Fetal Swallowing as a Protective Mechanism Against Oligohydramnios and Polyhydramnios in Late Gestation Sheep

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Robert A. Brace, PhD^{1,2}, Debra F. Anderson, PhD², and Cecilia Y. Cheung, PhD^{1,2}

Abstract

Our objectives were to (1) quantify the relationship between daily swallowed volume and amniotic fluid volume (AF volume) in late gestation ovine fetuses and (2) use the resulting regression equation to explore the role of swallowing in regulating AF volume. Daily swallowed volume ranged from 36 to 1963 mL/d while experimental AF volume ranged from 160 to 6150 mL ($n = 115$). Swallowed volume was near zero when AF volume was far below normal, a maximum of 635 ± 41 (standard error) mL/d when AF volume was 1682 ± 31 mL and did not increase further with higher AF volumes. Computer simulations predicted that fetal swallowing would (1) return AF volume to normal in 5 to 6 days following an acute volume change in the absence of changes in other amniotic inflows or outflows and (2) stabilize AF volume in 4 to 8 days following sustained alterations in amniotic inflows or outflows other than swallowing. Conclusions: The volume of AF swallowed each day by the fetus is a strong function of AF volume and reaches a maximum when mild polyhydramnios develops. With deviations in AF volume from normal, changes in fetal swallowing protect against oligohydramnios and polyhydramnios because the changes in swallowing over time reduce the extent of the AF volume change. However, with experimental changes in AF volume stabilizing in 1 to 2 days, it appears that swallowing is not the major regulator of AF volume.

Keywords

sheep fetus, amniotic fluid volume regulation, fetal swallowing, polyhydramnios, oligohydramnios

Introduction

Although it is generally assumed that swallowing of amniotic fluid (AF) by the fetus contributes to the regulation of AF volume,¹⁻⁵ the vice versa also occurs in that alterations in AF volume induce changes in fetal swallowed volume. Experimental studies in fetal sheep have shown that swallowed volume is reduced when AF volume is reduced⁶ and increased when AF volume is elevated.⁷ However, the changes in fetal swallowing over the full range of AF volumes have not been explored. Furthermore, although past studies suggest that fetal swallowing may be a function of AF volume, the contribution of fetal swallowing to the regulation of AF volume has not been studied except when swallowing is blocked.⁸⁻¹¹

The objective of this study was to determine the potential role that fetal swallowing plays in maintaining AF homeostasis over a wide range of experimental AF volumes. In order to achieve this objective, we first sought to develop an equation describing fetal swallowing over the full range of possible AF volumes. Computer simulations were used to integrate the resulting regression equation over time in order to predict the contribution of fetal swallowing to the regulation of AF volume for acute alterations in AF volume and for changes in amniotic inflows and outflows.

Materials and Methods

Ethical Approval

These studies were approved by our Institutional Animal Care and Use Committee (IACUC), and we followed the National Research Council's *Guide for the Care and Use of Laboratory Animals*.¹²

Because of large interanimal and intraanimal variability in daily swallowed volumes and the broad range of possible AF volumes, a large number of experimental observations were required in order to characterize the relationship between swallowing and AF volume. Thus, we combined data from ongoing experiments with unpublished data from previous studies^{13,14} in order to obtain 115 paired measurements of AF volume and daily swallowed volume.

¹ Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland, OR, USA

² The Heart Research Center, Oregon Health and Science University, Portland, OR, USA

Corresponding Author:

Robert A. Brace, Department of Obstetrics and Gynecology, Oregon Health and Science University, 3181 SW Sam Jackson Park Rd, Portland, OR 97239, USA.
Email: bracer@ohsu.edu

Experimental Preparations

Twenty-two late gestation singleton ovine fetuses were catheterized at 117 to 123 days of gestation as detailed elsewhere.¹³⁻¹⁵ Briefly, using inhalation anesthesia and aseptic techniques, polyvinyl catheters were placed in a fetal artery and vein. A catheter was placed in the fetal urinary bladder and the urachus was ligated to prevent urine entry into the allantoic sac. A flow probe (Transonic Systems, Ithaca, New York) was secured around the mid-cervical esophagus for measuring fetal swallowing.^{3,13,14,16} Additional catheters were attached to the fetal skin to allow access to the AF. In 9 fetuses, the trachea was catheterized through a mid-cervical incision, hence all secreted lung liquid entered the AF as it exited the trachea.

