Published in final edited form as:

Am J Obstet Gynecol. 2009 March ; 200(3): 313.e1–313.e6. doi:10.1016/j.ajog.2008.10.025.

Amniotic Fluid Volume Responses to Esophageal Ligation in Fetal Sheep: Contribution of Lung Liquid

Juanita K. JELLYMAN, PhD¹, Cecilia Y. CHEUNG, PhD^{1,2}, and Robert A. BRACE, PhD^{1,2}

¹ Division of Perinatal Medicine, Department of Reproductive Medicine, University of California San Diego

² Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Oregon Health and Science University

Abstract

Objective—To determine the amniotic fluid volume (AFV) response to fetal esophageal ligation with and without fetal lung liquid entering the amniotic sac.

Study Design—AFV was measured in 3 groups of late gestation ovine fetuses: time controls, tracheo-esophageal shunted, and esophageal ligated.

Results—One day postsurgery, AFV was similar in all groups, averaging $1,064 \pm 66$ ml. On postsurgical day 9, AFV was unchanged in control fetuses, increased to $3,025 \pm 294$ ml in fetuses with esophageal ligation and lung liquid shunted into the fetal stomach, and to $3,437 \pm 430$ ml in fetuses with esophageal ligation and no shunting.

Conclusions—AFV expanded gradually following esophageal ligation to the highest volume thus far reported in non-infused ovine fetuses. Lung liquid entry into the amniotic sac altered neither the time course nor the extent of the AFV increase following esophageal ligation.

Keywords

ovine fetus; amniotic fluid volume regulation; lung liquid; swallowing; intramembranous absorption

INTRODUCTION

Fetal esophageal ligation or obstruction has been studied in experimental animals to gain insight into the regulation of amniotic fluid (AF) volume. In fetal sheep, esophageal ligation or obstruction has been reported to increase, decrease or have no effect on AF volume^{1,2,3}. Currently, there is no explanation for the differences. In recent esophageal ligation studies, fetal lung liquid was diverted from the AF into the fetal stomach with a shunt between the trachea and esophagus^{4,5,6}. Although unknown, lung liquid diversion may alter the AF volume response to esophageal ligation because late gestation ovine fetuses secrete a volume of lung liquid equal to 10% of body weight each day, half of which normally enters the amniotic sac and the other half is swallowed as it exits the trachea⁷. In addition to a direct volume contribution to the AF, fetal lung liquid may contain a substance(s) that modulates the rate of

Correspondence: Robert A. Brace, PhD, Department of Ob/Gyn, Mail Code L-458, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland OR 97239; email: E-mail: bracer@ohsu.edu.
Reprints: Not Available

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

intramembranous absorption. This possibility is suggested indirectly by observations that, in ovine fetuses, the AF volume response to continuous washout of AF with Ringer's solution depended upon the presence or absence of a tracheo-esophageal shunt that diverted lung liquid from the amniotic cavity into the fetal stomach^{5,8}. The possibility of a substance in the AF that modulates the rate of intramembranous absorption is further supported by the experimental observations that fetal polyuria and intra-amniotic saline infusions are associated with increased rates of intramembranous absorption. If present, the substance may be derived from fetal lung liquid^{9,10,11}.

In order to determine whether fetal lung liquid entry into amniotic sac alters the AF volume response to esophageal ligation, we conducted studies in chronically catheterized fetal sheep to test two hypotheses: 1) that AF volume would increase over time following either esophageal ligation or placement of a trachea-esophageal shunt, and 2) that the volume changes over time in shunted and ligated fetuses would be dependent on lung liquid entry. We also estimated the intramembranous absorption rate in order to explore whether changes in absorption rate might explain the differences in AF volume.

