Regulatory response to washout of amniotic fluid in sheep

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Regulatory response to washout of amniotic fluid in sheep. Am J Physiol Heart Circ Physiol 288:H1339–H1343, 2005. First published October 28, 2004; doi:10.1152/ajpheart.00740.2004.—To test the hypothesis that a substance present in the amniotic fluid could serve as a regulator of amniotic fluid volume, we drained and discarded amniotic fluid while replacing it with lactated Ringer solution that was isotonic to amniotic fluid. Seven ewes with singleton fetuses at 119 days of gestation (mean ± SE) were instrumented with multiple indwelling catheters in the pedal artery, pedal vein, and amniotic cavity. During the exchange periods, an average of 3,019 ± 171 ml/day of lactated Ringer solution was infused into the amniotic cavity while an equal amount of amniotic fluid was pumped out and discarded. During the control period, amniotic fluid composition and volume were not altered. Exchange and control periods started with the same amniotic fluid volume, lasted 3 or 4 days, and were randomized with regard to order. Amniotic fluid volume measured by vacuum drainage was 556 ± 98 ml at the end of the control period and 986 ± 209 ml (P = 0.03) at the end of the exchange period. Fetal arterial blood gases, hemodynamic parameters and the osmolality gradient between fetal plasma and amniotic fluid were not altered by the exchange process. A linear relationship between the control amniotic fluid volume and the volume at the end of the exchange period (P = 0.003) suggests that the animals with larger control volumes responded to isovolumic dilution with a larger volume increase. We conclude that amniotic fluid may contain a substance that regulates amniotic volume.

Furthermore, intramembranous absorption of amniotic fluid shows a large regulatory response to an abnormal inflow of exogenous amniotic fluid (7).

The term regulatory response is used for a response that is in a direction that counteracts the initial change and is of sufficient magnitude to assist in restoring volume to its proper value. Although lung fluid, urine flow, and swallowing are regulated by the fetus, esophageal ligation does not routinely result in increased amniotic fluid volume (16), and neither lung fluid production nor swallowing is necessary for an adequate homeostatic response to abnormal production of amniotic fluid (7). Furthermore, lung fluid production and urine flow often do not change or change in the opposite direction necessary to restore amniotic fluid volume (6, 12, 17, 19).

Although intramembranous absorption does appear to function as a regulator of amniotic fluid volume (7), it is not known what modulates this process. It is also unclear which quantity is regulated: total uterine volume or amniotic fluid volume. If amniotic fluid volume is regulated, which is part of our hypothesis, then we should test the hypothesis that a substance present in the amniotic fluid serves as a regulator. We therefore measured amniotic fluid volume, replaced it with 1 1 of lactated Ringer solution, and then slowly drained and discarded amniotic fluid while replacing it with an equal flow of Ringer solution that was isotonic to amniotic fluid. An isovolumic exchange was chosen to dilute any substance present in the amniotic fluid without altering amniotic volume. We reasoned that a change in the volume of amniotic fluid after an isovolumic dilution period compared with a control period would be consistent with a change in concentration of a regulatory substance that affects fluid absorption in the intramembranous pathway.

METHODS

Surgical protocols. Seven time-bred ewes, all carrying single fetuses, were obtained from a licensed source. Surgeries were performed at 119 ± 1 days of gestation (mean ± SE). All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee. Anesthesia was induced with 400 mg iv of ketamine and 10 mg iv of diazepam and was continued after intubation with ~1% halothane or 1% isoflurane in a mixture of oxygen and nitrous oxide. The fetus was completely anesthetized. Surgeries were performed using sterile procedures. An incision was made on the abdomen of the ewe to expose the uterus. A second incision was made in the uterus over the fetal head. The chorionamnion was sutured to the amnion and chorion (8, 9). This microvasculature is extensive and is uniquely situated as a sensor. In full-term sheep, 17% of the amnion and 50% of the chorion are covered by microvessels (3), and blood flow to the chorion and amnion averages 6–10% of total umbilical blood flow (10, 13).

Intramembranous absorption is thought to be important in the regulation of amniotic fluid volume and has been shown to increase from 200 to >1,000 ml/day during conditions of prolonged hypoxia (19) or after infusion of large volumes of lactated Ringer solution directly into the amniotic cavity (7).
This catheter consisted of tubing connected to a small, plastic, 10-ml screw-top vial. Numerous holes were drilled in the walls of the vial to minimize the chance of occluding the tubing with membranes during amniotic fluid drainage. The fetal head was returned to the uterus, and the incision was repaired. Special care was taken to close the uterus in layers to maintain a watertight closure.