Experimental Procedures

Experiments began 5 to 6 days after surgery. The AF volume and average daily swallowed volume were measured over 1-, 2-, or 3-day intervals for a period of 5 to 12 days. Seven fetuses were monitored for 5 days under control conditions; 9 fetuses were subjected to 4 days of hypoxia after initial control measurements; and 6 fetuses were subjected to alternating 2 to 3 day control and intra-amniotic infusion periods. A total of 115 paired values of AF volume and daily swallowed volume were obtained, 62 under control conditions, 17 during intra-amniotic fluid infusion to increase AF volume (2 or 4 L/d for 2 or 3 days¹³), and 36 during fetal hypoxia.¹⁴ Fetal hypoxia was produced by infusing sufficient nitrogen gas supplemented with 1% to 2% carbon dioxide into the maternal trachea in order to reduce fetal blood oxygen content to half normal.¹⁴ The AF volume was measured either by drainage or by indicator dilution.^{13,14}

The esophageal flow signal was sampled at 100 Hz. The daily swallowed volume was calculated by integrating both positive and negative deviations from baseline in the esophageal flow signal over each 1-, 2-, or 3-day protocol and averaging over 24 hours. No agents were administered to experimentally stimulate or inhibit fetal swallowing.

The animals were euthanized at the end of the study using an IACUC approved intravenous euthanasia solution.

Predicted Contribution of Swallowing to AF Volume Homeostasis

The potential contribution of fetal swallowing to the maintenance of AF volume (AFV) was explored using an exponential equation derived from daily swallowed volumes and AF volumes. First, we calculated the changes in AF volume that would occur over time due to changes in swallowing following either a 50% increase or 50% decrease in AF volume assuming no other changes in amniotic inflows or outflows occurred. Second, we calculated the changes in AF volume over time following a sustained 200 mL/d increase or 200 mL/day decrease in nonswallowing amniotic flows. For these calculations, we integrated over time (t) the equation

$$AFV = AFV_0 + \int (SW - SW_0)dt + \int (UF - UF_0)dt$$

where SW is the swallowing rate (mL/time), UF the urine flow rate (mL/time) and the subscript 0 represents the initial value at time = 0. Predicted AF volume changes for a 200 mL/d increase in urine flow are the same as those for a 200 mL/d increase in lung liquid secretion or a 200 mL/d decrease in intramembranous absorption and vice versa.

From the computer calculations, the time for 95% of the response to occur was taken as an estimate of the time required for the response to be complete.

Data Presentation and Statistics

Experimental data were divided into control, hypoxia, and intra-amniotic infused groups. For purposes of comparison, the volume swallowed per 24 hours within each of the 3 groups was divided into subgroups for each 500 mL increment in AF volume (0-500, 500-1000, 1000-1500 mL, etc). A one-factor analysis of variance was used to compare mean swallowed volumes within the infused or hypoxic group with values in the control fetuses. Data were combined when there were no statistically significant differences among means.

Least squares regression was used to determine the statistical relationships between variables. Nonlinear least squares regression was used to fit either an exponential function or 2 interconnected straight lines to the daily swallowed volume and AF volume data. The exponential function was used because it provides a smooth function that may better represent underlying physiology. The term breakpoint is used to describe the swallowed volume and AF volume at which the 2 interconnected straight lines intersect. The breakpoint function was used because it provides a statistical value above which no further change occurs.

In order to normalize distributions and reduce skewing, daily swallowed volumes were base 10 logarithmically transformed and AF volumes were square root transformed prior to regression analysis. Statistical significance was accepted at $P \leq .05$.