MATERIALS AND METHODS

These studies were approved by our Institutional Animal Care and Use Committees (IACUC) and were conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Surgical Preparation

Twenty pregnant sheep of a mixed western breed carrying a singleton fetus at 121 ± 1 (mean $\pm$ SE) days gestation (term=145–150 days) were instrumented with maternal and fetal arterial, fetal urinary bladder and amniotic catheters using previously described techniques^{4–7,9,12}. Anesthesia was induced with intravenous anesthetics and maintained with inhalation anesthetics in oxygen through an endotracheal tube. Midline abdominal and uterine incisions exposed the fetal hindquarters for placement of bilateral femoral arterial catheters and a suprapubic bladder catheter. Urachal ligation at the base of the umbilical cord prevented entry of fetal urine into the allantoic sac. Amniotic catheters were sutured to the fetal skin. The fetal membranes and uterus were closed in a watertight manner. Three fetuses underwent no additional surgery or instrumentation. In 4 fetuses, the fetal trachea and esophagus were exposed without placement of a shunt or ligation. Because statistical testing found no differences, these 7 animals were combined to form a control group. In 7 fetuses, the pulmonary end of the fetal trachea was connected to the gastric end of the esophagus with a short tube and the cranial ends of the trachea and esophagus were ligated^{4–6}. This shunt allowed liquid egress from the lungs into the fetal stomach without allowing lung fluid to enter the amniotic sac and simultaneously prevented fetal swallowing of AF. In 6 fetuses, the esophagus was ligated with a permanent suture. This ligation prevented not only the swallowing of AF but also the swallowing of lung liquid as it exited the trachea, resulting in twice the normal amounts of lung liquid entering the amniotic sac. All incisions were closed. A maternal femoral artery was catheterized. All catheters were exteriorized through the maternal flank and housed in a pouch sutured to the maternal skin. Ewes received intravenous fluids during surgery (lactated Ringer's solution with 5% dextrose). To replace potential AF loss during surgery, 500 ml of lactated Ringer's solution were infused into the amniotic sac shortly after surgery. Vascular catheters were flushed daily with a heparin-sodium solution (100 U/ml). Ewes received intramuscular antibiotics at the time of surgery (1,500,000 U penicillin G procaine). Antibiotics (500 mg ampicillin) were administered to the fetus via the amniotic cavity at surgery and for 4 days postsurgery.

Experimental Design

Animals were housed in individual pens with access to food and water *ad libitum* for the duration of the experiment. All fetuses were studied on postsurgery days 1, 3, 5, 7, and 9. AF volume was determined using indicator dilution techniques^{10,13} with technetium ^{99m}Tc. Maternal and fetal arterial blood, AF and fetal urine were sampled and analyzed for sodium, potassium, chloride, bicarbonate, glucose and lactate concentrations (Radiometer Model 725, Copenhagen, Denmark). Arterial pH, partial pressure of carbon dioxide (PCO₂), partial pressure of oxygen (PO₂), and hematocrit were determined in the blood samples (Radiometer Model 725). Fetal urinary flow rate was determined by gravity drainage for approximately one hour between 8 and 10 a.m. At this time of day, urine flow rates are not different from the 24 hour mean¹⁴. The daily urinary volume was calculated as the hourly volume multiplied by 24. Fluid compositions and urinary output were monitored in order to determine whether the differences in AF volumes could be related to variations in compositions and/or flows over the 9 day study.

On day 9, the animals were euthanized using an IACUC approved procedure. The total amount of fluid in the uterine cavity was collected and measured. Extensive effort was made to ensure membrane integrity, including careful inspection of the uterus and membranes and the absence of leakage of indigo carmine dye injected 30 minutes prior to autopsy. Animals with leakage were excluded from the study. The fetuses were weighed and autopsied. The neck incisions were examined to ensure integrity of the esophageal ligation and tracheo-esophageal shunts. Towards the latter part of the study, a few fetuses were noted to have unilateral or bilateral hydronephrosis at autopsy. As this has been reported to be a consequence of urachal ligation in male ovine fetuses,¹⁵ and because urinary output and composition were within normal ranges, these fetuses were included in the present study.

