Published in final edited form as:

Reprod Sci. 2009 January ; 16(1): 88–93. doi:10.1177/1933719108324888.

Responses of Amniotic Fluid Volume and Its Four Major Flows to Lung Liquid Diversion and Amniotic Infusion in the Ovine Fetus

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Abstract

We designed experiments to allow direct measurement of amniotic fluid volume and continuous measurement of lung liquid production, swallowing, and urine production in fetal sheep. From these values, the rate of intramembranous absorption was calculated. Using this experimental design, the contribution of lung liquid to the control of amniotic fluid volume was examined. Fetuses were assigned to 1 of 4 protocols, each protocol lasting 3 days: control, isovolemic replacement of lung liquid, supplementation of amniotic fluid inflow by 4 L/day, and supplementation of amniotic inflow during isovolemic replacement of lung liquid. We found no effect of lung liquid replacement on any of the known flows into and out of the amniotic fluid. Although intramembranous absorption increased greatly during supplementation, the amniochorionic function curves were not altered by isovolemic lung liquid replacement. We conclude that lung liquid does not appear to contain a significant regulatory substance for amniotic fluid volume control.

Keywords

Fetal lung liquid; swallowing; intramembranous absorption

INTRODUCTION

Derangements of amniotic fluid volume often result in poor obstetric outcome.^{1–3} For example, severe oligohydramnios is associated with renal disorders, fetal malformations, and pulmonary hypoplasia whereas polyhydramnios is associated with premature delivery and fetal hypoxia due to severe fetal anemia. Because amniotic fluid volume derangements occur in less than 10% of the population,^{4,5} it is believed that amniotic fluid volume must be regulated. This concept is supported by a variety of studies in experimental animals.^{6–10} Although the mechanisms are currently unknown, amniotic fluid volume may be regulated by varying the rate of fetal urination, lung liquid secretion, swallowing, and/or intramembranous absorption.^{7,11} Of these 4 flows, modulation of intramembranous absorption appears to be the primary regulator of amniotic fluid volume.^{6,8,12}

Although numerous studies have been performed in an attempt to elucidate the factors responsible for the regulation of amniotic fluid volume, they have all been limited by the necessity to either eliminate 1 or more of the amniotic inflows or outflows or to make assumptions about their values. We have developed a preparation in which fetal urination,

swallowing, lung liquid secretion, and amniotic fluid volume are measured directly, allowing calculation of the rate of intramembranous absorption. Using this technique, we determined the contribution of lung liquid to the regulation of amniotic fluid volume.

MATERIALS AND METHODS

Ethical Approval

All experimental and surgical procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Oregon Health and Science University.

Surgical Procedures

Eight time-bred ewes with singleton fetuses were obtained from a local breeder. Surgeries were performed at approximately 120 days of gestation. After giving an intramuscular injection of 7.5 mg atropine, we administered 400 mg of ketamine and 10 mg of diazepam intravenously. The ewe was intubated and anesthesia maintained using a mixture of oxygen and nitrous oxide (3:1) with 2% isoflurane. This regimen also anesthetizes the fetus. Surgery was performed using sterile techniques. A midline abdominal incision was made to expose the uterus and an incision was made in the uterine wall over the fetal head. The amniochorion was sutured to the uterus and the fetal head was exposed. After a midline incision of the fetal neck, catheters were placed in a jugular vein and carotid artery and each catheter advanced 7 to 8 cm toward the fetal chest. One end of a 500-cm long catheter [internal diameter (ID) 2.5 mm] was placed in the fetal trachea. A modified "Wiffle ball"^{12,13} covered the opposite end and was attached locally to the fetal skin to allow continuous and unobstructed flow of lung liquid into the amniotic cavity until the time of the experiment. The Wiffle ball creates a porous sphere around the tip of the drainage catheter, preventing obstruction of the catheter tip by the fetus or fetal membranes. A perivascular flow probe (Transonic Systems, Ithaca, NY) was secured around the esophagus. The neck incision was closed and a catheter was sutured to the fetal neck for later measurement of amniotic fluid pressure.

