Chronic in utero mitral inflow obstruction unloads left ventricular volume in a novel late gestation fetal lamb model

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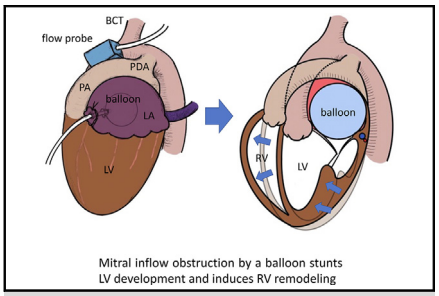
ABSTRACT

Objective: The in utero no flow/no grow hypothesis postulates that reduced inflow of blood into the left ventricle due to a stenotic mitral valve could lead to ventricular hypoplasia and hypoplastic left heart syndrome. This has been demonstrated in chick embryos, but less so in large animals. We investigated the impact of mitral obstruction on left and right ventricular growth in fetal lambs.

Methods: Twelve pregnant ewes, most bearing twins, were instrumented at 119 ± 1 days gestational age. Carotid artery and jugular vein catheters, an ascending aorta flow probe, and a left atrial deflated balloon catheter were implanted into 1 fetus (left atrial balloon group), and the twin remained an uninstrumented control. The balloon was inflated gradually over 8 days until net antegrade aortic flow was eliminated. Fetal transesophageal echocardiography was performed at the time of surgery and just before termination in both groups.

Results: Terminal fetal body weights were comparable between groups. Terminal heart/body weight ratio was higher in left atrial balloon group fetuses (6.9 ± 0.8 g/kg) compared with controls (5.9 ± 0.6 g, $P = .0126$). The left ventricular/right ventricular weight ratio was 24% ($P = .0077$) lower in left atrial balloon group fetuses than in controls. Left ventricular/heart weight (0.24 ± 0.04 g/g vs 0.30 ± 0.04 g/g, $P = .0009$), left ventricular end-diastolic volume (2.3 ± 0.7 mL vs 7.1 ± 0.8 mL; $P = .0012$), and left ventricular end-systolic volume (1.01 mL [0.95-1.95 mL] vs 3.38 mL [3.28-3.57 mL], $P = .0042$) were lower in left atrial balloon group fetuses compared with controls. Right ventricular weight (g/kg), right ventricular end-diastolic volume, and right ventricular end-systolic volume were similar between groups.

Conclusions: In this late-gestation fetal lamb model, in utero obstruction of mitral inflow slowed left ventricular growth and caused right ventricular remodeling. (JTCVS Open 2023;16:698-707)



Mitral inflow obstruction by a balloon stunts LV development and induces RV remodeling.

CENTRAL MESSAGE

We have established a reliable large animal model of obstructed mitral inflow into the LV that allows for mechanistic and therapeutic studies regarding HLHS.

PERSPECTIVE

Lack of physiological models hinder mechanistic studies for HLHS. Pregnant sheep are the premiere animal model for translatable perinatal studies, and fetal size and maturation permit chronic in vivo studies and therapeutic innovation. We describe a model of reduced fetal mitral inflow, which retards LV growth and induces RV remodeling.

See Discussion on page 708.

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Abbreviations and Acronyms

EDV = end-diastolic volume
EF = ejection fraction
ESV = end-systolic volume
HLHS = hypoplastic left heart syndrome
LA = left atrium
LAB = left atrial balloon group
LV = left ventricle
MV = mitral valve
OHSU = Oregon Health & Science University
RA = right atrium
RV = right ventricle

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Approximately 40,000 babies are born with a congenital heart defect in the United States each year, of which hypoplastic left heart syndrome (HLHS) is one of the common lesions.[1,2] HLHS is a critical life-threatening condition at birth characterized by in utero underdevelopment of the left side of the heart, resulting in a small and nonfunctional left ventricle (LV). The survival of a newborn with HLHS requires 3 staged palliative operations over several months to years. The resulting "Fontan" circulation passively redirects venous return into the lungs, bypassing the right side of the heart; the fully-developed right ventricle (RV) is surgically transposed to function as the systemic ventricle. These operations have increased survival to 65% at 5 years and 55% at 10 years, but result in the development of cardiac and multiorgan dysfunction with time.[3-5]

New innovations in therapies for HLHS have been few, and advancements have been lacking partly due to a dearth of animal models that mimic this pathological condition. The primary abnormalities diagnosed in utero associated with the development of HLHS include aortic and mitral atresia or stenosis, lending circumstantial support to the hypothesis that reduced or complete blockade of blood flow into the LV stunts signaling necessary for normal growth of the LV chamber. This "no-flow, no-grow" hypothesis has been demonstrated in chick embryos, where mitral valve (MV) obstruction by ligating the left atrium (LA) resulted in a small LV and an atretic aorta.[6-9] However, such small animal models are not suitable for surgical innovation or device development, necessitating the development of a larger animal model of HLHS. Fishman and colleagues[10] successfully obstructed the MV by implanting a rubber balloon into the LA of fetal lambs (0.6-0.8 gestation), but all fetuses died 2 to 7 days after the surgery. Wong and colleagues[11] developed a percutaneous method of occluding the foramen ovale in lambs (0.7-0.75 gestation), demonstrating a degree of LV hypoplasia. More recently, Reuter and colleagues[12] have reported a newer percutaneous fetal sheep model in which mitral inflow was obstructed using coils at 0.5 gestation, inducing a hypoplastic LV. Similar to the earlier studies, mitral inflow was instantaneously obstructed in this model, and the investigations continued to have a high (56%) mortality, with a 27% success rate for the development of hypoplastic LV.[12]

In this study, we sought to build on these previous efforts to obstruct MV inflow to develop a reduced LV growth model as follows: (1) gradually inflate the LA balloon over several days, reducing the high mortality rate; (2) assess fetal cardiac function and ventricular chamber hemodynamics by transesophageal echocardiography at baseline and at termination; and (3) compare loaded and unloaded chamber volumes and geometries.

