Investigation of a chronic single-stage sheep Fontan model

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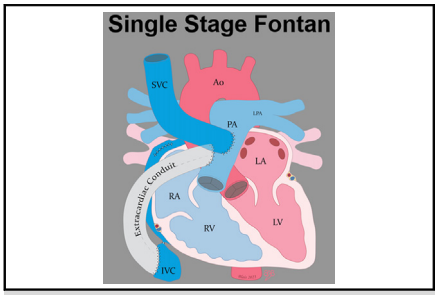
ABSTRACT

Objectives: Our goal was to conduct a hemodynamic analysis of a novel animal model of Fontan physiology. Poor late-term outcomes in Fontan patients are believed to arise from Fontan-induced hemodynamics, but the mechanisms remain poorly understood. Recent advances in surgical experimentation have resulted in the development of a chronic sheep model of Fontan physiology; however, detailed analysis of this model is lacking.

Methods: We created a single-stage Fontan model in juvenile sheep with normal biventricular circulation. The superior vena cava was anastomosed to the main pulmonary artery, and the inferior vena cava was connected to the main pulmonary artery using an expanded polytetrafluoroethylene conduit. Longitudinal hemodynamics, including catheterization and magnetic resonance imaging were evaluated.

Results: Four out of 12 animals survived, with the longest surviving animal living 3 years after single-stage Fontan. We showed a significant era effect regarding survival (1 out of 8 and subsequently 3 out of 4 animals surviving beyond 2 months) attributed in large part to the procedural learning curve. Key characteristics of Fontan hemodynamics, namely systemic venous hypertension and low normal cardiac output, were observed. However, recapitulation of passive human Fontan hemodynamics is affected by volume loading of the right ventricle given an anatomic difference in sheep azygous venous anatomy draining to the coronary sinus.

Conclusions: A significant learning curve exists to ensure long-term survival and future surgical modifications, including banding of the main pulmonary artery and ligation of the azygous to coronary sinus connection are promising strategies to improve the fidelity of model hemodynamics. (JTCVS Open 2024;21:268-78)



Creation of a chronic large animal model of Fontan palliation.

CENTRAL MESSAGE

Large animal model of systemic venous hypertension and low normal cardiac output. There is a steep learning curve in initiating the model and future modifications would enhance the clinical relevance.

PERSPECTIVE

Fontan patients experience significant comorbidities that are largely attributed to the abnormal hemodynamics of Fontan palliation. We investigated the hemodynamics of a novel chronic animal model of Fontan physiology. Our investigation defines the challenges with establishing the model and further surgical modifications for iterative improvements in model design.

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Abbreviations and Acronyms

3D = 3 dimensional
4D = 4 dimensional
IVC = inferior vena cava
MPA = main pulmonary artery
MRA = magnetic resonance angiography
MRI = magnetic resonance imaging
SVC = superior vena cava

▶ Video clip is available online.

Fontan palliation is the final of 3 surgical procedures in children born with single-ventricle heart defects. Although this treatment strategy is lifesaving, it is palliative. Patients cannot expect a normal quality or duration of life, with significant complications arising from cardiovascular, gastrointestinal, renal, and lymphatic abnormalities.[1,2] These complications are believed to arise in response to Fontan-induced changes in hemodynamic parameters: chronically elevated central venous pressure and decreased cardiac output. Our current understanding of the associated pathobiology leading to these adverse outcomes and potential treatment options beyond heart transplantation remains limited.

Despite an improved recognition of underlying causes of congenital heart disease, the complexity of the genetics and relatively low penetrance has made routine study in small animals difficult.[3] Acute models of single-ventricle palliation in animals with normal biventricular anatomy have been studied, but long-term analysis is limited by poor animal survival.[4] Recent advances in surgical experimentation have, for the first time, enabled development of a Fontan survival model in sheep born with normal cardiac anatomy and function.[5] A detailed longitudinal study of this model has not been performed. Herein, we present our experience with this model providing detailed in vivo imaging and hemodynamic measurements. To our knowledge, this study represents the longest reported survival (3 years after single-stage Fontan completion) in an animal model of the Fontan circulation. The focus of this article is 2-fold: first, to characterize our experience in establishing the model and, second, to define the relevance of the model hemodynamics.