The uterine incision and the amniochorion were closed with interrupted mattress sutures and a second running layer to maintain a watertight closure. A second uterine incision was made over the fetal hindquarters. The amniochorion was sutured to the uterus and the fetal hindquarters were exposed. The fetal abdomen and peritoneum were incised and the urachus was ligated to prevent urine inflow into the allantoic sac. A catheter with a silastic tip was placed into the fetal bladder and held in place using a purse string suture. The fetal peritoneal and abdominal incisions were repaired. A second Wiffle ball catheter and second amniotic fluid catheter were sutured to the fetal abdominal skin. A third Wiffle ball catheter and a third amniotic fluid catheter were attached to a hind leg. The membranes and uterine incision were closed in 2 layers as described above. The catheters were routed under the maternal skin to the flank where they emerged and were stored in a pouch attached to the skin of the ewe. The fascia transversa and the adherent peritoneum were closed with a running vertical mattress suture. The subcutaneous tissue was approximated with a running suture and the skin was closed with clips. A single dose of 1 million units of penicillin was injected into the amniotic fluid. The vascular catheters were filled with a saline solution containing 100 U of heparin/mL. Each ewe was given twice daily doses of 0.6 mg buprenorphine for 2 days for analgesia.

Experimental Protocols

After 4 to 5 days of postoperative recovery, the animals were placed in stanchions with free access to water and food. Experiments began the following day with fetal arterial blood samples for blood gases and pH. Fetal arterial, venous, and amniotic fluid pressures were measured for approximately 3 hours. The esophageal flow probe was connected to a Transonic flow meter

and continuous swallowing data were recorded for the duration of the experiment at a sampling rate 100/s. The manufacturer specifies an accuracy of $\pm 2\%$.

Animals were assigned to 1 of 4 protocols: (1) a control period during which lung liquid entered the amniotic sac; (2) external diversion of lung liquid away from the amniotic fluid and replacement by an equal flow of lactated Ringer's solution; (3) supplementation of amniotic fluid inflow with 4 L per day of lactated Ringer's solution; or (4) supplementation of amniotic fluid inflow with 4 L per day of lactated Ringer's solution during isovolemic replacement of lung liquid. The order of the protocols was randomized. Each of the 4 protocols lasted 3 days. In all, 4 fetuses completed 2 protocols and 4 completed all 4 protocols.

After completion of the experiments, the animals were killed by an intravenous injection of euthanasia solution (Euthasol, Virbac Corporation, Fort Worth, TX), as approved by the IACUC.

Measurements

Urine and lung liquid flow rates were measured continuously using a previously described technique.⁶ Briefly, to measure urine flow, the bladder catheter was connected to a small sterile bottle with 2 electrodes. The bottle was placed 6 in below the stanchion floor so all of the fetal urine would drain into the bottle. When the urine level rose high enough to contact both electrodes and complete an electrical circuit, a pump (Minipuls-3 roller pump, Gilson, Middleton, WI) was triggered and urine was pumped back into the amniotic cavity via the amniotic catheter attached to the fetal abdomen. A parallel circuit in the same roller pump moved an equal volume of fluid from a reservoir into a beaker for daily measurement of the average fetal urine flow rate. We calibrated random samples of the Gilson tubing and found them to be identical within 2%. The fluid volumes collected in the beakers were accurate within 5 mL.

To measure lung liquid flow rate, the tracheal catheter was cut at its midpoint. Lung liquid was drained from the tracheal end into a similar collection bottle with electrodes, which was positioned at the level of the stanchion floor. Using a second roller pump, the lung liquid was reinfused into the amniotic cavity through the Wiffle ball end of the catheter. The daily lung liquid volume was measured using a parallel circuit similar to that for measuring urine flow rate. During the period of lung liquid replacement, rather than returning fetal lung fluid to the amniotic fluid, it was diverted to a collection bottle. An equal volume of sterile lactated Ringer's solution was pumped into the amniotic fluid in its place through a parallel circuit in the roller pump. Thus, there was no change in the volume of fluid entering the amniotic sac during diversion of the lung liquid.