MATERIAL AND METHODS

Animal Care and Use

Animal experiments were conducted as part of a collaboration between Emory University and Oregon Health & Science University (OHSU). All animal work was conducted at OHSU, which is accredited by AAALAC International, was approved by OHSU's Institutional Animal Care and Use Committee, and was carried out in accordance with the Guide for the Care and Use of Laboratory Animals.[13]

Experimental Design

Twelve time-bred pregnant ewes of mixed Western breed were obtained from local breeders (AGNA LLC, Oregon; Oregon State University). Surgery was performed at 119 ± 1 days gestational age (0.8 gestation). Ten of these ewes were carrying twins, and 2 were carrying singleton fetuses. In ewes with twins, only 1 fetus underwent sterile surgery (left atrial balloon group [LAB]) and the other was left uninstrumented (control). Baseline echocardiographic imaging was conducted at the time of surgery exclusively in the LAB group because of the procedure's requirement for hysterotomy incision and fetal manipulation for transesophageal echocardiography of the fetus. After recovery from surgery, each ewe was housed in a stanchion to monitor the instrumented fetus throughout the study period. The LA balloon was gradually inflated over several days to reduce blood flow from the LA to the LV until net-positive antegrade aortic flow was eliminated. At the conclusion of the in vivo study (8 days, 134 ± 1 days gestational age), ewes were anesthetized for echocardiographic imaging of both the LAB and Control fetuses, and the tissues were collected for histology and banked for later gene array analysis. Figure 1 is a graphical summary of the study.

Surgical Technique and Creation of Model

Anesthesia. The ewes were anesthetized as previously described[13]: anesthesia was induced with ketamine (400 mg, intravenous) and diazepam (10 mg, intravenous), and a surgical level of anesthesia was maintained with isoflurane (1.5%-2%) and nitrous oxide (0.7 L/min) in oxygen. Ewes were mechanically ventilated and continuously monitored (electrocardiogram, pulse oximetry, end-tidal carbon dioxide, and body temperature) throughout the procedures.