Intramembranous Absorption Rate Calculation

Changes in AF volume (Δ AFV) over time are related to the 4 primary amniotic inflows and outflows by the equation Δ AFV = UO + LL – SW – IM where UO = urine production rate, LL = rate of lung liquid entry into the AF, SW = volume rate of swallowing of AF, and IM = rate of intramembranous absorption. Because IM cannot be measured directly, it was calculated using the equation IM = UO + LL – SW – Δ AFV. In the shunted animals, the calculation provides an accurate determination of IM because both SW and LL were zero and UO as well as Δ AFV were measured. In the animals with esophageal ligation, SW was zero and we assumed LL equaled the average published value in ovine fetuses of 10% of body weight/day¹. For the control fetuses, we assumed SW equaled the average published value of 20% body of weight per day, and LL equaled 5% of body weight per day¹⁶. The difference between 10% versus 5% per day occurs because normally half of the secreted lung liquid is swallowed as it exits the trachea and does not enter the AF⁷ whereas all secreted lung liquid enters the amniotic sac with esophageal ligation. Daily fetal body weights were calculated from autopsy weights assuming a fetal growth rate of 3.4% per day¹⁷. Because of the use of mean values, the standard errors of the calculated SW and LL will underestimate the true variability because they result from variability in fetal body weights and do not include the normal physiologic inter-animal variations in flows.

Data Presentation and Statistical Analysis

Data are presented as mean $\pm$ SE. Bivariate regression analysis was used to determine whether AF volume was related to the volume of urine that entered the amniotic cavity or to AF or fetal osmolality or concentrations. One and 3 factor analyses of variance (ANOVA) were used to compare mean values over the 9 days within and among the 3 groups. A difference in response over time among groups was indicated by a significant interaction between time and treatment group. If the null hypothesis was rejected, Fisher's least significant difference for multiple

comparisons was used for post-hoc testing. Many maternal blood, fetal blood, AF, and fetal urinary variables normally change over time as the animals recover from surgery, with steady values typically beginning 3–5 days after surgery¹⁸. In this study, only changes that differed among the 3 groups over time are reported except as noted. Statistical significance was accepted at $P \leq 0.05$.

RESULTS

On day 1 postsurgery, AF volume was similar in all 3 groups, averaging $1,064 \pm 66$ ml (Figure 1). Volume did not change significantly with time in control fetuses and was 1151 ± 199 ml on day 9. In contrast, AF volume in shunted fetuses rose progressively to $3,025 \pm 294$ ml on day 9. This was significantly higher than the volume in the control group ($P < 0.001$). In the ligated animals, a similar gradual rise in AF volume to $3,437 \pm 430$ ml on day 9 occurred. This was not different from the volume increase in the shunted animals ($P = 0.34$) but was different from that in the control animals ($P < 0.001$).

Fetal urine flow rate increased over time ($P = 0.0084$) but the changes were not different among groups ($P = 0.43$). The total urine volume that entered the amniotic cavity between day 1 and day 9 was $5,928 \pm 504$ ml, $4,848 \pm 384$ ml and $3,960 \pm 384$ ml in the control, shunted, and ligated groups, respectively ($P = 0.027$). With post hoc testing, only the difference between the control and ligated fetuses was significant. Neither the change in AF volume from day 1 to day 9 nor the absolute AF volume on day 9 correlated significantly with the volume of urine that entered the amniotic cavity in the shunted and ligated groups.

The calculated intramembranous absorption rate for all three groups increased over time from 279 ± 45 ml/day on day 1 to 521 ± 62 ml/day on day 9 ($P = 0.0021$), but the changes over time were not different among the 3 groups ($P = 0.078$). The total volume of intramembranous absorption between days 1 and 9 in the control ($2,960 \pm 576$ ml), shunted ($3,136 \pm 480$ ml) and ligated ($3,568 \pm 384$ ml) fetuses did not differ significantly ($P = 0.69$).

The estimated volumes of lung liquid that entered the amniotic cavity over the 9 day study were $1,016 \pm 80$ ml, 0 ± 0 ml and $2,080 \pm 116$ ml, in control, shunted, and ligated fetuses, respectively. The estimated volumes of AF swallowed over 9 days were $4,080 \pm 328$ ml, 0 ± 0 ml, and 0 ± 0 ml in the control, shunted, and ligated fetuses, respectively.