METHODS

Single-Stage Fontan Procedure

The Institutional Animal Care and Use Committee at the Abigail Wexner Research Institute approved the use of animals for this experiment (AR20-00121; December 7, 2022). All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.[6] Research was conducted at a US Department of Agriculture-licensed and Association for Assessment and Accredidation of Laboratory Animal Care (AALAC) accredited facility. An overview of the experimental design is shown in the graphical abstract and Figure 1. Baseline hemodynamics and cardiac function were assessed preoperatively in 6-month-old (juvenile) sheep by cardiac catheterization. Then in 12 sheep, the single-stage Fontan model was created by anastomosing the superior vena cava (SVC) to the superior/anterior wall of the main pulmonary artery in an end-to-side fashion (bidirectional Glenn). Following completion of the superior cavopulmonary connection, the short main pulmonary artery (MPA) segment is separated from the ascending aorta. A partial occluding clamp in placed as proximal as possible ensuring that the recently constructed Glenn flows unimpeded. An end graft to side MPA anastomosis is constructed with a 16-mm ring-enforced expanded polytetrafluoroethylene graft (distal anastomosis). The graft is measured twice for optimal length and lay and cut to the desired length and beveled. In ovine species, the intrathoracic portion of the inferior vena cava (IVC) is typically long (>5 cm). This anatomic variant allows placement of a side-biting clamp on the anterior wall of the IVC a few millimeters from the IVC/right atrial junction. An end graft to side IVC anastomosis is carried out. After de-airing maneuvers, the various clamps are removed and the IVC/right atrial junction is doubly ligated with 0-silk (See Videos 1-3 showing the completion of the single-stage Fontan operation). The continuity of the right ventricle to the MPA was kept, allowing egress of coronary sinus flow from the right ventricle to the PAs. Further details of the surgical methodology can be found elsewhere.[7] Two pleural chest tubes were placed in the right and left chest at the time of surgery. Continuous clinical assessment for the first 2 weeks postoperatively was undertaken. Intravenous fluids were provided for the first 2 days following surgery and diuretics typically started on the second postoperative day. In the final 4 animals, esmolol was administered if the heart rate in recovery exceeded 180 beats per minute. Enoxaparin was administered for 2 weeks postoperatively. If an animal died following Fontan completion, a necropsy was performed within 2 hours of the time of death to determine the cause of death.

Magnetic Resonance Imaging

Cardiac magnetic resonance imaging (MRI) used a 3-Tesla magnet (Siemens Medical Solutions) with a 32-channel phased-array cardiac coil at approximately 6-month intervals postoperatively. Animals were sedated with propofol (20-45 mg/kg/minute) and intubated for mechanical ventilation for all MRI studies. Two-dimensional velocity-encoded phase contrast MRI was performed through the ascending aorta, brachiocephalic artery, descending aorta, Glenn, Fontan, MPA, right PA, and left PA. Four-dimensional velocity-encoded phase contrast (4D flow) imaging covering the thorax and high-resolution 3-dimensional (3D) magnetic resonance angiogram (MRA) using a navigator respiratory gated, electrocardiogram gated, inversion-recovery prepped 3D fast gradient echo sequence. Contrast (gadobutrol 0.1 mmol/kg body weight) was injected intravenously for MRA and 4D flow sequences.

Funded by the Additional Ventures Cures Collaborative, Palo Alto, Calif.

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The Institutional Animal Care and Use Committee at the Abigail Wexner Research Institute approved the use of animals for this experiment (AR20-00121; December 7, 2022).

Received for publication Jan 30, 2024; revisions received May 16, 2024; accepted for publication June 4, 2024.

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2666-2736

https://doi.org/10.1016/j.xjon.2024.06.018

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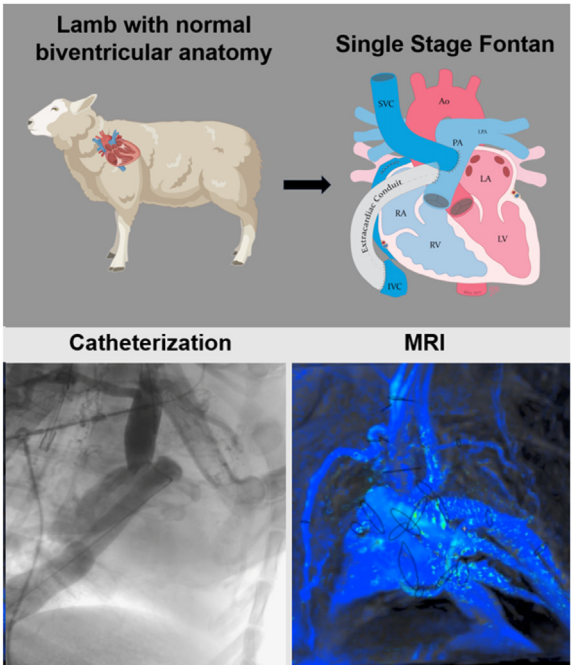