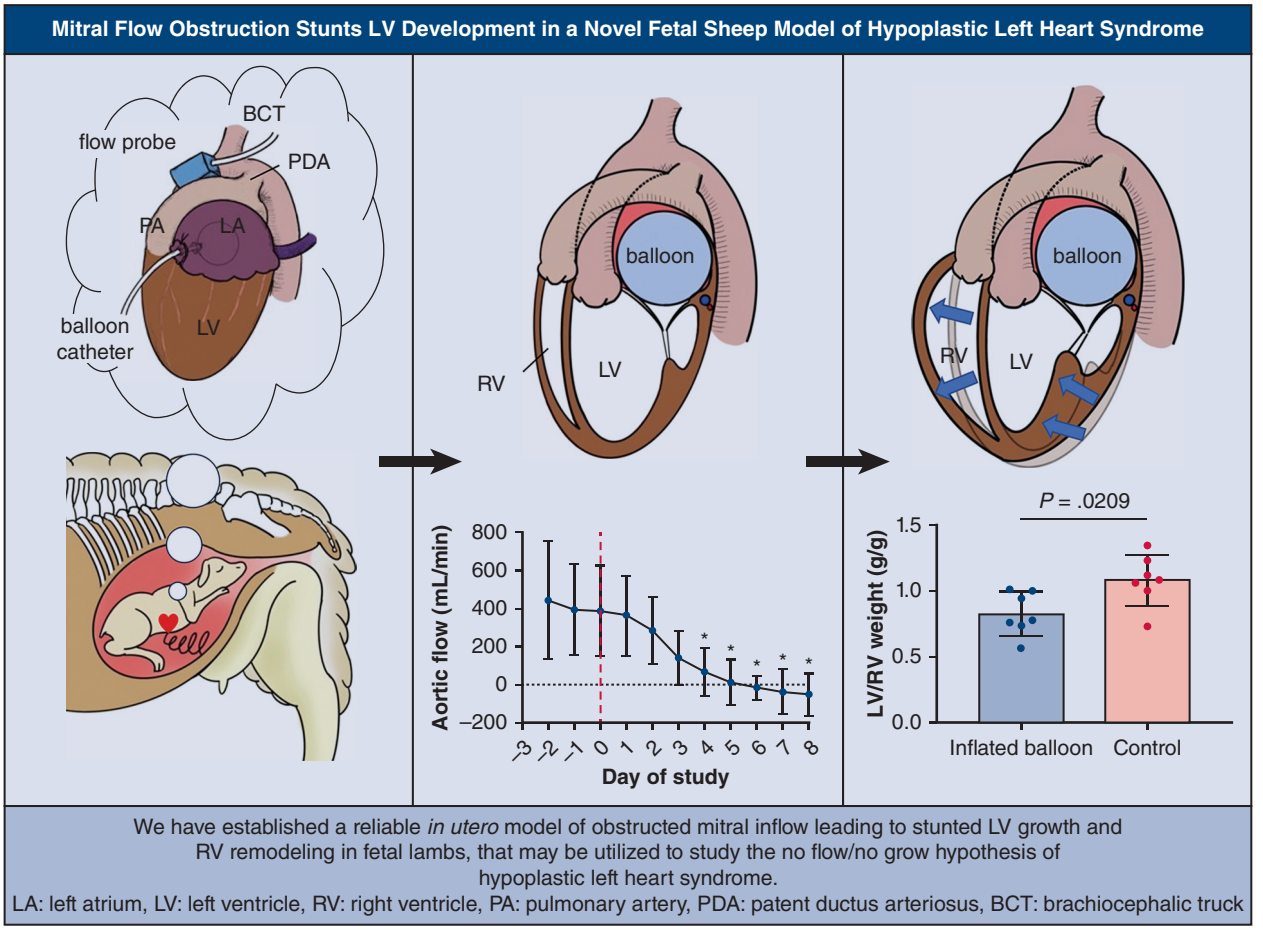

FIGURE 1. Summary of the methods and results in this experiment as the graphical abstract. *BCT*, Brachiocephalic trunk; *PDA*, patent ductus arteriosus; *PA*, pulmonary artery; *LA*, left atrium; *LV*, left ventricle; *RV*, right ventricle.

Surgical procedure. Surgery was performed as previously described,[14] with modifications (Figure 2). The upper body of 1 fetus was exposed through a hysterotomy, and polyvinyl chloride catheters were inserted into a carotid artery (for blood sampling and arterial pressure monitoring) and the ipsilateral jugular vein (for central venous pressure monitoring). A left thoracotomy (fourth intercostal space) was then performed to expose the heart, followed by a pericardiotomy. The plane between the aorta and the main pulmonary artery was dissected, and a

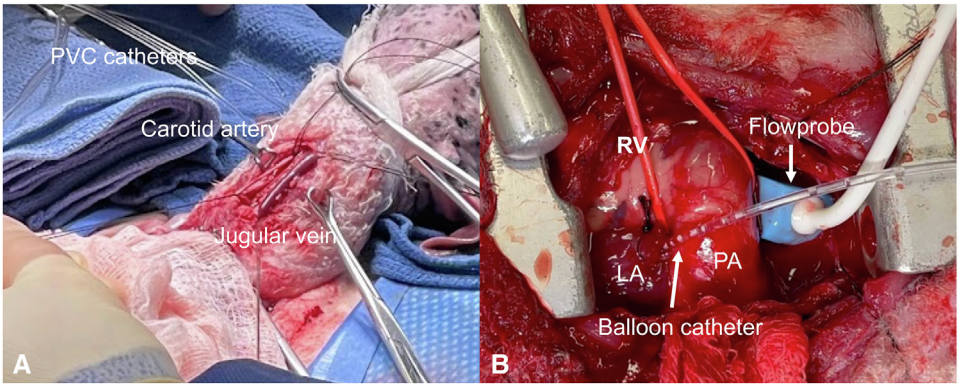

FIGURE 2. A, An externalized fetal neck: The left carotid artery and jugular vein are exposed for the placement of vascular catheters. B, The heart is exposed via a left thoracotomy for the placement of a balloon catheter in the LA and the flow probe around ascending aorta. *PVC*, Polyvinyl chloride; *RV*, right ventricle; *LA*, left atrium; *PA*, pulmonary artery.

transit time flow probe (8-mm Perivascular Flowprobe, Transonic Systems Inc) was implanted around the ascending aorta. A deflated, custom-made latex balloon connected to a catheter was inserted through a small incision in the LA appendage and secured with a silk suture. All fetal incisions were closed using 2-0 silk, and an additional amniotic catheter was sutured on the chest wall. All catheters and the flow probe cable were secured at multiple sites on the fetal skin to avoid kinking and entanglement with the fetal limbs, externalized through the hysterotomy incision, and then tunneled through the ewe's abdominal wall and exteriorized to a fabric pouch sutured to the ewe's flank. All maternal incisions were closed with absorbable sutures in layers.

Postoperative care. Ciprofloxacin (2 mg) and penicillin G (1 million units) were administrated into the fetal amniotic sac. Ewes received subcutaneous Buprenex (0.3 mg) and buprenorphine sustained-release (0.05 mg/kg) immediately after surgery for analgesia. The ewes were monitored postoperatively, with unrestricted access to food and water. After 3 to 5 days recovery, they were moved to individual housing stanchions for the remainder of the study.

Daily measurements and inflation of left atrial balloon. Arterial, central venous and amniotic fluid pressures, and aortic flow were monitored as previously described.[14] Daily arterial blood samples were obtained to assess pH, partial pressures of oxygen, carbon dioxide, oxygen content, hemoglobin, hematocrit, lactate, glucose, and plasma protein levels. The LA balloon was inflated slowly over several hours each day for several days with sterile 30% glycerin to gradually reduce blood flow from the LA to the LV while minimizing induced arrhythmias. Aortic flow was continuously monitored with the goal of approximately 30% daily reduction in aortic flow. Once antegrade flow was stopped, typically after 4 days, the balloon was not inflated further. Pressure and flow data were averaged over approximately 23 hours per day, excluding data only during transient signal interference. Arterial blood was sampled, and blood gases and contents were measured at approximately the same time each day.

