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We have established a reliable in utero model of obstructed mitral inflow leading to stunted LV growth and RV remodeling in fetal lambs, that may be utilized to study the no flow/no grow hypothesis of hypoplastic left heart syndrome.

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

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DISCLOSURES

Dr. Padala (MP) reports stock ownership, board directorship and employment in Nyra Medical Inc. MP also receive personal consulting fees from Heart Repair Technologies, and research grants from Heart Reparit Technologies. MP led this work when he was at Emory University. Dr. Onohara received personal consulting fees from Nyra Medical Inc. None of the other authors have any other financial disclosures.

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Central Picture: 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 left ventricle that allows for mechanistic and therapeutic studies regarding hypoplastic left heart syndrome.

Perspective statement

Lack of physiological models hinder mechanistic studies for hypoplastic left heart syndrome. 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 left ventricular growth and induces right ventricular remodeling.

**ABBRVIATIONS AND ACRONYMS**

- **BW** = body weight
- **dGA** = days gestational age
- **EDV** = end-diastolic volume
- **EF** = ejection fraction
- **ESV** = end-systolic volume
- **HLHS** = hypoplastic left heart syndrome
- **HW** = heart weight
- **LA** = left atrium
- **LAB** = left atrial balloon group
- **LV** = left ventricle
- **MV** = mitral valve
- **PA** = pulmonary artery
- **PCO$_2$** = partial pressure of carbon dioxide
- **PO$_2$** = partial pressure of oxygen
- **PVC** = polyvinyl chloride
- **RA** = right atrium
- **RV** = right ventricle
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 to hypoplastic left heart syndrome (HLHS). This has been demonstrated in chick embryos, but less so in large animals. We investigated the impact of mitral obstruction on left (LV) and right ventricular (RV) 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 one fetus (LAB), while the twin remained an uninstrumented control. The balloon was inflated gradually over eight days until net antegrade aortic flow was eliminated. Fetal transesophageal echocardiography was performed at the time of surgery and just prior to termination in both groups.

Results: Terminal fetal body weights were comparable between groups. Terminal heart/body weight ratio was higher in LAB fetuses (6.9±0.8g/kg) compared to Controls (5.9±0.6g, p=0.0126). The LV/RV weight ratio was 24% (p=0.0077) lower in LAB fetuses than Controls. LV/heart weight (0.24±0.04g/g vs. 0.30±0.04g/g, p=0.0009), LV EDV (2.3±0.7ml vs. 7.1±0.8ml; p=0.0012), and LV ESV (1.01ml (0.95 to 1.95ml) vs. 3.38ml (3.28 to 3.57ml), p=0.0042) were lower in LAB fetuses compared with Controls. RV weight (g/kg), RV EDV and RV ESV were similar between groups.

Conclusion: In this late-gestation fetal lamb model, in utero obstruction of mitral inflow slowed LV growth and caused RV remodeling.

Word count: 247/250

Keywords: large animal model, fetal lamb model, hypoplastic left heart syndrome
INTRODUCTION

Approximately 40,000 babies are born with a congenital heart defect in the US each year, of which hypoplastic left heart syndrome (HLHS) is one of the common lesions.\textsuperscript{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 non-functional left ventricle (LV). Surviving a newborn with HLHS requires three 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 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 multi-organ dysfunction with time.\textsuperscript{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 \textit{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.\textsuperscript{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 et al. successfully obstructed the MV by implanting a rubber balloon into the LA of fetal lambs (0.6-0.8 gestation), but all fetuses died 2-7 days after the surgery.\textsuperscript{10} Wong et al developed a percutaneous method of occluding the foramen ovale in lambs (0.7-0.75 gestation), demonstrating a degree of LV hypoplasia.\textsuperscript{11} More recently, Reuter et al. 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 upon these previous efforts to obstruct MV inflow to develop a reduced LV growth model as follows: (a) gradually inflate the LA balloon over several days, reducing the high mortality rate; (b) assess fetal cardiac function and ventricular chamber hemodynamics by transesophageal echocardiography at baseline and at termination; and (c) compare loaded and unloaded chamber volumes and geometries.

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, Oregon). Surgery was performed at 119±1 days gestational age (dGA; 0.8 gestation). Ten of these ewes were carrying twins, two were carrying singleton fetuses. In ewes with twins, only one fetus underwent sterile surgery (LAB) while the other was left uninstrumented (Control). Baseline echocardiographic imaging was conducted at the time of surgery exclusively in the LAB group, due to 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 dGA), 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: anesthetic was induced with ketamine (400mg, intravenous [iv]) and diazepam (10mg, iv) 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.