A second uterine incision was made over the fetal hindquarters. After the chorioamnion was sutured to the uterine wall, the fetal hindquarters were removed from the uterus. Polyvinyl catheters were placed in the pedal arteries and veins and advanced ~22 cm toward the aorta and the vena cava. A catheter for measuring amniotic fluid pressure and a second catheter for amniotic fluid drainage were attached to the fetal hindlimb. Finally, a midline incision was made on the fetal abdomen. The urachus was exposed, freed of the umbilical arteries, and ligated. The fetal incision was repaired. A third set of catheters for measuring amniotic fluid pressure and amniotic fluid drainage were attached to the fetal abdomen. The fetus was returned to the uterus, and the uterine incision was carefully repaired. Vascular catheters were filled with a one-third heparin solution. Penicillin G (1,000,000 U) was infused into the amniotic fluid. No other antibiotics were routinely given to the fetus.

Experimental protocols. Ewes were given 6 ± 1 days for postoperative recovery. After recovery, the ewes were placed in a stanchion in the laboratory where they remained for the duration of the experiment. The amniotic fluid was drained by connecting the three large-bore drainage tubes to evacuation bottles (Baxter Healthcare; Deerfield, IL). After the amniotic fluid was completely drained, 1 l of warm bore drainage tubes to evacuation bottles (Baxter Healthcare; Deerfield, IL). After the amniotic fluid was completely drained, 1 l of warm lactated Ringer solution was returned to the uterus. The composition of the lactated Ringer solution was (in meq/l) 130 Na⁺, 110 Cl⁻, 28 lactate, 4 K⁺, and 3 Ca²⁺. Thus all fetuses began the study with the same volume and composition of amniotic fluid.

Experiments consisted of two protocols that included a control period and an exchange period. All fetuses participated in both protocols and alternated between beginning with a control period and beginning with an exchange period. The control period lasted for 3 or 4 days during which no interventions were performed. At the end of the control period, blood pressures were measured, blood and amniotic fluid samples were collected, and amniotic fluid volumes were measured.

The exchange period also lasted for 3 or 4 days. During this time, amniotic fluid was continuously exchanged with lactated Ringer solution. A Gilson Minipuls3 roller pump (Gilson Medical Electronics; Villiers le Bel, France) with two parallel tubing systems was used. One tube was used to infuse lactated Ringer solution into the uterus using the amniotic fluid catheter at the fetal head. The second tube was used to simultaneously withdraw amniotic fluid at the same rate using the catheter at the fetal hindlimbs. The 3- or 4-day protocols were not mixed. If the initial control or exchange study was chosen to last 3 days, the corresponding exchange or control study was also for 3 days. The subsequent control and exchange period was then 4 days. This schedule was selected to limit weekend studies. All fetuses completed both a control period and an exchange period, and four of the seven fetuses completed two exchange and two control periods. In these four fetuses, values from the two control periods and the two exchange periods were averaged before any further analyses were performed. Each control or exchange period began with 1 liter of warm, lactated Ringer solution being returned to the uterus. We chose to standardize the volume and composition at the start of the control and exchange periods, because amniotic fluid volumes vary, and an exchange rate of ~3 l/day would result in different turnover rates if we did not start with identical volumes.

At the end of the experiment, the ewe and fetus were killed by means of a commercial euthanasia solution. An immediate necropsy was performed. The fetuses were inspected, and the catheter positions were verified.

Analytical methods. Fetal arterial, venous, and amniotic catheters were connected to calibrated sterile Abbott Transpac IV transducers. Pressures were measured and recorded using an InstruNet system operated by SuperScope software. Arterial and venous pressures are reported with respect to amniotic fluid pressure. Heparinized fetal arterial blood samples were obtained for determination of blood gas quantities, pH values, oxygen contents (IL306 and IL482; Instrumentation Laboratories), and hematocrit levels. Plasma and amniotic fluid samples were collected for later determination of Na⁺, K⁺, and Cl⁻ concentrations (Beckman Labyte 810 electrode system) and freezing point depression osmolarities (model 3MO Advanced Micro Osmometer; Advanced Instruments). Additional arterial blood samples were collected in EDTA for later radioimmunoassays of circulating angiotensin I concentrations and plasma renin activity levels as previously described (7).