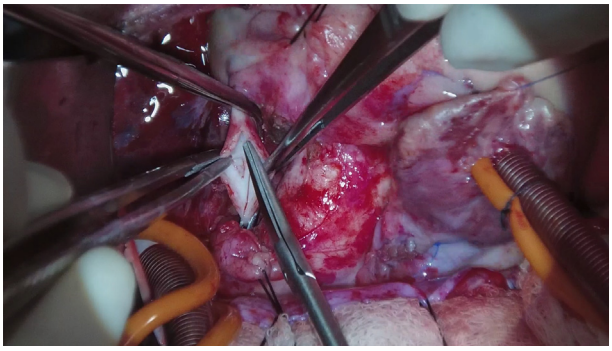
FIGURE 1. Overview of single-stage Fontan model and experimental approach. The single-stage Fontan model is created by anastomosing the superior vena cava to the pulmonary artery trunk in an end-to-side fashion (bidirectional Glenn) and by connecting the inferior vena cava to the pulmonary artery trunk with a synthetic expanded polytetrafluoroethylene graft (modified extracardiac Fontan). Detailed hemodynamic analysis demonstrates physiology that captures the salient features of Fontan physiology, namely systemic venous hypertension and decreased cardiac output. *SVC*, Superior vena cava; *Ao*, aorta; *PA*, pulmonary artery; *LPA*, left pulmonary artery; *LA*, left atrium; *RA*, right atrium; *RV*, right ventricle; *LV*, left ventricle; *IVC*, inferior vena cava; *MRI*, magnetic resonance imaging.

Catheterization

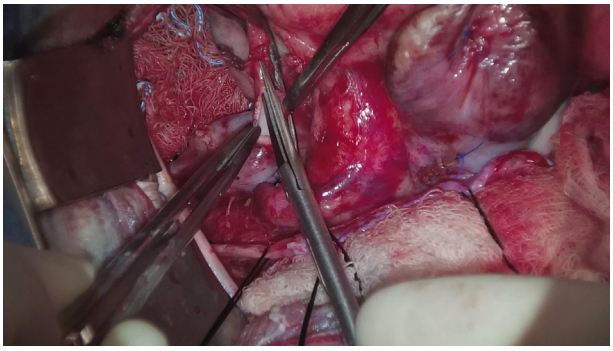
Postoperative catheterizations were performed at approximately 6-month intervals. After sedation and intubation, sheep were placed in a left lateral decubitus position. The right internal jugular vein was cannulated, and a 9 Fr sheath (Terumo Medical Corporation) was inserted followed by an intravenous bolus of heparin (150 U/kg). Pressures were measured in the Glenn, Fontan, MPA, distal PA, and pulmonary capillary wedge. Dedicated angiograms were obtained if venovenous collaterals were noted.

Statistics

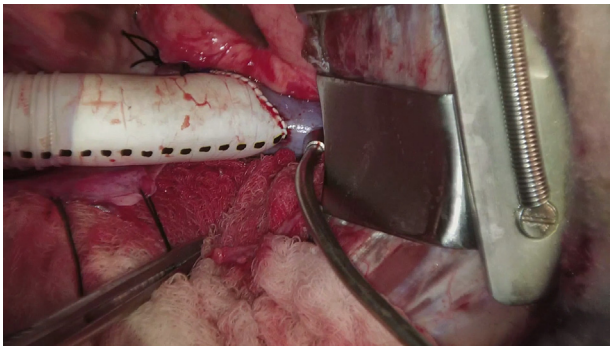
Statistical significance was determined with paired *t* test in comparing baseline to postoperative hemodynamic measures by catheterization and MRI.



VIDEO 1. Pulmonary artery incision for Glenn anastomosis and Glenn anastomosis to pulmonary artery completion. Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.



VIDEO 2. Pulmonary artery incision for Fontan anastomosis and Fontan anastomosis to pulmonary artery completion. Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.



VIDEO 3. Fontan anastomosis to inferior vena cava. Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.

RESULTS

Surgical Model

An intraoperative photo of the surgical procedure is shown in Figure 2, *A*. A 3D rotational angiogram from a postoperative catheterization and associated 3D reconstruction demonstrates the relationship of the Glenn and Fontan pathways (Videos 4 and 5).

Outcome of Single-Stage Fontan Surgeries

The survival outcomes of all animals are detailed in Table 1. Intraoperative survival was ~92% (11 out of 12). Approximately 33% (4 out of 12) of the sheep are still living, at ~3 years, 10 months, 7 months, and 2 months after single-stage Fontan completion. We note a significant increase in animal survival throughout the duration of the study, with 12.5% survival for the first 8 surgeries and 75% survival for the last 4 surgeries. Approximately 33% (4 out of 12) died from procedural complications and 33% (4 out of 12) died from low cardiac output with associated abdominal ascites, pleural effusions, and 1 case of acute kidney injury.