**Surgical procedure:** Surgery was performed as previously described, with modifications (Figure 2). The upper body of one 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 (4th intercostal space) was then performed to expose the heart, followed by a pericardiotomy. The plane between the aorta and main pulmonary artery was dissected and a transit time flow probe (8mm Perivascular Flowprobe, Transonic Systems Inc, Ithaca, NY) 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, then were 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.

Post-operative care: Ciprofloxacin (2mg) and Penicillin G (1 million units) were administered into the fetal amniotic sac. Ewes received subcutaneous buprenex (0.3mg) and buprenorphine Sustained-Release (0.05mg/kg) immediately after surgery for analgesia. The ewes were monitored post-operatively, with unrestricted access to food and water. After 3-5 days recovery, they were moved to individual housing stanchions for the remainder of the study.

Daily measurements and inflation of LA balloon: Arterial, central venous and amniotic fluid pressures, and aortic flow, were monitored as previously described. Daily arterial blood samples were obtained to assess pH, partial pressures of oxygen (PO2), carbon dioxide (PCO2), 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 ~30% daily reduction in aortic flow. Once antegrade flow was abolished, typically after 4 days, the balloon was not inflated further. Pressure and flow data were averaged over ~23 hours per day, excluding data only during transient signal interference. Arterial blood was sampled, and blood gases and contents 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 endpoint echocardiographic data are compared to LAB baseline and Control endpoint (there is no Control baseline). A sterile 10Fr intracardiac echo probe (AcuNav 10Fr; Biosense Webster, Irvine, CA) 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 side 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. TEE data are only available for four LAB fetuses due to one fetal death subsequent to maternal anesthesia and prior to the final imaging study, poor image quality in one study, and theft of equipment containing data for two studies. Additionally, during the terminal study only, M5Sc-rs probe (GE Precision Healthcare LLC, Cincinnati, OH) 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 (10,000U) followed by saturated potassium chloride to arrest the heart in diastole (administered via venous catheter (LAB) or direct injection to the umbilical vein (Control)).
Following removal of both fetuses, each ewe was euthanized with sodium pentobarbital while still anesthetized (SomnaSol, 80mg/kg, i.v.). 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 either 4% paraformaldehyde or 2% paraformaldehyde + 3% glutaraldehyde at mean arterial pressure. Following overnight immersion fixation in either solution, the heart was photographed, re-weighed, 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 seven months) before transfer to 10% non-buffered 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 2x, 4x, 10x, and 20x and qualitatively evaluated for histological tissue remodeling. Ultrastructural images at 500x, 1000x, 1500x, and additional images at 2500x were evaluated for myofibrillar and interstitial alterations.

**Statistical methods**

Data analysis was conducted using Prism 9.0 (GraphPad Software Inc, San Diego, CA). Data is presented as mean ± standard deviation (normally distributed data) or median with interquartile range (non-normally distributed data). Repeated measures one-way ANOVA 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 vs. Control terminal, or for LAB baseline vs. 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 an <0.05 for the family of tests, and therefore at p<0.025 for each individual test.

RESULTS

Procedural outcomes

Twelve pregnant ewes with 22 fetuses were used for the experiments. Five fetuses died prior to the terminal procedure. Only one fetus died during surgery, due to bleeding from the superior vena cava while positioning the flow probe. Two fetuses died post-operatively related to umbilical torsion on the 1st day or compression on the 14th day. The other two died for unknown reasons on the 5th and 12th post-operative days (study days 0 and 6, respectively). The overall fetal mortality rate in this series was 42%. Survival increased as our experience increased; we had 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 eight days of LA balloon inflation (Figure 4a, b). On the 4th day, and thereafter, mean aortic flow was significantly lower compared to aortic flow prior to balloon inflation on day 0 (Figure 4c). Heart rate gradually decreased, as expected for this stage of gestation, and was significantly lower on day 8 than day 0 (Figure4d). Arterial pH 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 5e). There were no changes in arterial PO2, PCO2, total hemoglobin, hematocrit, protein, or lactate over the study (Figure 5a-d, f, g). Arterial glucose levels were significantly lower on the last day of the study compared to day 0 (Figure 5h).
Changes in ventricular volume and systolic function

LV EDV was significantly decreased in the LAB fetuses at termination compared to baseline LAB (p=0.0017), and terminal LAB EDV was 32% of terminal Control EDV (p=0.0012). 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=0.0042). LV EF was not different between terminal and baseline values within the LAB group, or between LAB and Control fetuses at termination.