Statistics. Data are presented as means ± SE. Statistical significance (P < 0.05) was determined using the Wilcoxon signed rank test or linear regression analysis. When using the Wilcoxon signed rank test, the differences between data collected during the control and exchange periods were compared with zero (GraphPad Prism 3.02 for Windows; GraphPad Software; San Diego, CA).

RESULTS

Fetuses were of comparable age at the beginning of the control period (131 ± 2 days) and the exchange period (132 ± 1 days). The durations of both the control and exchange periods were 3.6 ± 0.3 days. During the control period, mean arterial blood pressure was 46 ± 1 mmHg, mean venous blood pressure was 5.0 ± 1.0 mmHg, and heart rate was 154 ± 6 beats/min. None of these variables showed a statistically significant change during the exchange period (Table 1). Results from analyses of arteriotomy during the control period were pH, 7.332 ± 0.014; PO₂, 55.7 ± 2.1 mmHg; PO₂, 18.1 ± 1 mmHg; hematocrit, 34.2 ± 2%; and oxygen content, 6.5 ± 0.8 ml O₂/100 ml of blood. After the exchange period, PO₂ increased to 22.1 ± 1 mmHg (P = 0.03), hematocrit decreased to 32.1 ± 1% (P = 0.03), and oxygen content increased to 8.0 ± 0.7 ml O₂/100 ml of blood (P = 0.03). Plasma renin activity, 8 ± 2 mg·ml⁻¹·h⁻¹ (n = 6) and angiotensin I concentration, 3.6 ± 1.3 ng/ml (n = 6) did not change as a result of the exchange of amniotic fluid. Twenty-two experiments were performed.

Table 1. Results for variables measured after control and exchange periods

<table>
<thead>
<tr>
<th>Variable</th>
<th>End of Control Period</th>
<th>End of Exchange Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal age, days</td>
<td>131 ± 0.3</td>
<td>131 ± 0.3</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>46 ± 1</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Mean venous blood pressure, mmHg</td>
<td>5.0 ± 1.0</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>154 ± 6</td>
<td>163 ± 5</td>
</tr>
<tr>
<td>pH</td>
<td>7.332 ± 0.014</td>
<td>7.324 ± 0.021</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>55.7 ± 2.1</td>
<td>53.8 ± 1.1</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>18 ± 1</td>
<td>22 ± 1*</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>34 ± 2</td>
<td>32 ± 1*</td>
</tr>
<tr>
<td>Oxygen content, ml O₂/100 ml</td>
<td>6.5 ± 0.8</td>
<td>8.0 ± 0.7*</td>
</tr>
<tr>
<td>Plasma renin activity, ng·ml⁻¹·h⁻¹</td>
<td>8 ± 2</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>Angiotensin I, ng/ml</td>
<td>3.6 ± 1.3</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Gradient osmolality, mosmol/kgH₂O</td>
<td>15.1 ± 0.5</td>
<td>13.0 ± 0.5</td>
</tr>
<tr>
<td>Na⁺, mM</td>
<td>1.1 ± 0.4</td>
<td>4.4 ± 3.9</td>
</tr>
<tr>
<td>K⁺, mM</td>
<td>0.3 ± 0.2</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>Cl⁻, mM</td>
<td>7.0 ± 2.9</td>
<td>3.7 ± 2.1</td>
</tr>
<tr>
<td>Amniotic fluid volume, ml</td>
<td>556 ± 98</td>
<td>986 ± 209*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 sheep except for plasma renin activity and angiotensin I evaluations, in which n = 6 sheep. Durations of both control and exchange periods were 3.6 ± 0.3 days. Gradient denotes fetal plasma-amniotic fluid gradient. *P ≤ 0.03.
performed on seven ewes with singleton fetuses. All animals completed a randomized control and washout period. Four of the seven animals completed an additional control and washout period of either 3 or 4 days duration as detailed (see METHODS).