Measured Hemodynamics

Evaluation of the hemodynamics by catheterization and MRI demonstrates sustained and significant elevation of systemic venous blood pressure, altered PA pulsatility, and low resting cardiac output (Figure 2, *B-D*). Assessment of the 4D flow data derived from MRI showed approximately 75% to 90% of the blood flow supplied to the lungs is coming from the SVC (Glenn) and IVC (Fontan) pathways (Figure 3, *D*), whereas the remaining is supplied via antegrade flow from the right ventricle. Table E1 compares the hemodynamics in this preclinical sheep model with those typically observed clinically in children. 4D-flow MRI confirmed a left azygos vein directly connecting to the coronary sinus, which is a known anatomic difference between sheep and humans[8] (Figure 3, *A*, where the left azygos vein outlined in red, and Video 6). This results in a volume load to the coronary sinus in addition to the expected coronary venous blood flow. Longitudinal quantification of blood flow through the azygous vein demonstrates a clear trend of diminished flow and an expected increase in the percent of passive blood flow to the lungs followed by an increase in flow through the azygos system at late term follow-up (Figure 3, *D* and *E*). The decompression of the azygous network is explained by the formation of venovenous collaterals. These collaterals can be seen angiographically, draining to the pulmonary venous and coronary venous systems (Figure 3, *B* and *C*, and Videos 7 and 8). The venovenous collaterals draining to the pulmonary venous system result in right-to-left shunting (blood bypassing the lungs) and systemic cyanosis (Figure 3, *F* and *G*).

DISCUSSION

The development of a preclinical survival model of single-ventricle physiology has the potential to identify new mechanisms of disease underlying the significant complications associated with Fontan palliation and provides a testbed for evaluating the safety and efficacy of new treatments. Building on the experience of others, we sought to investigate a novel sheep Fontan model to assess the hemodynamics in greater detail and define surgical modifications that may enhance the relevance of the model. The model induces hemodynamic changes that reflect salient features of Fontan physiology; namely, systemic venous hypertension, preload deprivation, and low normal cardiac output. The degree of elevation in systemic venous pressure and decreased cardiac output is consistent with hemodynamics that portend the worst outcomes for patients with central venous pressure >15 mm Hg and cardiac output of 2.5 (L/minute/m^2).[9]

The most significant deviation of the model hemodynamics from human Fontan hemodynamics is the persistent connection of the right ventricle to the PAs. We found that the right ventricle induces a pulse pressure of approximately 2 to 3 mm Hg and contributes approximately 10% to 30% of the pulmonary blood flow to the pulmonary vasculature. We also noted a variation in the systemic venous anatomy between sheep and humans that was not reported in the initial evaluation of this sheep Fontan model but is a known anatomic difference in sheep and several other large animals[5]; that is, a left azygous vein draining to the coronary sinus, which represents an additional volume load to the right ventricle beyond coronary venous flow.[10,11] In our longest surviving animal, we found that flow through the left azygous vein connection decreased significantly and then slowly increased over time. This is explained by the formation and dynamic change in flow distribution between venovenous collaterals from the SVC, which decompresses to the pulmonary veins and to the coronary sinus. The formation of such collaterals is seen in human patients undergoing Fontan, where they develop due to the high central venous pressure and tend to balance the venous pressure difference. Antegrade flow within the Glenn shunt is impeded by the pulsatile flow