Echocardiographic Imaging

Ultrasound imaging was performed under anesthesia (described above) at the time of instrumentation surgery (baseline) after placing vascular catheters and before inserting the deflated balloon into the LA, and a second ultrasound study was performed at the conclusion of the 8-day in vivo experiment. LAB end point echocardiographic data are compared with LAB baseline and Control end point (there is no Control baseline). A sterile 10F intracardiac echo probe (AcuNav 10F; Biosense Webster) with a GE Vivid iq 4D (GE Ultrasound) system were used to obtain transesophageal cardiac B-mode, color Doppler, pulse wave, and continuous wave imaging of the left and right sides of the heart. Two-dimensional, B-mode images were obtained from a high esophageal view, and LV and RV volumes were calculated by tracing the ventricular cavity on the long-axis view. Pulsed and continuous-wave Doppler imaging was performed to measure transvalvular flow. Transesophageal echocardiography data are only available for 4 LAB fetuses due to 1 fetal death subsequent to maternal anesthesia and before the final imaging study, poor image quality in one study, and theft of equipment containing data for 2 studies. Additionally, during the terminal study only, M5Sc-rs probe (GE Precision Healthcare LLC) was used for transabdominal cardiac imaging of the fetal heart. Two-dimensional transthoracic B- and M-mode images were obtained to measure the ventricular shape, size, and function (Figure 3). End-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) were derived by the Simpson disk summation method.[15]

Necropsy and Histopathology

At the conclusion of the second echocardiographic study, each fetus was anticoagulated with heparin (10,000 U) followed by saturated potassium chloride to arrest the heart in diastole administered via venous catheter (LAB) or direct injection to the umbilical vein (Control). After removal of both fetuses, each ewe was euthanized with sodium pentobarbital while still anesthetized (SomnaSol, 80 mg/kg, intravenous). Fetal body and standardly trimmed heart weights were recorded. Myocardial biopsies were obtained from the LV and RV for future RNA analysis. The fresh heart was perfused with saline followed by 4% paraformaldehyde or 2% paraformaldehyde + 3% glutaraldehyde at mean arterial pressure. After overnight immersion fixation in either solution, the heart was photographed, reweighed, then dissected into the LA, right atrium (RA), LV, RV, and interventricular septum and weighed. A fresh:fix weight ratio was calculated for each heart to back-calculate fresh component weights. Fresh whole heart and back-calculated chamber weights are reported. Heart components were stored in the immersion fixation solution (up to 7 months) before transfer to 10% nonbuffered formalin or 3% glutaraldehyde. Fixed tissues were paraffin embedded and cut at 5 μm. Sets of slides were stained with hematoxylin and eosin, or Masson's Trichrome. Images were viewed at 2×, 4×, 10×, and 20× and qualitatively evaluated for histological tissue remodeling. Ultrastructural images at 500×, 1000×, 1500×, and additional images at 2500× were evaluated for myofibrillar and interstitial alterations.

Statistical Methods

Data analysis was conducted using Prism 9.0 (GraphPad Software Inc). Data are presented as mean ± standard deviation (normally distributed data) or median with interquartile range (non-normally distributed data). Repeated-measures 1-way analysis of variance with Tukey's multiple comparison test or Kruskal–Wallis test was used to compare the daily arterial blood values and hemodynamics. Comparisons of data (echocardiographic, morphological) for LAB terminal versus Control terminal, or for LAB baseline versus LAB terminal, were performed by paired *t* test for normally distributed data, otherwise by Wilcoxon signed-rank test; for echocardiographic comparisons, the value at which *P* was significant was set at less than .025 for each individual test.

RESULTS

Procedural Outcomes

Twelve pregnant ewes with 22 fetuses were used for the experiments. Five fetuses died before the terminal procedure. Only 1 fetus died during surgery, due to bleeding from the superior vena cava while positioning the flow probe. Two fetuses died postoperatively related to umbilical torsion on the first day or compression on the 14th day. The other 2 died for unknown reasons on the fifth and 12th postoperative days (study days 0 and 6, respectively). The overall fetal mortality rate in this series was 42%. Survival increased as our experience increased; there was 17% mortality in the latter half of our cases.

Impact of Chronic Flow Disturbance in the Fetus

There were no differences in the mean aortic or central venous pressures over the 8 days of LA balloon inflation (Figure 4, *A* and *B*). On the fourth day, and thereafter, mean aortic flow was significantly lower compared with aortic flow before balloon inflation on day 0 (Figure 4, *C*). Heart rate gradually decreased, as expected for this stage of gestation, and was significantly lower on day 8 than day 0 (Figure 4, *D*). Arterial pH

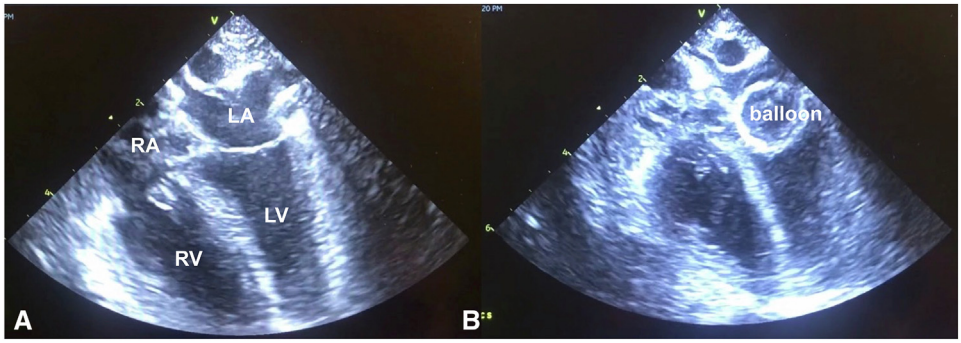

FIGURE 3. Four-chamber view with esophageal echocardiography at (A) baseline and (B) after 8 days inflation of a balloon in the LA. *LA*, Left atrium; *RA*, right atrium; *LV*, left ventricle; *RV*, right ventricle.