During the 9 day study, maternal blood compositions were not different among the 3 groups (Table 1) and there were no significant differences in the changes over time. Similarly, there were no differences among the groups and no differences in the changes with time for fetal arterial pH, blood gases, electrolyte concentrations, or osmolalities (Table 1). The same pattern was true for AF compositions for the 3 groups. Although the changes over time were similar among the 3 groups, urinary sodium concentration was higher ($P = 0.014$) in the control fetuses (61.0 ± 6.4 mmol/L) than in the shunted (47.0 ± 4.9 mmol/L) and ligated (37.1 ± 2.8 mmol/L) fetuses while urinary potassium concentrations were lower in the controls (9.3 ± 2.0 mmol/L) compared to the shunted (20.2 ± 4.2 mmol/L) and ligated (32.2 ± 3.9 mmol/L) fetuses. Other urinary concentrations were similar among groups.

At autopsy on day 9, fetal weights in the control ($2,901 \pm 233$ g), shunted ($2,844 \pm 302$ g) and ligated ($2,963 \pm 165$ g) groups were comparable ($P = 0.94$). In addition, there was excellent agreement between the AF volume determined with indicator dilution techniques and the volume of AF collected at autopsy approximately 5 hours after the indicator dilution determination (Figure 2). The slope of the regression equation was not different from unity whereas the Y axis intercept of 188 ml was significantly greater than zero ($P < 0.01$).

COMMENTS

The AF volumes on postsurgical day 9 of approximately 3 liters in fetuses with either a tracheo-esophageal shunt or a ligated esophagus are unique in that they are the highest mean volumes so far reported in non-infused ovine fetuses. Such high volumes were not expected for several reasons. First, previous esophageal ligation, occlusion and shunt studies reported much lower AF volumes^{1,2,3}. Second, large increases in intramembranous absorption were expected to minimize the increase in AF volume. In normal (non-shunted and non-ligated) ovine fetuses, intra-amniotic infusions of physiologic saline or intravascular saline infusions with a concomitant polyuria are associated with large increases in intramembranous absorption^{9,10,11}. In shunted fetuses, when the AF was replaced with lactated Ringer's solution followed by Ringer's infusion into the amniotic sac, intramembranous absorption increased to an average of over 5,000 ml/day¹⁹ even though AF volumes were less than those observed in the shunted fetuses of the present study. This absorption rate is an order of magnitude greater than that of the shunted fetuses in the present study. The large difference in intramembranous absorption rates between the non-infused fetuses of the present study and the infused fetuses of previous studies¹⁸ is an important observation. A logical explanation would be that the AF contains a factor that inhibits intramembranous absorption and was diluted with infusion. However, the present study does not support the idea that the fetal lungs are the source. Further, if such an inhibitor exists, it would likely be a minor contributor because AF washout studies^{5,8} found only a modest change in AF volume in response to washout of AF with lactated Ringer's solution. Alternatively, swallowing may remove an inhibitory factor present in the AF that might accumulate following esophageal ligation. More studies are needed in order to decipher the cause of the large increases in intramembranous absorption rate during intra-amniotic infusion in contrast to the maintained low intramembranous absorption rate in fetuses not subjected to infusion.

In contrast to many reports of increased intramembranous absorption under various experimental conditions, there are only a few studies in addition to the present one in which intramembranous absorption rate changes minimally, leading to development of polyhydramnios. Intravascular lactate infusion in the ovine fetus produced polyuria and polyhydramnios that cannot be mimicked by intra-amniotic lactate infusion²⁰. Intravenous infusion of high but not low doses of Angiotensin I or Angiotensin II induced significant polyhydramnios in fetal sheep²¹⁻²⁴. These agents share a commonality in that lactate and angiotensin are believed to act at the placenta to attract fluid from the mother and produce excess amounts of fluid in the fetus^{21,22,25}, with lactate acting osmotically to attract water and angiotensin presumably attracting water due to precapillary vasoconstriction.

Similar to the rate of AF volume expansion in the present study, fetal esophageal occlusion beginning 5 days after surgery increased AF volume by 1 liter over in 3 days¹. The increase in AF volume after esophageal occlusion and the current data differ from previous reports that AF volume was unchanged following esophageal ligation³ and differ from our previous finding that AF volume decreased². At present we are unable to reconcile these differences and are unable to reproduce our previous results. We do note, however, that extreme care was taken in the present study to ensure that the fetal membranes were intact as the present study suggests a lack of membrane integrity in our previous study.