To initiate an experimental protocol, amniotic fluid was drained by evacuation through the 3 Wiffle ball catheters for 20 minutes. Previous necropsies demonstrated that this was sufficient time for complete drainage of amniotic fluid.^{6,8,12,13} After drainage, 1 L of warm lactated Ringer's solution containing 250 000 units of penicillin or 5 mg of gentamicin was returned to the amniotic compartment. Thus, all protocols started with the same volume of fluid. The rates of lung liquid flow and urine flow were measured daily for 3 days. We made a continuous record of swallowing. At the end of the 3-day period, fetal arterial blood samples were collected; arterial, venous, and amniotic fluid pressures were measured; and amniotic fluid volume was measured as described above.

Arterial blood pH, PCO₂, PO₂, and oxygen content were analyzed with an IL306 and IL482 system (Instrumentation Laboratories, Lexington, Mass) at 39°C. Hydrostatic pressures were measured using Transpac transducers (Abbott, Abbott Park, Ill) and a Macintosh-based recording system. The transducers were calibrated at the start of every protocol period against

a mercury manometer and zeroed at regular intervals. Intravascular pressures are reported with respect to amniotic fluid pressure. Heart rates were derived from arterial pressure pulsations. Continuous esophageal flow was measured using a T106 small animal flow meter (Transonic Systems Inc, Ithaca, NY) and recorded and stored using a Macintosh computer system. The volume of amniotic fluid swallowed by the fetus was calculated by integrating the area under the esophageal flow curve with baseline correction every 20 minutes.

Calculations

Intramembranous absorption rate was calculated as the difference between the inflows into the amniotic cavity (urine, lung liquid, and any added lactated Ringer's solution) and the outflow from the amniotic cavity (swallowing) and the change in amniotic fluid volume over the 3-day period.

Amniochorionic Function Curves

Amniochorionic function curves were generated by plotting the rate of intramembranous absorption versus amniotic fluid⁸ volume at the ends of the control and supplementation periods. To examine the role of lung liquid, a second set of function curves was generated by plotting intramembranous absorption versus amniotic fluid volume during diversion of lung liquid (and isovolemic replacement with lactated Ringer's solution) once with and once without supplementation.

Statistics

Statistical calculations were performed with a commercial software package (SPSS, Inc). Paired and unpaired *t* tests were performed as appropriate. If variances were unequal and the higher means also had higher variances, the data were log transformed. Data are reported as means $\pm$ SEM and differences were judged to be statistically significant when $P < .05$.

RESULTS

The fetuses maintained stable hemodynamic and arterial blood gas values over the duration of the study (Table 1).

Effect of Diversion of Fetal Lung Liquid from the Amniotic Compartment and Isovolemic Replacement with Lactated Ringer's Solution

We found no statistically significant differences in the rates of intramembranous absorption, urine flow, lung liquid flow, or swallowing when mean values over the 3-day control period were compared to data collected during the 3-day replacement of lung liquid. In addition, the amniotic fluid volume measured at the end of the control period did not differ from that measured during replacement of lung liquid (Table 2).

Effect of Supplementation of Amniotic Fluid Volume

Supplementation of amniotic fluid inflow with 4 L/day of lactated Ringer's solution led to statistically significant increases in urine flow ($P < .003$), intramembranous absorption rate ($P < .002$), and amniotic fluid volume ($P < .02$) when compared to control but the rate of lung liquid production was unaltered. Similarly, when we compared the data after replacement of lung liquid alone to the data after replacement of lung liquid and supplementation of amniotic fluid inflow, we found statistically significant increases in urine flow ($P < .001$), intramembranous absorption ($P < .004$), and amniotic fluid volume ($P < .03$) again with no change in lung liquid production. In both cases, the mean rate of swallowing increased during supplementation, but the increases were comparatively small and not statistically significant.

