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Ovine Amniotic Fluid Volume Response to Intra-amniotic Balloon Filling

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Although ovine placentation is markedly different from human, the ovine model has been extensively used to study the regulation of amniotic fluid volume because the majority of the experiments has been performed in the chronically catheterized fetal sheep. However, there are considerable similarities between the human fetus and the sheep fetus with regard to amniotic fluid volumes, fetal urination and fetal swallowing. In both species, there is a continual turnover of amniotic fluid throughout gestation. In sheep, urine and lung fluids enter the amniotic fluid while fluid is removed through swallowing and intramembranous absorption across the amniochorion [1,2]. Because the amniotic fluid volume remains constant at about 0.5–1.0 l in spite of a turnover of about 1 l per day, the volume must be regulated. Regulation of the amniotic fluid may be mediated by a mechanical sensor (such as stretch of the uterus or membranes) or a chemical sensor (such as an alteration in the concentration of an effector substance in amniotic fluid). Described below are results from our experiments examining the response of amniotic fluid volume to changes in total intrauterine volume induced by filling of an intra-amniotic balloon.

The protocol received approval of the OHSU Institutional Animal Care and Use Committee. Surgeries were performed on 6 pregnant sheep (5 singletons and 1 twin, only one of which was instrumented) at 120 days of gestation. The details of these preparations have been previously described [3,4]. Briefly, catheters were placed in the femoral artery and vein of each fetus. The urachus was ligated at the neck of the bladder to prevent urine from entering the allantoic space. Three catheters for draining amniotic fluid and two catheters for measuring the amniotic fluid pressure were attached to the fetal body. Finally, one or two

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inflatable balloons of our own design were positioned along the back of the fetus. The balloons were constructed from a folded sheet of silicon rubber glued together on three sides, with one end of a 2.5 m long polyurethane tubing glued in place inside the balloon. All catheters were exteriorized and stored in a nylon pouch until use.

Six days after catheterization, control arterial blood gas analyses and vascular pressures measurements (referenced to amniotic fluid pressure) were made in each fetus. Amniotic fluid samples were collected for later analysis. The amniotic fluid was drained into evacuated bottles. The amniotic fluid volume was measured and returned to the uterus. Fetuses were randomly assigned to one of two protocols: (1) filling of the intrauterine balloon(s) with normal saline or (2) empty balloon(s). Each experimental period lasted three days. After three days, the initial measurements were repeated and the amniotic fluid volume was measured. The animal then completed the remaining protocol and the initial measurements were repeated at the completion of this period. All fetuses completed the one filled/empty cycle and four of the six fetuses completed two cycles. Amniotic fluid volumes in fetuses with duplicate measurements were averaged before statistical testing. Data are reported as means ± SEM. Statistical comparisons were by paired and unpaired *t* test and ANOVA (Graphpad Prism).

On the initial day of the experiment, fetal arterial pH = 7.363 ± 0.009, Pco_2 = 51.5 ± 0.8 mm Hg, Po_2 = 20.9 ± 0.8 mm Hg, oxygen content = 6.5 ± 0.4 vol/100 ml blood and hematocrit = 32 ± 2%. None of these variables changed statistically over the six day or twelve-day duration of the experiments with the exception that hematocrit increased to 35 ± 1% (P = 0.02, paired *t* test). Similarly, there were no statistically significant changes in arterial blood pressure (47 ± 2 mm Hg) or venous blood pressure (4.3 ± 0.4 mm Hg) over the duration of the experiments. However, heart rate decreased from 168 ± 4 bpm at the beginning of the initial experiment to 147 ± 3 bpm at the end of the final experiment (P = 0.003, paired *t* test).

After filling, intrauterine volume in fetuses with one balloon (n = 2) increased by 659 ± 1 ml and in fetuses with two balloons (n = 4) increased by 1331 ± 50 ml. After three days with filled balloons, amniotic fluid volume was 1426 ± 395 ml. After three days with empty balloons, amniotic fluid volume was 1452 ± 298 ml. Clearly, amniotic fluid volume was unaffected by total intrauterine volume (P = 0.90). We concluded that these results argue against a mechanical stimulus for amniotic fluid volume regulation over the range of volumes studied.

Comparison of amniotic fluid composition during the control period (before the initiation of the experiment), the period after the balloons were filled and the period with the balloons empty demonstrated no statistically significant changes for K^+, Na^+ Ca^{2+}, Cl^-, glucose or lactate concentrations (ANOVA).

Initial experiments used two balloons to increase total intra-uterine volume. While amniotic fluid volume was independent of balloon inflation, we did detect a steady increase in amniotic fluid volume over the duration of the experiment, prompting us to instrument each of the next two fetuses with one rather than two balloons. The response of fetuses with one

or two intrauterine balloons was similar ($P = 0.58$). A similar trend in amniotic fluid volume has been reported in fetal sheep with a shunt between the oesophagus and trachea studied over a similar duration of time [5]. However, no shunt was present in the fetuses described in the present study.

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