In the present study, although the rate of intramembranous absorption was directly determined in the shunted fetuses, the rates in the control and ligated fetuses were estimated. The resulting values should be interpreted cautiously because they are based on the mean values of lung liquid secretion and swallowing rates of normal fetuses. In control fetuses, the intramembranous absorption rate would be expected to remain stable after recovery from surgery so that the assumption of normal rates of swallowing and lung liquid secretion would

be logical when applied to data interpretation for that group. Further, the calculated rates of intramembranous absorption are similar to those recently measured in control fetuses²⁶. In the ligated group, the estimated intramembranous absorption rate depended largely on the value for lung liquid secretion rate. Although not previously measured in ligated fetuses, fetal lung liquid secretion rates during experimentation have been reported to only decrease or remain unchanged rather than increase²⁷. If secretion had indeed decreased in the present study as compared to our assumption of no change, then the calculated rate of intramembranous absorption would be reduced. This would strengthen rather than invalidate the conclusion that polyhydramnios occurred in the ligated group because the increase in intramembranous absorption rate was insufficient to offset the inhibition of fetal swallowing of AF.

In humans, fetal esophageal atresia or blockage has long been associated with polyhydramnios but there are exceptions²⁸. In monkeys, fetal esophageal ligation produced a transient polyhydramnios that resolved over time²⁹. In fetal sheep, AF volume was normal 3 weeks after esophageal ligation³. Although the exceptions in humans and the resolution in monkeys and sheep may be due to increased rates of intramembranous transport, the present study suggests that such increases do not occur within the first 9 days after esophageal ligation. Further study is needed to resolve possible increases in intramembranous transport over time following inhibition of fetal swallowing.

The present conclusions regarding AF volume changes following either fetal esophageal ligation or esophageal ligation with a trachea-esophageal shunt depend strongly on the accuracy of AF volume measurements. A comparison of indicator dilution volumes and autopsy volumes showed that the indicator dilution methodology is highly accurate over the entire range of volumes, with the dilutional volume being slightly larger by 188 ml than that collected at autopsy. This small difference likely is due to fluid remaining in the fetal wool at the time of autopsy, the volume of AF removed for sampling over the course of the experiment, and the small amount of fluid in the amniotic catheters.

In summary, the placement of a trachea-esophageal shunt that diverts secreted lung liquid into the fetal stomach and prevents fetal swallowing of AF produces a gradual but extensive polyhydramnios over 9 days. A similar gradual increase in AF volume occurs following esophageal ligation that prevents fetal swallowing of AF while allowing twice normal amounts of lung liquid to enter the amniotic cavity. Polyhydramnios occurs because intramembranous absorption does not increase sufficiently to offset the blockage of fetal swallowing. This contrasts with the large increase in intramembranous absorption rate that occurs during experimentally induced fetal polyuria or intra-amniotic fluid infusion. The stimulus that promotes the increase in intramembranous absorption in the latter studies and its absence in the present study has yet to be identified and is an important area for further studies.

Acknowledgments

Financial Support: National Institute of Child Health and Human Development Award HD 35890

We thank Richard Lee, Emilio Ramos and Jesse Trujillo for their technical assistance.

References

1. Fujino Y, Agnew CL, Schreyer P, Ervin MG, Sherman DJ, Ross MG. Amniotic fluid volume response to esophageal occlusion of fetal sheep. *Am J Obstet Gynecol* 1991;165:1620–6. [PubMed: 1750455]
2. Matsumoto LC, Cheung CY, Brace RA. Effect of esophageal ligation on amniotic fluid volume and urinary flow rate in fetal sheep. *Am J Obstet Gynecol* 2000;182:699–705. [PubMed: 10739533]