Effect of Replacement of Lung Liquid During Supplementation of Amniotic Fluid Volume

Neither amniotic fluid volume nor any of the 4 amniotic flows were altered by replacement of lung liquid during supplementation of amniotic fluid with lactated Ringer's solution (Table 2).

Effect of Lung Liquid Replacement on Amniochorionic Function Curves

Amniochorionic function curves for the 4 fetuses that completed all 4 protocols are shown in Figure 1. The relationship between amniotic fluid volume and the rate of intramembranous absorption was maintained within each fetus independent of the presence of lung liquid in the amniotic fluid. The average slopes of the function curves (control = 0.23 ± 0.06 and replacement = 0.24 ± 0.13) did not differ significantly.

Necropsy

The animals were euthanized at 137 ± 1 days of gestation. Five fetuses were male and 3 fetuses were female. The average fetal weight was 4524 ± 293 g. Two animals had unilateral right hydronephrosis. Because these animals had urine flows similar to the other fetuses, their data were included.

DISCUSSION

There are 2 major aspects to the present study. First, we have developed an animal model in which fetal urine flow, lung liquid flow, and swallowing can be measured continuously and simultaneously. Because there are only 2 major amniotic inflows and 2 major outflows, this model along with measured changes in amniotic fluid volume allows intramembranous absorption rates to be directly calculated. In previous studies, intramembranous absorption has been determined either during elimination of 1 or more of the normal amniotic flows (eg, by ligation of the esophagus) or by assuming that mean published values for some of the flows remained valid under the conditions of the experiment.

The second major aspect of the present study is that it directly addresses the possibility that fetal lung liquid contains a substance involved in the regulation of amniotic fluid volume.^{6, 13, 14} Making use of our ability to directly measure amniotic fluid volume, urine flow, lung liquid flow, and swallowing, we were able to demonstrate that there was no change in the amniotic fluid volume during isovolumic replacement of lung liquid. This was true under both control conditions and when the fetuses were challenged by supplementation of amniotic fluid with large volumes of lactated Ringer's solution. Thus, we conclude that lung liquid does not contribute a significant regulatory substance to the amniotic fluid. This conclusion is further supported by the lack of effect of lung liquid replacement on the amniochorionic function curves, that is, the relationship between the intramembranous absorption rate and amniotic fluid.

In general, the flows and volumes we have measured are similar to those values obtained by other investigators. Our amniotic fluid volumes obtained under control conditions and during lung liquid replacement are comparable to those measured by others.^{13–15} As expected, supplementation of the amniotic fluid led to an increase in amniotic fluid volume, similar to what we have demonstrated in the past.⁸ A similar increase in amniotic fluid volume occurs when the supplemental fluid volume is added to the fetal vascular compartment through intravascular supplementation,¹⁶ associated with an increase in urine production. Our rates of fetal urine production are comparable to what have been previously reported^{13, 14, 17} and also show large increases in response to addition of fluid to the fetal compartment.¹⁶ Our rate of lung liquid production is greater than that reported by Harding's group.^{18, 19} This difference is attributable to the larger size of our fetuses because, when expressed as a percentage of body weight, our lung liquid production rate of 9.3% per day is comparable to that of Harding's

group. Although our rate of fetal swallowing agreed with that measured by Fujino et al,¹⁵ they were less than those measured by other investigators in fetal sheep at comparable gestational ages.^{17,20–22} Although hypoxia has been shown to decrease fetal swallowing,^{17,23} our fetuses were not hypoxic and demonstrated no statistically significant changes over the course of the experiment in any parameter of arterial blood that we measured. A possible explanation could be fetuses normally swallow about half of the secreted lung liquid as it exits the trachea and enters the back of the mouth.¹⁷ Our catheter system delivered all of the lung fluid to the amniotic sac, making it unavailable for swallowing. There is also the possibility that the presence of both the flow sensor and the tracheal catheter in the fetal neck acted to diminish swallowing.