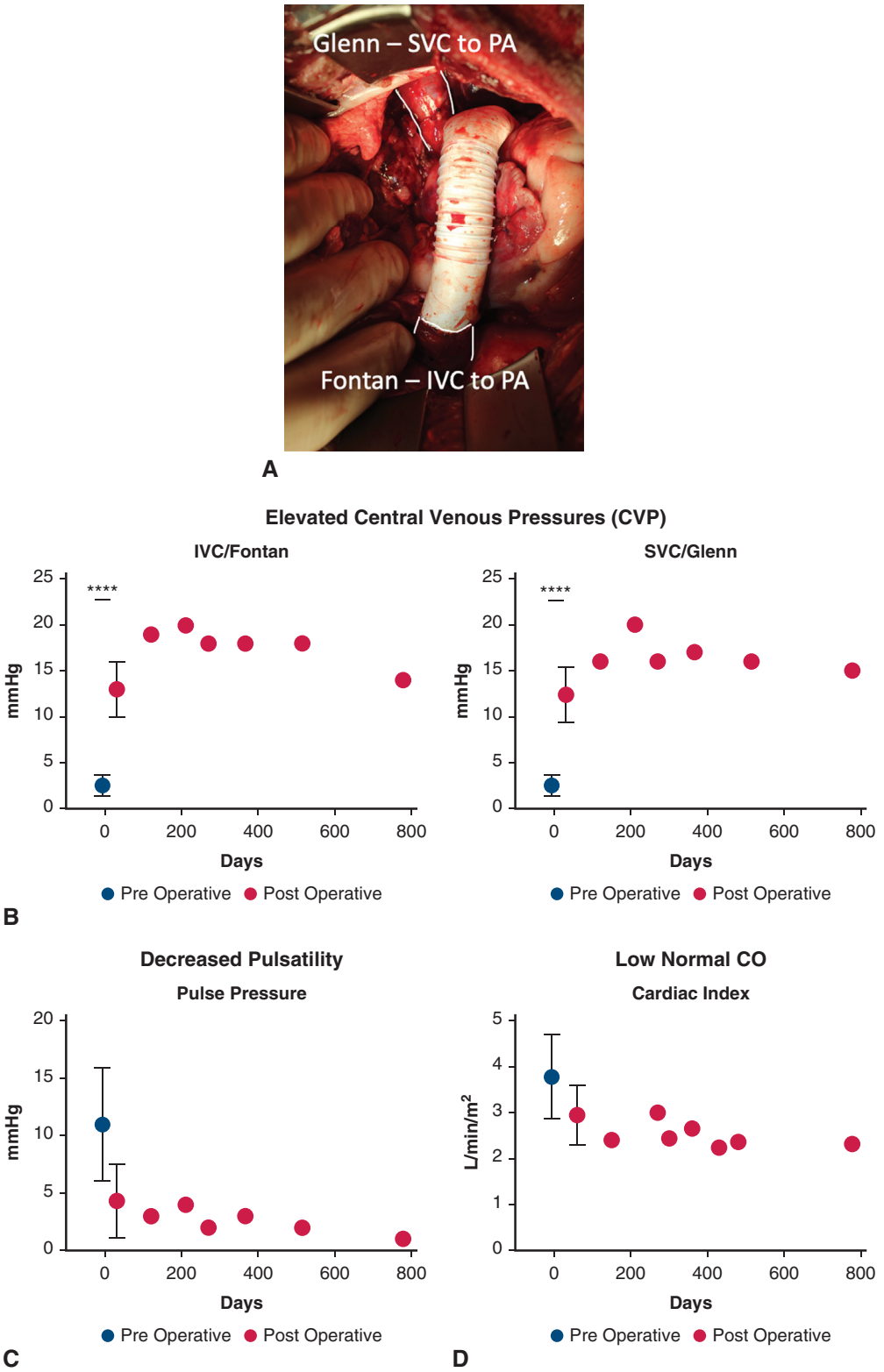
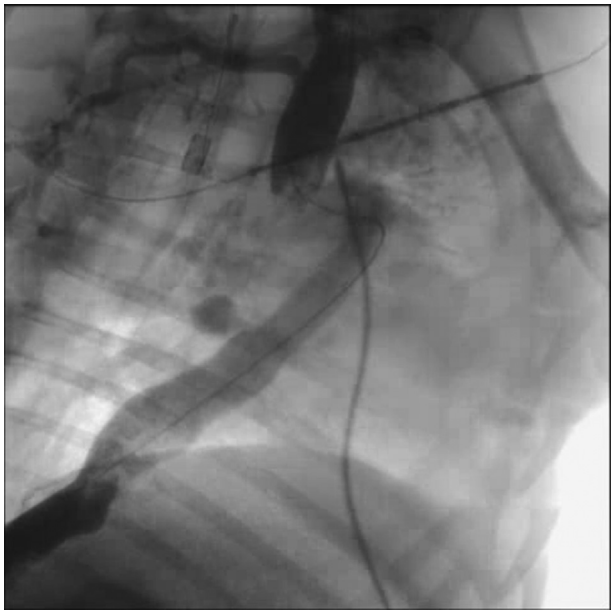
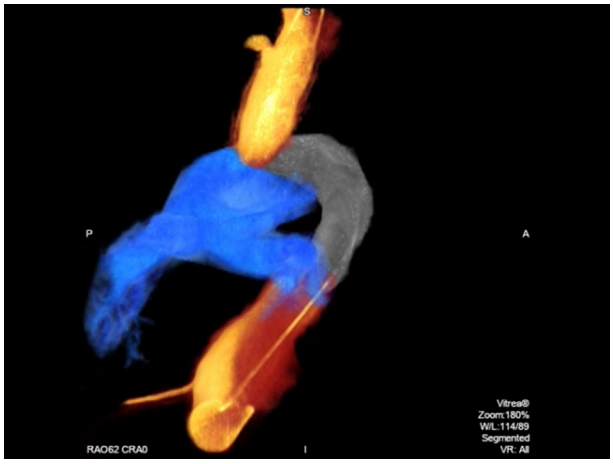


FIGURE 2. Single-stage fontan surgical model and hemodynamics. A, Intraoperative photo showing the Glenn (superior vena cava [*SVC*] to pulmonary artery [*PA*] connection) and Fontan (inferior vena cava [*IVC*] to PA connection with an expanded polytetrafluoroethylene graft). The right ventricle remains connected to the main PA to allow egress of coronary sinus flow. B, Significant and sustained elevation in systemic venous pressures. C, Decreased PA pulsatility. Pulse pressure is equal to the difference in systolic and diastolic pressures. D, Low normal cardiac index. Cardiac index is equal to the cardiac output normalized to body surface area. The sample was 9 preoperative, 4 <180 days postoperative, and 1 >180 days postoperative. Statistical significance determined with paired *t* test. ****$P < .0001$.