Osmolalities. Intramembranous absorption is dependent upon the gradients existing between the fetal plasma and the amniotic fluid (8). During the control period, gradients existed between the fetal plasma and the amniotic fluid for osmolality (15 mosmol/kgH2O), Na+ (1.1 ± 3.4 mM), Cl− (7.0 ± 2.9 mM), and K+ (−0.3 ± 0.2 mM). None of these changed significantly after the exchange period. Changes in the composition of the amniotic fluid for these same variables were also examined. After the exchange of amniotic fluid with lactated Ringer solution, only Cl− composition showed a statistically significant change, increasing from 102.4 ± 3.6 to 108.6 ± 3.4 mM (P = 0.008).

Amniotic fluid volume. During the exchange periods, an average of 3.019 ± 171 ml/day of lactated Ringer solution was infused into the amniotic cavity while an equal amount of amniotic fluid was pumped and discarded. Amniotic fluid volume was 556 ± 98 ml after the control period and increased significantly to 986 ± 209 ml (P = 0.03) after the exchange period (Table 1). Linear regression analysis was used to compare the amniotic fluid volume after the control period to that after isovolumic exchange (Fig. 1). A highly significant relationship existed (P = 0.003), which indicates that the animals with larger control volumes responded to isovolumic dilution with a greater volume increase.

**DISCUSSION**

This study was designed to test the hypothesis that a regulatory substance or substances present in amniotic fluid affect fluid volume. In this study, the order of the control or exchange periods was randomized, and the start of either period coincided with the end of the other period. Importantly, hemodynamic parameters were similar to other studies of chronically instrumented fetal sheep (7, 8) and were not altered by the washout periods. The major finding was that exchanging amniotic fluid with an equal volume of lactated Ringer solution over a 3- to 4-day period resulted in a nearly 1.8-fold increase in amniotic fluid volume.

Amniotic fluid volume homeostasis is maintained by the balance between inflow (fetal urine and lung secretion) and outflow (swallowing and intramembranous absorption) of fluid in the amniotic cavity (2, 7, 9). In the present study, the contribution of urine, lung fluid, and swallowing were not measured and were not surgically affected; thus intramembranous absorption cannot be determined. A 3- or 4-day period was chosen to study amniotic fluid dynamics during nearly steady-state conditions. During the washout periods, the average exchange rate of lactated Ringer solution was 3.0 l/day, and the final amniotic fluid volume was 986 ml. Thus the amniotic fluid volume can be estimated to have turned over nearly every 8 h or 9–12 times during the experimental dilution period. Changes in urine and lung fluid volumes or swallowing rates should not have affected amniotic fluid volume by the third or fourth day under steady-state conditions. A second observation was that the amniotic fluid volume in the control state correlated with the volume measured at the end of the exchange period. The larger the initial control volume, the greater the amniotic fluid volume after the washout period. The mechanisms responsible remain unknown, but this implies a regulatory process. This would be consistent with a secreted substance or chemical mediator being part of the regulatory process of intramembranous absorption.

Under basal conditions, amniotic osmolality is lower than fetal blood osmolality, and the difference is believed to be a driving force for intramembranous absorption (4, 8). In the present study, it is unlikely that crystalloid osmotic pressure altered amniotic fluid absorption, because the osmolality gradient between the fetal plasma and the amniotic fluid remained unchanged after the infusion of lactate Ringer solution. Furthermore, although the osmolality gradient does affect the rate of amniochorionic absorption, the effect is very small and amounts to 16 ml/day (per mmol/kg; Ref. 8). We did observe that the fetal plasma-amniotic fluid gradient for Na+ in this study was less than previously reported (7, 11). This was because of a higher Na+ concentration in amniotic fluid, which may in part be due to the use of 11 of lactated Ringer solution at the start of the study as well as during the washout periods. Lactated Ringer solution has a Na+ concentration of 130 mM. In any case, the amniotic fluid-fetal plasma gradient was the same in both groups and thus would not have increased the amount of amniotic fluid absorption.