RV EDV significantly increased in LAB fetuses between baseline and terminal study (p=0.0069), but was not different between terminal LAB and terminal Control fetuses. The increase in RV ESV within LAB fetuses between baseline to terminal study was not different when the p value was adjusted for multiple comparisons (p=0.0033), 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=0.0346), 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=0.0065). 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=0.0478).

Fetal morphology

Fetal body weight at termination was not different between groups (p=0.8823). Hearts from the LAB group were 19% heavier than hearts from the Control group (p=0.0470). Heart to body weight ratios were similarly greater (p=0.0126). 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=0.0469). The ratio of LV to RV weight was less in LAB hearts compared with Controls (p=0.0077). The LV to heart weight ratio was significantly lower in LAB compared to Control (p=0.0009), but there was no difference in the RV to heart weight ratio (p=0.5790). No difference was observed in the septal weight between the groups. LAB LA weight was significantly greater than Control (p=0.0156); similarly, the LAB LA to heart weight ratio was significantly greater than Control (p=0.0377). The LAB RA weight was also significantly greater than Control (p=0.0111), as was RA to heart weight (p=0.0068) (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 to the control group (Figure S1). 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.

Animal model of HLHS have long been sought. Harh et al. \(^6\) 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 et al.\(^7\)\(^,\)\(^8\) reduced LV filling by LA ligation in chicken embryos, with a 25-30% survival at 4.5 days. In 1978, Fishman et al.\(^10\) 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 two to seven days (mean four days). In 2020, Wong et al.\(^11\) 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 et al.\(^12\) 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 rate of 58%.

Our study data demonstrated that mitral inflow obstruction for eight 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 to twin controls), with less relative change in the RV volume, indicating asymmetrical ventricular effects due to the altered flow dynamics. Similarly, 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 eight-day study of graded mitral blockade and Wong et al.’s 21-day study of foramen ovale blockade. 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. However, similar to previous studies, 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 pro-fibrotic myocardium describe by Reuter et al. Other anatomical characteristics of HLHS have been described in animal models but not yet fully described in this model, including a small aortic and mitral valve annuli, and narrowed aorta.

Fetal monitoring data collected during balloon inflation provided insights into the adaptations and development of fetuses with HLHS during gestation. As also described by Fishman et al., despite largely abolishing aortic flow, systemic pressure was maintained and stable. In contrast to Fishman et al., 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. There was, however, 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.

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
following 3-week foramen ovale occlusion,\textsuperscript{11} although not as profound as occurs 6 weeks after LA coiling,\textsuperscript{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\textsuperscript{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 between 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 utilized as a novel animal model to gain insights into the pathophysiology of and advancement of new therapeutic strategies for HLHS patients.
Table 1. Fetal ventricular volumes and systolic function as determined by transesophageal ultrasound. 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 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). *p<0.05 vs. terminal LAB by paired t-test (normally distributed data) or Wilcoxon signed rank test (non-normally distributed data).
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<th>Control (n=7)</th>
<th>LAB (n=8)</th>
<th>p-value</th>
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<td>4.5±0.6</td>
<td>4.6±0.9*</td>
<td>NS</td>
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<td>Heart weight (g)</td>
<td>26.4±2.4</td>
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<td>Heart/body (g/kg)</td>
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<td>RV (g)</td>
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<td>8.35g (7.73-11.25)</td>
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<td>Septum (g)</td>
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<tr>
<td>LA (g)</td>
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<td>2.67 (2.36-3.41)</td>
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<td>RA (g)</td>
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</tbody>
</table>

Table 2. Fetal body and heart weights from LAB (n=7-8* and age-matched controls (n=7). Data are mean ± SD (normally distributed data) or median with interquartile range (non-normally distributed data). * means the total sample numbers were 8, and the others were 7 in the LAB column.
FIGURE LEGENDS

Figure 1. These illustrations summarize the methods and results in this experiment as the graphical abstract. LA: left atrium, LV: left ventricle, RV: right ventricle, PA: pulmonary artery, PDA: patent ductus arteriosus, BCT: brachiocephalic trunk.