VIDEO 4. Three-dimensional rotational angiogram with simultaneous injection in the superior vena cava and inferior vena cava at catheterization demonstrates the single-stage Fontan anatomy. Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.



VIDEO 5. Three-dimensional (3D) reconstruction from the 3D angiogram showing the inferior vena cava and superior vena cava (*orange*), expanded polytetrafluoroethylene extracardiac conduit (*grey*) and pulmonary arteries (*blue*). Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.

delivered by the right ventricle and results in flow reversal within the Glenn pathway, worsened by the development of the systemic venous collaterals arising from systemic venous tributaries of the SVC. We suggest future surgical modifications, including placement of a MPA band and ligation of the azygous to coronary sinus connection to reduce the pressure transmitted to the PAs/Glenn/Fontan from the right ventricle and volume loading of the right ventricle.

To our knowledge, the results reported represent the longest living animal model of the Fontan circulation at 3 years from the time of surgery. However, animal survival long-term was ∼33% (4 out of 12) and consistent with the earlier results reported with this model.[5] In our

TABLE 1. Single-stage Fontan outcomes and cause of death

Sheep No.	Age at surgery (d)	Weight at surgery (kg)	Alive/died	Current weight/ weight at death	Days postsurgery	Cause of death
1	405	45.00	Died	45.00	0	Pulmonary artery tore during surgery, died secondary to acute blood loss
2	402	44.50	Alive	80.00	1065	N/A
3	774	46.00	Died	46.00	1	Died suddenly, suspect pulmonary embolus given thrombus found within jugular catheter at necropsy
4	588	50.00	Died	49.00	8	Died following attempt at thoracentesis with lacerating injury to the liver and acute blood loss
5	616	50.00	Died	52.00	33	Persistent pleural effusions; found dead
6	865	50.50	Died	51.00	6	Died following attempt at thoracentesis with lacerating injury to the liver and acute blood loss
7	272	48.00	Died	50.00	4	Died following acute kidney injury
8	315	45.00	Died	45.00	0	Low cardiac output and respiratory failure with significant pleural effusions and abdominal ascites
9	90	30.50	Alive	47.00	309	N/A
10	217	24.00	Died	24.00	1	Died follow respiratory failure and acute kidney injury
11	252	29.00	Alive	47.50	211	N/A
12	406	37.50	Alive	51.00	57	N/A

N/A, Not available.