3. Wintour EM, Barnes A, Brown EH, Hardy KJ, Horacek I, McDougall JG, Scoggins BA. Regulation of amniotic fluid volume and composition in the ovine fetus. *Obstet Gynecol* 1978;52:689–93. [PubMed: 733137]
4. Faber JJ, Anderson DF. Absorption of amniotic fluid by amniochorion in sheep. *Am J Physiol Heart Circ Physiol* 2002;282:H850–4. [PubMed: 11834478]
5. Anderson D, Yang Q, Hohimer A, Faber J, Giraud G, Davis L. Intramembranous absorption rate is unaffected by changes in amniotic fluid composition. *Am J Physiol Renal Physiol* 2005;288:F964–8. [PubMed: 15821258]
6. Faber JJ, Anderson DF. Regulatory response of intramembranous absorption of amniotic fluid to infusion of exogenous fluid in sheep. *Am J Physiol* 1999;277:R236–42. [PubMed: 10409278]
7. Brace RA, Wlodek ME, Cock ML, Harding R. Swallowing of lung liquid and amniotic fluid by the ovine fetus under normoxic and hypoxic conditions. *Am J Obstet Gynecol* 1994;171:764 – 70. [PubMed: 8092226]
8. Yang Q, Davis L, Hohimer A, Faber J, Anderson D. Regulatory response to washout of amniotic fluid in sheep. *Am J Physiol Heart Circ Physiol* 2005;288:H1339–43. [PubMed: 15513961]
9. Gilbert WM, Brace RA. The missing link in amniotic fluid volume regulation: intramembranous absorption. *Obstet Gynecol* 1989;74:748–54. [PubMed: 2812652]
10. Brace RA. Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline. *Am J Obstet Gynecol* 1989;161:1049–54. [PubMed: 2529768]
11. Gilbert WM, Brace RA. Increase in fetal hydration during long-term intraamniotic isotonic saline infusion. *Am J Obstet Gynecol* 1988;159:1413–7. [PubMed: 3207117]
12. Gilbert WM, Brace RA. Novel determination of filtration coefficient of ovine placenta and intramembranous pathway. *Am J Physiol* 1990;259:R1281–8. [PubMed: 2260737]
13. Tomoda S, Brace RA, Longo LD. Fate of labeled albumin and erythrocytes following injection into amniotic cavity of sheep. *Am J Physiol* 1986;251:R781–6. [PubMed: 3766778]
14. Brace RA, Moore TR. Diurnal rhythms in fetal urine flow, vascular pressures, and heart rate in sheep. *Am J Physiol* 1991;261:R1015–21. [PubMed: 1928420]
15. Gobet R, Bleakley J, Peters CA. Premature urachal closure induces hydroureteronephrosis in male fetuses. *J Urol* 1998;160:1463–7. [PubMed: 9751394]
16. Ross MG, Brace RA. National Institute of Child Health and Development Workshop Participants. National Institute of Child Health and Development Conference summary: amniotic fluid biology--basic and clinical aspects. *J Matern Fetal Med* 2001;10:2–19. [PubMed: 11332413]
17. Brace RA, Gilbert WM, Thornburg KL. Vascularization of the ovine amnion and chorion: a morphometric characterization of the surface area of the intramembranous pathway. *Am J Obstet Gynecol* 1992;167:1747–55. [PubMed: 1471693]
18. Mellor, DJ. Investigations of fluid spaces of the sheep conceptus. In: Nathanielsz, PW., editor. *Animal Models In Fetal Medicine*. Elsevier; North Holland: 1980. p. 60-106.
19. Faber J, Anderson D, Hohimer R, Yang Q, Giraud G, Davis L. Function curve of the membranes that regulate amniotic fluid volume in sheep. *Am J Physiol Heart Circ Physiol* 2005;289:H146–50. [PubMed: 15749743]
20. Powell TL, Brace RA. Elevated fetal plasma lactate produces polyhydramnios in the sheep. *Am J Obstet Gynecol* 1991;165:1595–607. [PubMed: 1750453]
21. Anderson DF, Faber JJ. Animal model for polyhydramnios. *Am J Obstet Gynecol* 1989;160:389–90. [PubMed: 2644839]
22. Anderson DF, Borst CG, Faber JJ. Excess extrafetal fluid without demonstrable changes in placental concentration gradients after week-long infusions of angiotensin into fetal lambs. *Eur J Obstet Gynecol Reprod Biol* 1995;63:175–9. [PubMed: 8903774]
23. Moritz KM, Tangalakakis K, Wintour EM. Renal, hormonal, and cardiovascular responses to chronic angiotensin I infusion in the ovine fetus. *Am J Physiol* 1997;272:R1912–7. [PubMed: 9227607]
24. Moritz K, Koukoulas I, Albiston A, Wintour EM. Angiotensin II infusion to the midgestation ovine fetus: effects on the fetal kidney. *Am J Physiol Regul Integr Comp Physiol* 2000;279:R1290–7. [PubMed: 11003995]