before balloon inflation was 7.356 ± 0.015 and decreased over the course of the study, resulting in statistically significant reductions from the 6th day on, suggesting mild metabolic acidosis (Figure 5, *E*). There were no changes in arterial partial pressure of oxygen, partial pressure of carbon dioxide, total hemoglobin, hematocrit, protein, or lactate over the study (Figure 5, *A-D*, *F*, and *G*). Arterial glucose levels were significantly lower on the last day of the study compared with day 0 (Figure 5, *H*).

Changes in Ventricular Volume and Systolic Function

LV EDV was significantly decreased in the LAB fetuses at termination compared with baseline LAB ($P = .0017$), and terminal LAB EDV was 32% of terminal Control EDV ($P = .0012$). There was no change in LV ESV in LAB fetuses between baseline and terminal, but at termination, LAB ESV was 30% of terminal Control ($P = .0042$). LV EF was not different between terminal and baseline values within the LAB group or between LAB and Control fetuses at termination.

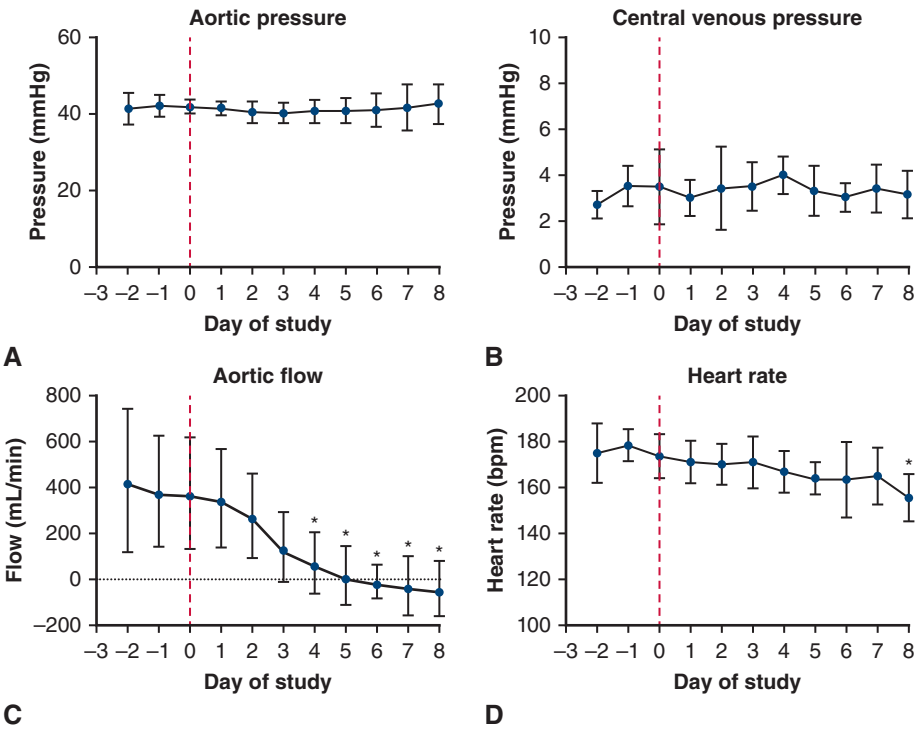

FIGURE 4. Mean aortic pressure (A), central venous pressure (B), ascending aortic flow (C) (Transonic flow probe), and heart rate (D) during the 8 days of LA balloon inflation. Data are mean ± SD. *$P < .025$ compared with baseline (before balloon inflation). Data are for LAB fetuses only, n = 8. The *dashed line* indicates the start of balloon inflation.