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 left atrium and the flowprobe around ascending aorta. PVC; polyvinyl chloride, RV; right ventricle, LA; left atrium, PA; pulmonary artery.

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

Figure 4. A) Mean aortic pressure, B) central venous pressure, C) ascending aortic flow (Transonic flow probe), and D) heart rate during the 8 days of LA balloon inflation. Data are mean ± SD. *p<0.05 compared to baseline (before balloon inflation). Data are for LAB fetuses only, n=8. The dashed line indicates the start of balloon inflation.

Figure 5. Arterial blood values during the 8 days of LA balloon inflation. Data are mean ± SD. *p<0.05 compared to baseline (before balloon inflation). Data are for LAB fetuses only, n=8. The dashed line indicates the start of balloon inflation.

Figure S1. Representative images of A) H&E 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 20x (scale bar = 100µm).
REFERENCES


9. deAlmeida A, McQuinn T, Sedmera D. Increased ventricular preload is compensated by myocyte proliferation in normal and hypoplastic fetal chick left ventricle. Circ Res 2007;100(9):1363-70.


Table 1. Fetal ventricular volumes and systolic function as determined by transesophageal ultrasound. 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 balloon inflation (terminal LAB, n=4) and in age matched terminal Controls. Data are mean ± SD (normally distributed data) or median with interquartile range (non-normally distributed data). *p<0.05 vs. Terminal LAB by paired t-test (normally distributed data) or Wilcoxon signed rank test (non-normally distributed data).
<table>
<thead>
<tr>
<th></th>
<th>Control (n=7)</th>
<th>LAB (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>4.5±0.6</td>
<td>4.6±0.9*</td>
<td>NS</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>26.4±2.4</td>
<td>31.4±5.6*</td>
<td>0.0470</td>
</tr>
<tr>
<td>Heart/body (g/kg)</td>
<td>5.92±0.62</td>
<td>6.93±0.83*</td>
<td>0.0126</td>
</tr>
<tr>
<td>LV (g)</td>
<td>7.9±1.1</td>
<td>7.4±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>RV (g)</td>
<td>7.59 (6.92-7.95)</td>
<td>8.35g (7.73-11.25)</td>
<td>0.0469</td>
</tr>
<tr>
<td>Septum (g)</td>
<td>5.1±0.7</td>
<td>5.5±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>LA (g)</td>
<td>2.03g (1.77-2.28)</td>
<td>2.67 (2.36-3.41)</td>
<td>0.0156</td>
</tr>
<tr>
<td>RA (g)</td>
<td>1.4±0.3</td>
<td>2.2±0.7</td>
<td>0.0111</td>
</tr>
<tr>
<td>LV/RV (g/g)</td>
<td>1.080±0.192</td>
<td>0.826±0.165</td>
<td>0.0077</td>
</tr>
<tr>
<td>LV/heart (g/g)</td>
<td>0.300±0.037</td>
<td>0.236±0.037</td>
<td>0.0009</td>
</tr>
<tr>
<td>RV/heart (g/g)</td>
<td>0.282±0.037</td>
<td>0.290±0.021</td>
<td>NS</td>
</tr>
<tr>
<td>LA/heart (g/g)</td>
<td>0.078±0.009</td>
<td>0.100±0.027</td>
<td>0.0377</td>
</tr>
<tr>
<td>RA/heart (g/g)</td>
<td>0.055 (0.049-0.063)</td>
<td>0.064 (0.061-0.083)</td>
<td>0.0068</td>
</tr>
</tbody>
</table>

Table 2. Fetal body and heart weights from LAB (n=7-8*) and age-matched controls (n=7). Data are mean ± SD (normally distributed data) or median with interquartile range (non-normally distributed data). * means the total sample numbers were 8, and the others were 7 in the LAB column.
We have established a reliable in utero model of obstructed mitral inflow leading to stunted LV growth and RV remodeling in fetal lambs, that may be utilized to study the no flow/no grow hypothesis of hypoplastic left heart syndrome.

LA: left atrium, LV: left ventricle, RV: right ventricle, PA: pulmonary artery, PDA: patent ductus arteriosus, BCT: brachiocephalic trunk
Mitral inflow obstruction by a balloon stunts LV development and induces RV remodeling.
Chronic In Utero Mitral Valve Obstruction Induces LV hypoplasia and RV Dilatation in a Late Gestation Fetal Lamb Model

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