A final result of this study is that, because we were able to monitor each of the 4 major amniotic inflows and outflows, it is possible to put in perspective the contribution of each of these flows to the regulation of amniotic fluid volume. During supplementation, 12 L of lactated Ringer's solution was infused into the amniotic compartment over 3 days. However, amniotic fluid volume increased by an average of only 2 L, demonstrating a powerful regulatory response. Clearly, the most important volume regulator was intramembranous absorption as the absorption rate increased by nearly 13 L over the 3 days of supplementation. On average, fetal swallowing increased above basal levels by 1 L over the 3-day supplementation, making only a minor contribution to amniotic fluid volume regulation. Lung liquid secretion made no contribution to volume regulation as that secretion rate was unaltered during supplementation. In contrast, fetal urine production increased by more than 4 L above basal rates over the 3-day period and hence only detracted from amniotic fluid volume regulation.²³

ACKNOWLEDGMENTS

These experiments could not be performed without the technical assistance of Robert Webber, Ed Hedge, and Loni Socha. Funding for these experiments was provided in part by NIH grants 5R01HD035890, 5R01HL045043, and P01HD034430.

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Figure 1.

Relationship between the amniotic fluid volume and the rate of intramembranous absorption in 4 fetal sheep completing all of the experimental periods. The solid lines represent control values, with and without supplementation. The dashed lines represent replacement of lung fluid, with and without supplementation. Each symbol (bar, triangle, square, or circle) represents 1 fetus.

Table 1Comparison of Parameters of Fetal Well-Being Between First and Last Experimental Day^a

	Initial	Final
pH	7.368 ± 0.008	7.381 ± 0.006
PCO ₂ , mm Hg	52.1 ± 1.0	52.5 ± 0.8
PO ₂ , mm Hg	21.4 ± 1.4	20.0 ± 1.0
Oxygen content, ml/100ml ^b	8.0 ± 0.5	7.7 ± 0.7
Hematocrit, %	35 ± 1	37 ± 2
Heart rate, bpm ^b	182 ± 5	172 ± 12
Arterial blood pressure, mm Hg ^b	43 ± 1	47 ± 2
Venous blood pressure, mm Hg ^b	2.7 ± 0.7	4.4 ± 1.1

^a Values from 8 fetuses on the first experimental day and final experimental day. There were no statistically significant differences by paired *t* test for any of the data sets. Data are reported as means ± SEM.

^b N = 7 due to equipment failure.

Table 2
Effect of Replacement of Lung Liquid With and Without Supplementation on Amniotic Flows and Volume^a

	Control (n = 7)	Replacement (n = 7)	P	Supplementation (n = 5)	Supplementation and Replacement (n = 5)	P
Intramembraneous absorption (mL/h)	42.5 ± 6.5	46.8 ± 8.4	.57	217.8 ± 25.4	214.5 ± 30.6	.84
Swallowing (mL/h)	15.6 ± 2.1	16.0 ± 3.0	.81	31.8 ± 8.2	30.0 ± 6.7	.50
Urine (mL/h)	37.2 ± 4.6	40.1 ± 4.5	.68	101.2 ± 11.2	96.8 ± 11.2	.65
Lung (mL/h)	16.6 ± 2.5	18.3 ± 1.7	.29	20.0 ± 4.4	18.6 ± 2.6	.64
Amniotic fluid volume (mL)	703 ± 224	702 ± 157	.99	2888 ± 1052	2523 ± 884	.29

^aFlows were averaged over 3 days and the amniotic fluid volumes were measured at the end of 3 days. Data are reported as means ± SEM.