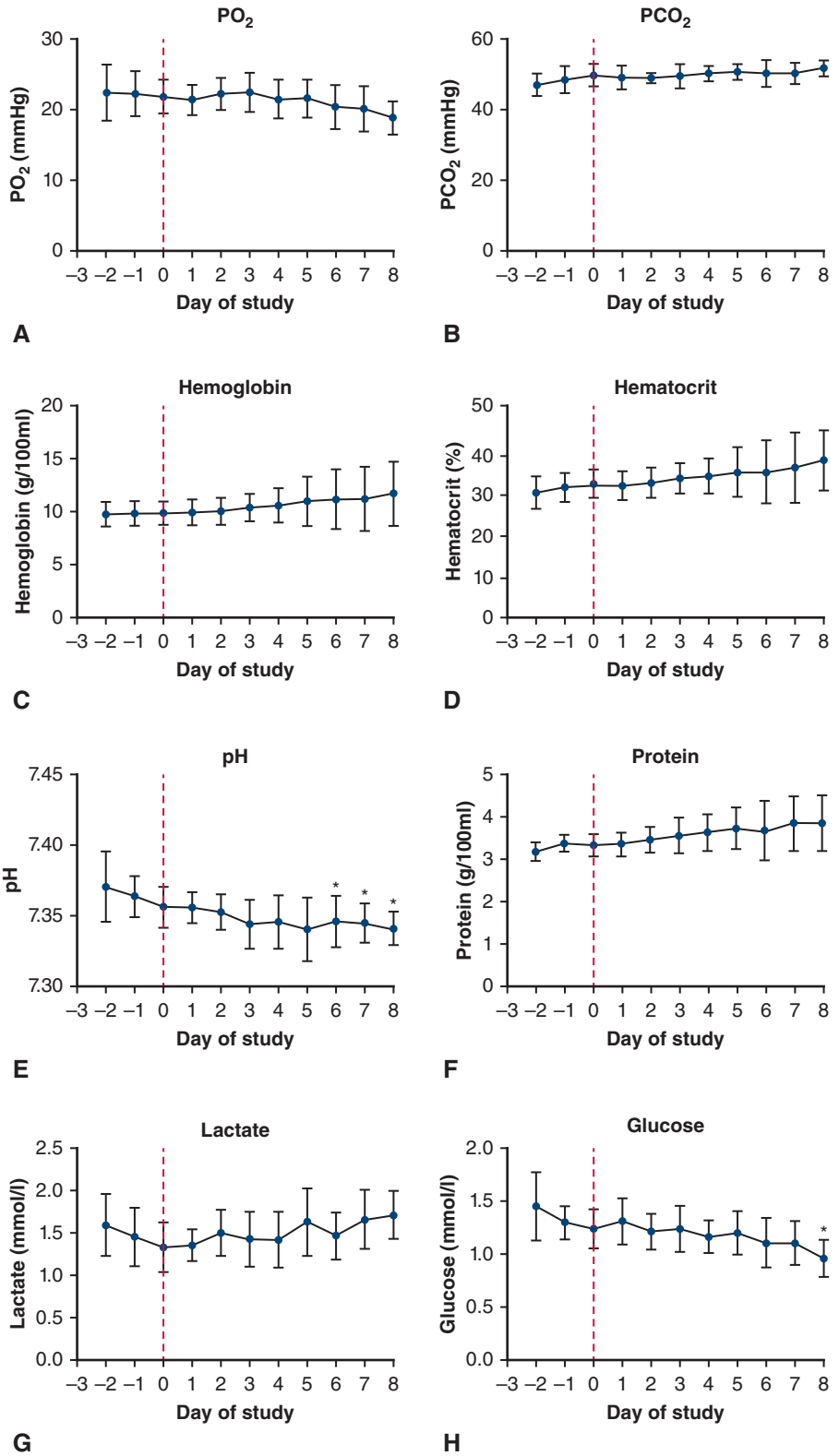

FIGURE 5. A-H, Temporal change of pO2, pCO2, hemoglobin, hematocrit, pH, protein, lactate, and glucose during the experiment. Arterial blood values during the 8 days of LA balloon inflation. Data are mean ± SD. *$P < .025$ compared with baseline (before balloon inflation). Data are for LAB fetuses only, n = 8. The *dashed line* indicates the start of balloon inflation.

RV EDV significantly increased in LAB fetuses between baseline and terminal study ($P = .0069$), but was not different between terminal LAB and terminal Control fetuses. The increase in RV ESV within LAB fetuses between baseline and terminal study was not different when the P value was adjusted for multiple comparisons ($P = .0033$), and was not different between terminal LAB and terminal Control fetuses. The increase in RV EF was not significant within LAB fetuses between baseline and terminal studies when adjusted for multiple comparisons ($P = .0346$) and was not different between terminal LAB and terminal Control fetuses (Table 1).

The LV/RV area ratio was calculated from cross-sectional images of the mid-level of the heart in both LAB and Control fetuses obtained at termination. In diastole, the LV/RV area ratio of LAB fetuses was 38% of the Control LV/RV area ratio (0.21 ± 0.06 vs 0.56 ± 0.05, $P = .0065$). In systole, the LV/RV area ratio of LAB fetuses was 28% of the Control LV/RV area ratio (0.15 ± 0.04 vs 0.53 ± 0.16, $P = .0478$).

Fetal Morphology

Fetal body weight at termination was not different between groups ($P = .8823$). Hearts from the LAB group were 19% heavier than hearts from the Control group ($P = .0470$). Heart to body weight ratios were similarly greater ($P = .0126$). No difference was observed in LV free wall weights (non-normalized) between groups, but RV free walls were significantly heavier in the LAB group compared with the Control group ($P = .0469$). The ratio of LV to RV weight was less in LAB hearts compared with Controls ($P = .0077$). The LV to heart weight ratio was significantly lower in LAB compared with Control ($P = .0009$), but there was no difference in the RV to heart weight ratio ($P = .5790$). No difference was observed in the septal weight between the groups. LAB LA weight was significantly greater than Control ($P = .0156$); likewise, the LAB LA to heart weight ratio was significantly greater than Control ($P = .0377$). The LAB RA weight was also significantly greater than Control ($P = .0111$), as was RA to heart weight ($P = .0068$) (Table 2).

Histopathology

Focal fibrosis and inflammatory infiltrates were observed in the myocardium of the LAB LA, indicating inflammation caused by the balloon insertion or with repeated abrasion between the balloon and the atrial endocardium. The LAB group exhibited mild inflammatory changes in the RA and focal inflammatory infiltrates with replacement fibrosis in the RV, suggesting right-sided heart remodeling due to volume overload compared with the control group (Figure E1). No differences were observed between the groups in the LV or interventricular septum. In transmission electron microscopy evaluation, the images examined showed preserved myofibrils with intact sarcomeres and there was no evidence of endocardial fibroelastosis in the LV.

