activation of amiloride-inhibitable Na+ channels (18, 23), other mechanisms have been identified that could mediate the clearance of lung liquid at birth. Egan et al. (11) found that the pore size of the pulmonary epithelium increased from 5 (21) to 34–56 Å at the initiation of breathing after birth. In association with the large increase in pulmonary blood flow and decrease in pulmonary vascular resistance (26), these changes may also contribute to the reabsorption of lung liquid at birth.

In summary, we have found that the volume and production rate of fetal lung liquid do not decrease before the onset of labor in fetal sheep that have been exposed to normal, uncomplicated pregnancies. However, in pregnancies complicated by oligohydramnios, fetal lung liquid volumes were markedly reduced.

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