Results

Values of swallowed volume ranged from 36 to 1963 mL/d while AF volume ranged from 160 to 6150 mL ($n = 115$). When subdivided for each 500 mL increment in AF volume, there were no statistically significant differences in daily swallowed volumes between the control and hypoxic fetuses or between the control and infused fetuses. Further, there were no differences in daily swallowed volumes under control conditions with or without tracheal catheterization, so all data were combined. The daily swallowed volume increased as AF volume increased above zero and then reached a plateau as AF volume increased further. Statistically, an exponential function (Figure 1A) or 2 straight lines (Figure 1B) fit the data equally well. The average maximum volume swallowed/d depended on

Figure 1. Daily swallowed volumes as a function of amniotic fluid volume. A, An exponential function fit to data: $Y = A + B 10^{(CX)}$; $A = 2.857$, $B = -2.042$, $C = -0.0315$. Plateau = 719 mL/d. B, A breakpoint function fit to data: $Y = A$ or $B + CX$; $A = 2.803$, $B = 1.917$, $C = 0.0216$. Breakpoint = 1682 mL, plateau = 635 mL/d. Y scale, logarithmic; X scale, square root. Solid lines are regression lines; dotted lines, 95% confidence intervals about the regression line; and dash lines, 95% prediction intervals about individual points.

the statistical model; the plateau in the exponential function was 719 mL/d compared with 635 mL/d for the breakpoint function.

Predicted Contribution of Swallowing to AF Volume Homeostasis

Figure 2 shows the computer-simulated changes in AF volume that would occur over time using the exponential equation from Figure 1A following a 50% increase or 50% decrease in AF volume assuming no changes in amniotic flows other than swallowing. For the increase in volume, 95% of the response was complete in 5.8 days. For the decrease in volume, 95% of the recovery occurred in 5.1 days.

The calculated changes in AF volume following a 200 mL/d increase or 200 mL/d decrease in net amniotic inflows and outflows other than swallowing are shown in Figure 3. Following a 200 mL/d decrease in urine production, decrease in lung liquid secretion or increase in intramembranous absorption, AF volume would decrease to zero in 3.5 days without compensation

Figure 2. Calculated changes in amniotic fluid volume following a 50% increase (A) or 50% decrease (B) in amniotic fluid volume assuming no change in amniotic inflows or outflows other than swallowing. Dashed lines show volumes without compensation by swallowing; dotted lines show basal values.

by reduced swallowing. In contrast, AF volume would expand indefinitely following the opposite changes in amniotic flows without swallowing compensation. With swallowing compensation, AF volume reached a stable value over time, with 95% of the volume change being complete in 8.4 days following net positive changes in flows compared with 4.0 days following net decreases in amniotic flows (Figure 3). For both changes in flows and changes in volume, the difference in times to 95% completion was due to the nonlinearity of the regression equation in Figure 1A, with the difference in times increasing for larger volume or flow disturbances and vice versa.

Discussion

There are several new findings in the present study. First, even with considerable day-to-day and fetus-to-fetus variability, there appears to be a fundamental underlying relationship between the volume of AF swallowed each day and the volume of AF that is present in the late gestation sheep fetus. Second, daily swallowed volume reaches a maximum as mild polyhydramnios develops and does not increase further with greater AF volumes. Third, the AF volume-dependent changes in fetal swallowing provide an effective mechanism for restoring AF volume to normal following a gain or loss of fluid as well as for stabilizing AF volume following a maintained alteration in fetal urine production, lung liquid secretion, or intramembranous absorption rate.

Figure 3. Calculated changes in amniotic fluid volume following a sustained 200 mL/d increase (A) or 200 mL/d decrease (B) in fetal urine production assuming no change in amniotic inflows or outflows other than swallowing and urine flow. Dashed lines show volumes without compensation by swallowing; dotted lines show basal values.

The conclusion that there is a functional relationship between daily swallowing volume and AF volume is a new observation made possible by the large range and number of values in the present study. Although important, there are clearly other factors involved in that AF volume explains only about 40% of the variations in daily swallowed volume.