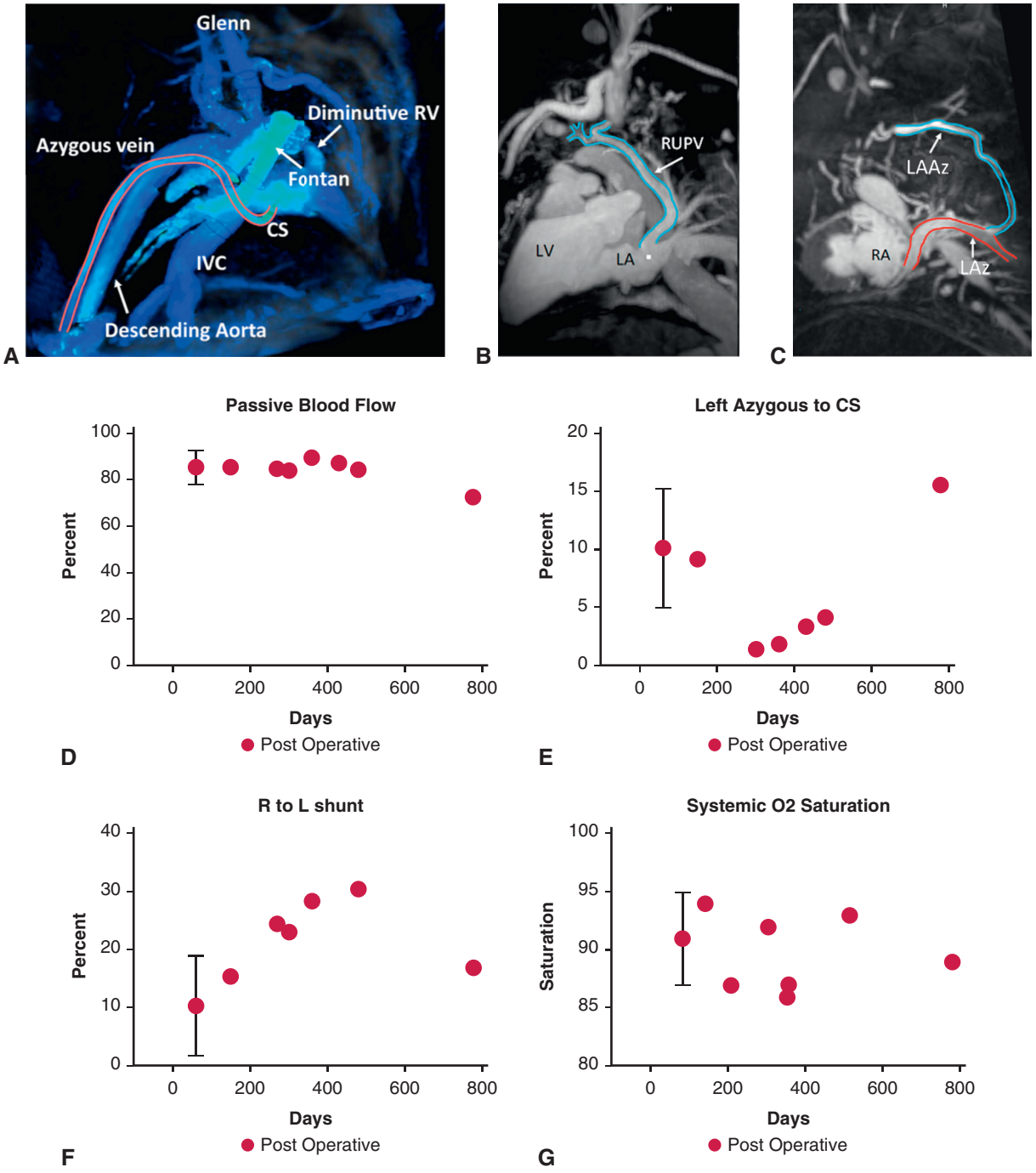
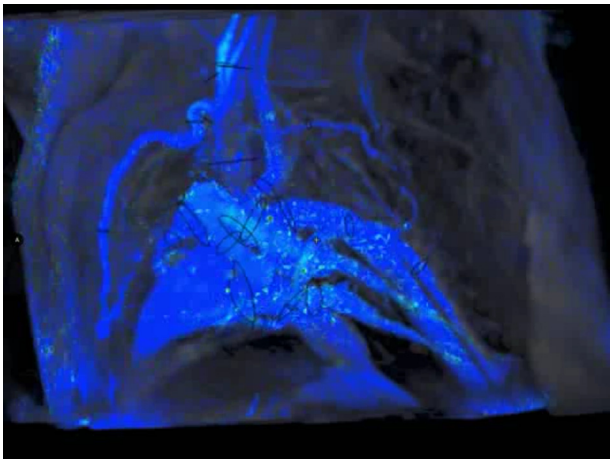
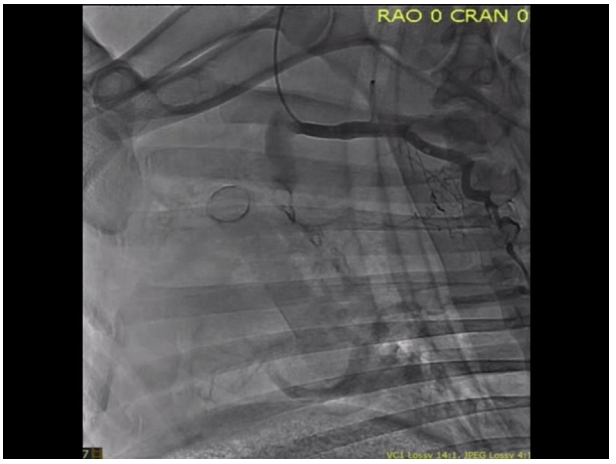


FIGURE 3. Percent passive pulmonary blood flow to the lungs and change in venovenous collateral blood flow. A, Three-dimensional rendering of 4-dimensinal flow data from magnetic resonance imaging. The left azygos (*LAz*) vein (*highlighted in red*) is draining to the coronary sinus (*CS*). B, Example of a systemic venous to pulmonary venous collateral (*highlighted in blue*) between the right thoracic vein and the right upper pulmonary vein. C, Example of a systemic venous to systemic venous collateral between the thoracic intercostal veins via a left accessory azygous (*LAAz*) vein to the LAz vein, which anatomically drains to the CS. D, Percent "passive" flow defined as ([Glenn + Fontan]/total flow to the pulmonary arteries) × 100. E, Flow through the LAz vein as a percentage of cardiac output. F, Quantification of the blood flow bypassing the pulmonary circulation, termed right-to-left shunting, over time. G, Degree of systemic arterial desaturation from right to left shunting over time. Sample was 4 <180 days postoperative and 1 >180 days postoperative. *Glenn*, Superior vena cava to pulmonary artery connection; *RV*, right ventricle; *Fontan*, inferior vena cava to pulmonary arteyr connection; *IVC*, inferior vena cava; *RUPV*, right upper pulmonary vein; *LV*, left ventricle; *LA*, left atrium; *RA*, right atrium.