Intramembranous absorption does occur independent of osmotic differences against a hydrostatic gradient and protein gradients (5, 8); however, we cannot estimate this, because we did not measure urine flow, swallowing rate, or tracheal secretion. Other investigators have shown that hypoxia (19), volume infusion (5), or esophageal ligation (15, 16) increase intramembranous absorption. Proposed mechanisms include aquaporin-mediated water channels (21) or vesicular transport mediated by vascular endothelial growth factor (4). The latter is present in the amnion, chorion, villous cytrophoblast of the placenta, and amniotic fluid (1, 16, 20). In this study, the fetal oxygen content actually increased minimally after the fluid exchange period; thus it is unlikely that the amount of vascular endothelial growth factor increased. We cannot, however, exclude that the loss of a regulatory substance(s) during the exchange period resulted in decreased intramembranous absorption.
which led to an increase in amniotic fluid volume. Whether the putative regulatory substance could downregulate vascular endothelial growth factor and thereby decrease intramembranous absorption remains unknown. Additional studies to directly measure intramembranous absorption are needed to clarify this hypothesis.

One of the concerns during the study was to determine whether an isovolumic exchange during the washout period was actually achieved. We verified the amount of fluid collected during the exchange period compared to the amount of lactated Ringer solution infused into each fetus in several ways. First, we compared the volume collected to the calculated volume infused for the seven fetuses, which was estimated by counting the number of 1-liter bags infused. The volume out-to-calculated volume in ratio was 0.92 ± 0.04. Comparing the expected ratio to 1 using a Wilcoxon test, there was no significant difference. One-liter bags of fluid, however, contain slightly more (~50 ml) than 1,000 ml, and estimates of less than a full bag may be inaccurate. To accurately determine the volumes infused, in two fetuses, we weighed all bags before and after the infusion. The ratio of volume infused to volume collected was 1.02. Finally, we verified the pump calibrations and tubing by weighing the infusion and withdrawal volumes and found the rates were within 1.7%. Because the pump tubing was randomized with regard to either infusion or withdrawal, the difference was negligible.

Another concern was that amniotic fluid volume may have increased due to a decrease in hematocrit (18). Although the hematocrit decreased 2% during the exchange period, the oxygen content increased from 6.5 ± 0.8 to 8.0 ± 0.7 ml/100 ml. Thus it is unlikely that renal oxygen consumption decreased or that plasma lactate levels increased. Other investigators have found that amniotic fluid did not change until the fetal hematocrit decreased below 25% (18). Thus we do not believe that the slight difference in hematocrit affected the results.

Investigators have used several methods to measure amniotic fluid volume. We feel that direct measurement of amniotic fluid volume by drainage (6, 8) as performed in this study has advantages compared with dilution techniques, which may overestimate amniotic fluid volume due to increases in swallowing or unequal mixing or to ultrasound techniques, which are only approximations.

Comparative studies are few. Daneshmand et al. (5) intravenously infused 7 liters of normal saline into fetal sheep over 3 days. These authors found that amniotic fluid volume averaged 1,266 ml at the start of the study and increased ~800 ml. Over the same period of time, estimated intramembranous absorption exceeded 4,276 ml. Thus the increase in amniotic fluid was 11% of the infused volume. Similarly, Faber and Anderson (7) infused nearly 1.2 l/day for 6 days into the amniotic cavity of fetal sheep and determined that with the exclusion of one fetus that developed polyhydramnios, the final average amniotic fluid volume was 812 ml. Taken together, these studies suggest that only a small portion of the excess volume infused into the fetus or the amniotic fluid cavity remained in the amniotic cavity. In the present study, we estimate this volume to be <100 ml assuming a volume out-to-volume in ratio of at least 0.92 and 11% retention of excess fluid.

The identity and the origin of a regulatory substance(s) remain unknown. It may be produced by the amnionchorion itself and directly released to the amniotic cavity to modulate amniotic fluid absorption. However, the intact pathways of fetal urination and lung secretion in the present study leave open the possibility that a regulatory substance(s) may come from fetal urine or/and lung fluid. The speculation of either a urine- or lung fluid-contained factor in amniotic fluid regulation was previously proposed by Matsumoto et al. (16). A urine-produced factor is problematic, because a 50% reduction in fetal urine flow did not change amniotic fluid volume (14), nor did a threefold increase in urinary flow affect amniotic fluid volume (16).

In summary, this study demonstrated a significant increase in amniotic fluid volume in ovine fetuses undergoing amniotic fluid dilution (exchange with an equal flow of lactate Ringer solution), which suggests that there is a regulatory substance(s) present in the amniotic fluid that affects fluid absorption. However, we do not know what the control mechanism is, and we cannot further speculate at this time. The identity of the regulatory factor(s) and the underlying mechanisms remain unknown and need to be further explored.

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