DISCUSSION

We successfully established an in utero hypovolemic LV fetal lamb model by gradually obstructing mitral inflow with an inflatable balloon catheter in the LA. Our primary objective was to ascertain whether LA balloon inflation affected cardiac morphological and functional development, rather than to exactly replicate the developmental timing of HLHS. We found that this is a promising experimental model for investigating how flow dynamics impact fetal cardiac development and for developing a more severe model of HLHS in animals, which can be leveraged for chronic physiological echocardiographic imaging studies, and which holds promise as a model in which to test the surgical interventions to reverse LV unloading such as a ventricular septal defect or aortic valve insufficiency.

An animal model of HLHS has long been sought. Harh and colleagues[6] first reported the induction of hypovolemic LV in an animal model (chicken embryo) by implanting a nylon device into the left atrioventricular canal in 1973, reporting a survival of 20% at 12 days. Sedmera and colleagues[7,8] reduced LV filling by LA ligation in chicken embryos, with a 25%-30% survival at 4.5 days. In 1978, Fishman and colleagues[10] were the first to effectively impair LV growth in the fetal lamb model, which they did by inflating a LA balloon with silicone rubber during the surgical implantation; fetuses all died within

TABLE 1. Fetal ventricular volumes and systolic function as determined by transesophageal ultrasound

	Baseline LAB	Terminal LAB	Terminal control
(n)	(11)	(4)	(5)
LV EDV (mL)	4.0 ± 0.5*	2.3 ± 0.7	7.1 ± 0.8*
LV ESV (mL)	1.95 (1.74-2.11)	1.01 (0.95-1.95)	3.38 (3.28-3.57)*
LV EF (%)	51.1 ± 2.9	46.1 ± 9.7	51.8 ± 3.6
RV EDV (mL)	5.6 ± 1.1*	12.0 ± 2.6	11.6 ± 3.8
RV ESV (mL)	2.4 ± 0.5*	4.8 ± 1.4	5.8 ± 1.7
RV EF (%)	56.7 ± 2.0	60.8 ± 3.9	57.0 ± 4.2

Baseline LAB measurements were taken during surgery (anesthetized), after placement of catheters and other instrumentation. A second imaging study (anesthetized) was performed after 8 days of balloon inflation (terminal LAB, n = 4) and in age-matched controls. Data are mean ± SD (normally distributed data) or median with interquartile range (non-normally distributed data). *LAB*, Left atrial balloon group; *LV*, left ventricular; *EDV*, end-diastolic volume; *ESV*, end-systolic volume; *EF*, ejection fraction; *RV*, right ventricular. *$P < .025$ versus terminal LAB by paired t test (normally distributed data) or Wilcoxon signed-rank test (non-normally distributed data).

TABLE 2. Fetal body and heart weights from left atrial balloon group (n = 7-8*) and age-matched controls (n = 7)

Values	Control (n = 7)	LAB (n = 8)	*P* value
Body weight (kg)	4.5 ± 0.6	4.6 ± 0.9*	NS
Heart weight (g)	26.4 ± 2.4	31.4 ± 5.6*	.0470
Heart/body (g/kg)	5.92 ± 0.62	6.93 ± 0.83*	.0126
LV (g)	7.9 ± 1.1	7.4 ± 1.8	NS
RV (g)	7.59 (6.92-7.95)	8.35 g (7.73-11.25)	.0469
Septum (g)	5.1 ± 0.7	5.5 ± 0.9	NS
LA (g)	2.03g (1.77-2.28)	2.67 (2.36-3.41)	.0156
RA (g)	1.4 ± 0.3	2.2 ± 0.7 g	.0111
LV/RV (g/g)	1.080 ± 0.192	0.826 ± 0.165	.0077
LV/heart (g/g)	0.300 ± 0.037	0.236 ± 0.037	.0009
RV/heart (g/g)	0.282 ± 0.037	0.290 ± 0.021	NS
LA/heart (g/g)	0.078 ± 0.009	0.100 ± 0.027	.0377
RA/heart (g/g)	0.055 (0.049-0.063)	0.064 (0.061-0.083)	.0068

Data are mean ± SD (normally distributed data) or median with interquartile range (non-normally distributed data). *LAB*, Left atrial balloon group; *LV*, left ventricle; *NS*, not significant; *RV*, right ventricle; *LA*, left atrium; *RA*, right atrium. *Means the total sample numbers were 8, and the others were 7 in the LAB column.

2 to 7 days (mean 4 days). In 2020, Wong and colleagues[11] published innovative transcatheter occlusions of the fetal ovine foramen ovale with a 76% survival at 21 days of those successfully implanted (50% survival of those attempted). More recently, Reuter and colleagues[12] occluded mitral inflow in fetal lambs by transcatheter delivery of coils into the LA, with 44% survival at 46 days. Common among all these models is the abrupt onset of LV inflow reduction at the time of procedure; ours is the first with a gradual onset that allows hemodynamic compensation with a survival of 58%.