Conceptually, the underlying relationship between swallowed volume and AF volume may be explained by the passive mechanical effects of fetal compression by the uterine wall as AF volume changes.¹⁷ That is, with low AF volume, the fetus would be compressed more than normal by the uterine wall and thus less able to open its mouth and ingest AF and vice versa. The finding that swallowed volume is at its maximal value when AF volume is far above normal is consistent with the concept that the fetus is free floating in AF without compression by the uterine wall when AF volume is high.¹⁷ Under this circumstance, the fetus is able to fully open its mouth to swallow AF. This potential mechanism differs only slightly from that proposed by Sherman et al¹⁸ in that it is not the availability of fluid (because fluid is available whenever AF volume is greater than zero) but rather it is the ability of the fetus to open its mouth combined with limited availability of AF that leads to the positive relationship between swallowed volume and AF volume. It is unknown whether a similar relationship exists between daily swallowed volume and AF volume in human fetuses although compression of the fetus by the uterine wall as AF volume decreases is well known.

There are few previous studies available for comparison with the current study and those that do exist compared swallowed volume for only one alteration in AF volume. Kullama et al⁶ measured the flow in the thoracic esophagus of fetal sheep and found that a reduction in AF volume from 415 to 157 mL produced by urine drainage reduced swallowing from 446 to 101 mL/d, values of swallowing approximately twice as high as predicted from the present study. This difference may be due to an intact urachus and the presence of allantoic fluid in their study compared with the absence of allantoic fluid after urachal ligation in our study. The presence of allantoic fluid would reduce compression of the fetus by the uterine wall and thus may allow swallowing of a larger volume of AF. Also, during long-term intravascular infusions of large volumes of physiologic saline into the ovine fetal circulation,⁷ AF volume increased modestly and the 24-hour swallowed volume increased more than would be predicted from the present study. However, when the infusion was terminated, AF volume remained elevated and swallowed volume decreased to values consistent with the present study. A possible explanation is that the intravascular fluid infusion may have induced increases in fetal swallowing activity.

Another new observation is that the fundamental relationship between fetal swallowing and AF volume allows for a quantitative understanding of the contribution that fetal swallowing makes to AF volume homeostasis in the absence of pathological conditions. From our calculations, following acute increases or decreases in AF volume, changes in swallowing would return AF volume to normal in 5 to 6 days assuming no other flow changes occur. Experimentally, AF volume returns to normal following acute volume disturbances in late gestation fetal sheep in 1 to 2 days.^{19,20} Although not studied in detail, alterations in AF volume following acute volume changes in human fetuses may follow a similar time course in view of the rapid loss of fluid following amnio-infusion for oligohydramnios²¹ and gain of fluid following amnio-reduction for polyhydramnios.²² Thus, the present calculations suggest that fetal swallowing contributes but is not the primary AF volume regulator. Instead, large changes in the rate of intramembranous absorption appear to be the predominant mechanism that regulates AF volume.^{13,15,23}

Integration of the regression equation for sustained moderate increases or decreases in fetal urine production also suggests that the AF volume-dependent changes in fetal swallowing would stabilize AF volume albeit at nonnormal volumes. Although we know of no comparable experimental studies, Wlodek et al²⁴ found that AF volume decreased from 803 to 165 mL after draining 5 to 11 L of fetal urine over 11 days. From our simple calculations, AF volume would be reduced from 700 to 392 mL when fetal urine production was reduced by 2 L over 10 days.

In summary, the volume of fluid swallowed daily by the late gestation ovine fetus is a strong function of AF volume and this relationship changes little under various steady-state, long-term experimental conditions. Changes in the volume swallowed contribute importantly, even though passively, to the regulation

of AF volume when AF volume is altered over the range from near zero to approximately 2000 mL. However, when the swallowed volume approaches its maximum at an AF volume of 2000 mL, further increases in AF volume are not associated with increases in the volume of AF swallowed by the fetus. These AF volume-dependent changes in fetal swallowing help return AF volume to normal following acute volume alterations and stabilize AF volume following sustained alterations in fluid entry into or exit from the amniotic compartment and thus are protective against oligohydramnios and polyhydramnios.