25. Powell TL, Brace RA. Fetal fluid responses to long-term 5 M NaCl infusion: where does all the salt go? *Am J Physiol* 1991;261:R412–9. [PubMed: 1877698]
26. Brace RA, Vermin ML, Huijssoon E. Regulation of amniotic fluid volume: intramembranous solute and volume fluxes in late gestation fetal sheep. *Am J Obstet Gynecol* 2004;191:837–46. [PubMed: 15467551]
27. Olver RE, Walters DV, Wilson SM. Developmental regulation of lung liquid transport. *Annu Rev Physiol* 2004;66:77–101. [PubMed: 14977397]
28. Liley, AW. Disorders of amniotic fluid. In: Assali, NS., editor. *Pathophysiology of Gestation*. New York: Academic Press; 1972. p. 57-206.
29. Minei LKJ, Suzuki R. Role of fetal deglutition and micturation in the production and turnover of amniotic fluid in the monkey. *Obstet Gynecol* 1976;48:177–81. [PubMed: 821026]

Figure 1.

Amniotic fluid volume as a function of time after fetal surgery. Values are mean $\pm$ SE for control (open circle, $n = 7$), shunted (square, $n = 7$) and ligated (filled circle, $n=6$) fetuses.

Figure 2.

Comparison of the amniotic fluid volumes determined by indicator dilution techniques with amniotic fluid volumes collected at autopsy on the same day. Solid line is the regression equation ($Y = 188 + 0.983 X$), dotted lines are the 95% confidence intervals about the regression line, dashed lines are the 95% confidence values for individual values. Diagonal line from lower left to upper right corner is line of identity.

Table 1

Mean $\pm$ SE* values over 9 days in maternal arterial blood, fetal arterial blood, amniotic fluid, and fetal urine.

variable (units)	maternal blood	fetal blood	amniotic fluid	fetal urine
PCO ₂ (mmHg)	37.0 $\pm$ 0.4	51.7 $\pm$ 0.5		
PO ₂ (mmHg)	109 $\pm$ 1	23.6 $\pm$ 0.6		
Hematocrit (%)	30.5 $\pm$ 0.4	31.8 $\pm$ 0.4		
pH	7.459 $\pm$ 0.004	7.342 $\pm$ 0.005	7.16 $\pm$ 0.02	6.98 $\pm$ 0.04
Sodium (mmol/L)	146.3 $\pm$ 0.2	139.3 $\pm$ 0.2	122.5 $\pm$ 1.9	47.0 $\pm$ 3.1
Potassium (mmol/L)	4.26 $\pm$ 0.04	4.36 $\pm$ 0.09	6.84 $\pm$ 0.49	17.6 $\pm$ 2.6
Chloride (mmol/L)	112.6 $\pm$ 0.5	103.3 $\pm$ 0.6	95.2 $\pm$ 1.8	25.6 $\pm$ 3.1
Glucose (mmol/L)	3.63 $\pm$ 0.06	1.19 $\pm$ 0.04	0.36 $\pm$ 0.03	0.25 $\pm$ 0.01
Lactate (mmol/L)	0.69 $\pm$ 0.05	1.53 $\pm$ 0.06	3.17 $\pm$ 0.16	0.35 $\pm$ 0.02
Bicarbonate (mmol/L)	24.7 $\pm$ 0.3	26.2 $\pm$ 0.3	19.8 $\pm$ 0.6	7.3 $\pm$ 0.08
Osmolality (mosm/kg)	299.4 $\pm$ 0.5	297.3 $\pm$ 0.5	282.4 $\pm$ 1.5	189 $\pm$ 7

* n = 20. Value in each animal taken as mean over 9 day study.