Our study data demonstrated that mitral inflow obstruction for 8 days in a fetal lamb during late gestation led to a significantly reduced LV volume (EDV smaller by 68% and ESV smaller by 70% compared with twin controls), with less relative change in the RV volume, indicating asymmetrical ventricular effects due to the altered flow dynamics. Likewise, reductions in LV chamber dimensions, or in the ratio of LV to RV dimensions, have been quantified in the previous animal models of impaired LV inflow.[7,11,12] Of particular note is the similar degree of morphological change in our 8-day study of graded mitral blockade and Wong and colleagues' 21-day study of foramen ovale blockade.[11] In our study, total heart weight (normalized and non-normalized to body weight) was significantly heavier in the instrumented fetuses, which contrasts with the normal or reduced total heart weights others have observed.[7,11] However, similar to previous studies,[7,10-12] we found undergrowth of the LV in relationship to the RV. Interestingly, our histological findings indicate a degree of fibrotic remodeling of the RV and atria, consistent with the profibrotic myocardium describe by Reuter and colleagues.[12] Other anatomic characteristics of HLHS have been described in animal models but not yet fully described in this model, including a small aortic and MV annuli, and narrowed aorta.[6,11,12]

Fetal monitoring data collected during balloon inflation provided insights into the adaptions and development of fetuses with HLHS during gestation. As also described by Fishman and colleagues,[10] despite largely abolishing aortic flow, systemic pressure was maintained and stable. In contrast to Fishman and colleagues,[10] who noted progressive and severe hypoxemia, blood gases were also largely stable in our study. Although an instrumented control group was not evaluated in this study, these values were within the normal range reported in our previous studies.[14,16,17] However, there was a continuous decrease in pH and glucose levels, which we speculate was due to the development of metabolic acidosis as a result of reduced cardiac output. Metabolic diseases, neonatal sepsis, and congenital adrenal hyperplasia cause metabolic acidosis, which is commonly seen in newborns and is caused by acid deposition in body fluids rather than bicarbonate loss, but congenital heart diseases such as HLHS also lead to metabolic acidosis, especially after birth.[18]

Study Limitations

Our study has several limitations, including the gestational timing of the intervention and a relatively short duration of mitral inflow obstruction. The differences we report from our 8-day intervention are comparable to the degree of changes in ventricular volumes and weights observed after 3-week foramen ovale occlusion,[11] although not as profound as occurs 6 weeks after LA coiling,[12] which suggest a longer period of restricted LV inflow will induce more distinct changes in this model. Our aim in this study was to develop a reproducible model of obstructed LV inflow with measurable outcomes (adapted from previous efforts[10]) at a gestational age range typical of our previous studies. The next step of developing this model will include

initiating this intervention earlier in gestation and obstructing LV inflow for longer. Future studies will include in-depth characterization of other parameters known to be affected by HLHS (including valve and aortic arch geometry), as well as investigation of factors that impact postnatal survival and resilience including coronary vascular growth and cardiomyocyte number.

CONCLUSIONS

In this late-gestation fetal lamb model, obstructing mitral inflow inhibited LV development and induced RV remodeling, providing evidence for a potential mechanistic link among blood inflow, ventricular filing, and LV volumetric growth in utero. The no flow/no grow hypothesis is supported in this large animal model, and this methodology can be used as a novel animal model to gain insights into the pathophysiology of and advancement of new therapeutic strategies for patients with HLHS.

Webcast

You can watch a Webcast of this AATS meeting presentation by going to: https://www.aats.org/resources/chronic-in-utero-mitral-valve-obstruction-induces-lv-hypoplasia-and-rv-dilatation-in-a-late-gestation-fetal-lamb-model.

Conflict of Interest Statement

M.P. reports stock ownership, board directorship, and employment in Nyra Medical Inc; receives personal consulting fees from Heart Repair Technologies and research grants from Heart Reparit Technologies; received Single Ventricle Blue Sky grant from Additional Ventures; and led this work when he was at Emory University. D.O. received personal consulting fees from Nyra Medical Inc. All other authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: fetal lamb model, hypoplastic left heart syndrome, large animal model

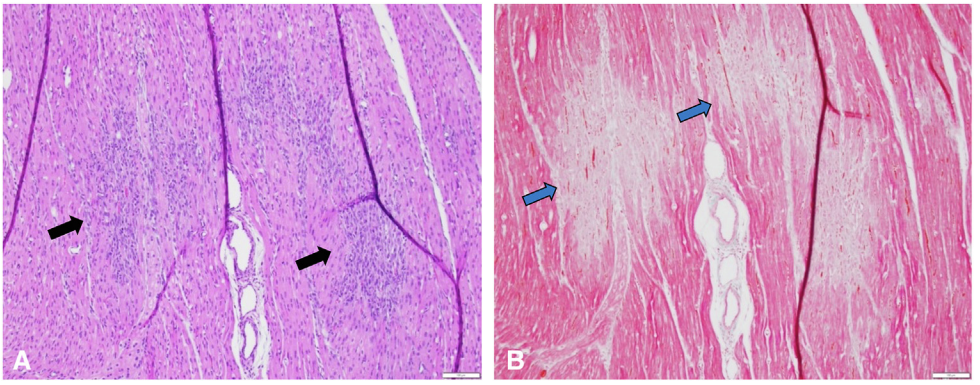

FIGURE E1. Representative images of (A) hematoxylin–eosin stain showing mild epicardial inflammation and hemorrhage (*black arrows*) in the RV of a LAB fetus and (B) Masson's Trichome showing fibrosis in the RV (*blue arrows*). Magnification for both images 20× (scale bar = 100 μm).