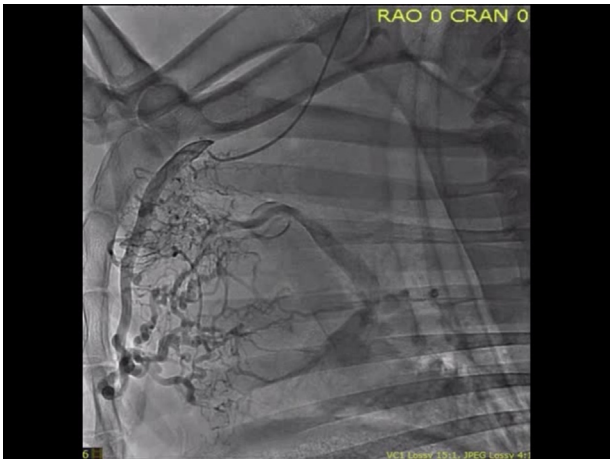


VIDEO 6. Four-dimensional flow with Ferumoxytol as a contrast agent. Note the left azygos vein coursing behind the descending aorta. Flow acceleration, depicted in green, is seen in the coronary sinus. Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.



VIDEO 8. Selective angiogram showing draining of venovenous collaterals arising from the thoracic intercostal veins and draining to the left azygos to coronary sinus connection. Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.

experience, 50% (4 out of 8) of the deaths were attributed to procedure-related complications associated with the learning curve of undertaking a complex surgical procedure and providing appropriate postoperative care with limited procedural complications. We found that performing the surgeries efficiently with limited blood loss contributed to improved survival, with long-term survival in 3 of the final 4 animals in our cohort. The poor survival is also evidence of the significant insult incurred with the abrupt initiation of Fontan hemodynamics in sheep with previously normal biventricular circulation with 50% (4 out of 8) of deaths resulting from Fontan failure during the early postoperative period. We found the administration of esmolol to be helpful in attenuating the tachycardia induced by Fontan palliation and we believe improved our animal survival. In summary, we believe an approximate 66% long-term survival is a reasonable expectation for the animal model in this current surgical configuration, but further study is required to confirm this finding.



VIDEO 7. Selective angiogram in the right thoracic vein shows a dense network of collaterals, which primarily drain to the right upper pulmonary vein and subsequently to the left atrium. Video available at: https://www.jtcvs.org/article/S2666-2736(24)00178-5/fulltext.

Study Limitations

The sheep in our study did not undergo the staged palliation process typically performed for human patients. In contrast to the usual Fontan anatomy, our model kept the right ventricle in continuity with the MPA. Consequently, the right heart introduced pulsatility into the pulmonary vasculature, leading to an incomplete representation of the passive pulmonary hemodynamics. Longitudinal analysis of the entire cohort was limited by poor animal survival.

CONCLUSIONS

We created a single-stage Fontan model in 12 juvenile sheep. Key characteristics of Fontan hemodynamics were observed, including systemic venous hypertension, reduced cardiac output, and development of venovenous collaterals. Placement of a MPA band and ligating the azygous vein to coronary sinus connection were the most effective strategies for improving the relevance of the model hemodynamics.

Conflict of Interest Statement

The 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.

The authors thank Dr Kirstie Keller for her work assembling and leading the Additional Ventures Cures Collaborative. The authors also thank Dr Filip Rega, KU Leuven, for generous advice based on an initial study of a Fontan model in sheep, Dr Jay Humphrey, Yale University, for technical advice, and Gabriel Freedman for editing of surgical videos.

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Key Words: Fontan, large animal model

E-Reference

E1. Ohuchi H. Where is the "optimal" Fontan hemodynamics? *Korean Circ J*. 2017; 47(6):842-857.

TABLE E1. Comparison of sheep and human Fontan Hemodynamics

	Human Fontan hemodynamics[E1]		
	Good hemodynamics	Poor hemodynamics	Sheep Fontan hemodynamics
Central venous pressure (mm Hg)	<10	>15	9-21
Pulmonary artery pulsatility (mm Hg)	∼0.5	∼0.5	2-3
Passive pulmonary blood flow (%)	100	100	75-90
Cardiac output (L/min/m^2)	>3	<2.5	2.3-3.4