BASIC SCIENCE: OBSTETRICS

Amniotic fluid volume responses to esophageal ligation in fetal sheep: contribution of lung liquid

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OBJECTIVE: The objective of the study was 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, tracheoesophageal shunted, and esophageal ligated.

RESULTS: One day after surgery, AFV was similar in all groups, averaging 1064 ± 66 mL. On postsurgical day 9, AFV was unchanged in control fetuses, increased to 3025 ± 294 mL in fetuses with esophageal ligation and lung liquid shunted into the fetal stomach, and to 3437 ± 430 mL in fetuses with esophageal ligation and no shunting.

CONCLUSION: AFV expanded gradually following esophageal ligation to the highest volume thus far reported in noninfused ovine fetuses. Lung liquid entry into the amniotic sac altered neither the time course nor the extent of the AFV increase following esophageal ligation.

Key words: amniotic fluid volume regulation, intramembranous absorption, lung liquid, ovine fetus, swallowing

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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.[1-3] 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.[4-6] 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 trachea.[7]

In addition to a direct volume contribution to the AF, fetal lung liquid may contain a substance(s) that modulates the rate of 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 on the presence or absence of a tracheoesophageal shunt, which 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 intraamniotic saline infusions are associated with increased rates of intramembranous absorption. If present, the substance may be derived from fetal lung liquid.[9-11]

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 2 hypotheses: (1) that AF volume would increase over time following either esophageal ligation or placement of a tracheoesophageal 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 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 ± 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.

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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 the swallowing of not only AF but also the 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 was 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 after surgery.

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-99 (^{99m}Tc). Maternal and fetal arterial blood, AF, and fetal urine were sampled and analyzed for sodium, potassium, chloride, bicarbonate, glucose, and lactate concentrations (model 725; Radiometer, Copenhagen, Denmark). Arterial pH, partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen (PO_2), and hematocrit were determined in the blood samples (model 725; Radiometer).

Fetal urinary flow rate was determined by gravity drainage for approximately 1 hour between 8 and 10 AM. At this time of day, urine flow rates are not different from the 24-hour mean.[14] The daily urinary volume was calculated as the hourly volume multiplied by 24. Fluid compositions and urinary output were monitored 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 killed 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 tracheoesophageal shunts. Toward the latter part of the study, a few fetuses were noted to have unilateral or bilateral hydronephrosis at autopsy. Because 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 (ΔAFV) over time are related to the 4 primary amniotic inflows and outflows by the equation $\Delta AFV = UO + LL - SW - IM$, where UO is the urine production rate, LL is the rate of lung liquid entry into the AF, SW is the volume rate of swallowing of AF, and IM is the rate of intramembranous absorption. Because IM cannot be measured directly, it was calculated using the equation $IM = UO + LL - SW - \Delta AFV$. In the shunted animals, the calculation provides an accurate determination of IM, because both SW and LL were zero and UO as well as ΔAFV 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 bodyweight per day.[1]

For the control fetuses, we assumed SW equaled the average published value of 20% bodyweight per day, and LL equaled 5% of bodyweight per day.[16] The difference between 10% vs 5% per day occurs because normally half of the secreted lung liquid is swallowed as it exits the trachea and does not enter the AF,[7] whereas all secreted lung liquid enters the amniotic sac with esophageal ligation. Daily fetal bodyweights were calculated from autopsy weights, assuming a fetal growth rate of 3.4% per day.[17] Because of the use of mean values, the SEs of the calculated SW and LL will underestimate the true variability, because they result from variability in fetal bodyweights and do not include the normal physiologic interanimal variations in flows.

Data presentation and statistical analysis

Data are presented as mean ± 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 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