Authors' Note

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Declaration of Conflicting Interests

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References

- Gitlin D, Kumate J, Morales C, Noriega L, Arevalo N. The turnover of amniotic fluid protein in the human conceptus. *Am J Obstet Gynecol.* 1972;113(5):632-645.
- Abramovich DR. Interrelation of fetus and amniotic fluid. *Obstet Gynecol Annu.* 1981;10:27-43.
- Harding R, Sigger JN, Poore ER, Johnson P. Ingestion in fetal sheep and its relation to sleep states and breathing movements. *Q J Exp Physiol.* 1984;69(3):477-486.
- Brace RA. Amniotic fluid volume and its relationship to fetal fluid balance: review of experimental data. *Semin Perinatol.* 1986;10(2):103-112.
- Ross MG, Nijland MJ. Fetal swallowing: relation to amniotic fluid regulation. *Clin Obstet Gynecol.* 1997;40(2):352-365.
- Kullama LK, Agnew CL, Day L, Ervin MG, Ross MG. Ovine fetal swallowing and renal responses to oligohydramnios. *Am J Physiol.* 1994;266(3 pt 2):R972-R978.
- Brace RA. Fetal blood volume, urine flow, swallowing and amniotic fluid volume responses to long term intravascular saline infusions. *Am J Obstet Gynecol.* 1989;161(4):1049-1054.
- Wintour EM, Barnes A, Brown EH, et al. Regulation of amniotic fluid volume and composition in the ovine fetus. *Obstet Gynecol.* 1978;52(6):689-693.
- Fujino Y, Agnew CL, Schreyer P, Ervin MG, Sherman DJ, Ross MG. Amniotic fluid volume response to esophageal occlusion in fetal sheep. *Am J Obstet Gynecol.* 1991;165(6 pt 1):1620-1626.
- 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(3):699-705.
- Jellyman JK, Cheung CY, Brace RA. Amniotic fluid volume responses to esophageal ligation in fetal sheep: contribution of lung liquid. *Am J Obstet Gynecol.* 2009;200(3):313.e1-e6.
- Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals, 8th ed. Washington, DC: National Academies Press. 2010.
- Robertson P, Faber JJ, Brace RA, et al. Responses of amniotic fluid volume and its four major flows to lung liquid diversion and amniotic infusion in the ovine fetus. *Reprod Sci.* 2009;16(1):88-93.
- Thurlow RW, Brace RA. Swallowing, urine flow, and amniotic fluid volume responses to prolonged hypoxia in the ovine fetus. *Am J Obstet Gynecol.* 2003;189(2):601-608.
- 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(1):H146-H150.
- Bradley RM, Mistretta CM. Swallowing in fetal sheep. *Science.* 1973;179(4077):1016-1017.
- Shields LE, Brace RA. Fetal vascular pressure responses to non-labor uterine contractions: dependence on amniotic fluid volume in the ovine fetus. *Am J Obstet Gynecol.* 1994;171(1):84-89.
- Sherman DJ, Ross MG, Day L, Humme J, Ervin MG. Fetal swallowing: response to graded maternal hypoxemia. *J Appl Physiol.* 1991;71(5):1856-1861.
- Tomoda S, Brace RA, Longo LD. Amniotic fluid volume regulation: basal volumes and responses to fluid infusion or withdrawal in sheep. *Am J Physiol.* 1987; 252(2 pt 2):R380-R387.
- Brace RA, Cheung CY. Amniotic fluid volume responses to amnio-infusion of amniotic fluid versus lactated Ringer's solution in fetal sheep. *J Soc Gynecol Investig.* 2004;11(6):363-368.
- Stefos T, Staikos I, Plachouras N, Dousias V. Serial saline amnioinfusion from 16th week of gestation resulted in successful outcome of pregnancy: report of two cases. *Eur J Obstet Gynecol Reprod Biol.* 2005;122(2):250-251.
- Moise KJ Jr, Dorman K, Lamvu G, et al. A randomized trial of amnioreduction versus septostomy in the treatment of twin-twin transfusion syndrome. *Am J Obstet Gynecol.* 2005;193(3 pt 1):701-707.
- Gesteland KM, Anderson DF, Davis LE, Robertson P, Faber JJ, Brace RA. Intramembranous solute and water fluxes during high intramembranous absorption rates in fetal sheep with and without lung liquid diversion. *Am J Obstet Gynecol.* 2009; 201(1):85.e1-e6.
- Wlodek ME, Harding R, Thorburn GD. Fetal-maternal fluid and electrolyte relations during chronic fetal urine loss in sheep. *Am J Physiol.* 1992;263(4 pt 2):F671-F679.