

Computational Fluid Dynamics of Fetal Left Ventricles and Aortic Stenosis: Impacts of Fetal Aortic Valvuloplasty and Application of Physics-Informed Neural Networks

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Statement of Originality

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Abstract

Critical aortic stenosis with evolving Hypoplastic Left Heart Syndrome (CAS-eHLHS) is a severe congenital condition marked by the narrowing of the aortic valve, which restricts blood flow and leads to the underdevelopment of the left side of the heart. Fetal aortic valvuloplasty (FAV) has emerged as an important in-utero intervention aimed at preventing CAS-eHLHS from progressing to the univentricular morphology seen in Hypoplastic Left Heart Syndrome (HLHS). FAV seeks to relieve aortic valve stenosis, enhance blood flow and promote left heart growth based on the "no flow, no grow" theory, which highlights the importance of blood flow for heart development. Despite some success, FAV's efficacy is inconsistent and carries significant procedural risks.

This thesis addresses gaps in understanding FAV's impact by investigating flow dynamics in the fetal left ventricle (LV) using computational fluid dynamics (CFD). Pre-FAV, flow is dominated by a high-velocity mitral inflow jet vortex, while post-FAV introduces further complexity with aortic valve regurgitation, creating chaotic secondary flow patterns and increasing wall shear stress. FAV also resulted in a highly inefficient flow field within the LV.

Traditional CFD models are time-consuming and technically demanding, limiting their clinical use. In this thesis, Physics-Informed Neural Networks (PINNs), a mesh-free method, are presented as a promising alternative by integrating clinical data and bypassing the need for precise flow boundary conditions. Tested within the thesis using synthetic Doppler data, PINNs successfully reconstructed detailed 3D flow fields, even when the data was sparse or noisy, outperforming conventional methods.

In conclusion, this thesis highlights significant differences in CAS-eHLHS LV flow dynamics compared to healthy LVs, even after FAV. While traditional CFD models are challenging to apply clinically, PINNs present a viable solution for reconstructing haemodynamic flows, addressing key limitations in current methods.

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List of Abbreviations

APEC	Association of European Paediatric and Congenital Cardiology
AS	Aortic stenosis
AV	Aortic valve
BMPs	Bone Morphogenetic Proteins
BSF	B-spline Fourier
BT	Blalock-Taussig
BV	Biventricular
CAS	Critical aortic stenosis
CAS-eHLHS	Critical aortic stenosis and evolving hypoplastic left heart syndrome
CART	Classification and Regression Tree
CDFI	Colour Doppler flow imaging
CFD	Computational fluid dynamics
CSF-PINN	Coupled Sequential Frames Physics-Informed Neural Network
CT	Computed Tomography
DA	Ductus arteriosus
DV	Ductus venosus
EF	Ejection fraction
EFE	Endocardial fibroelastosis
EndMT	Endocardial-to-mesenchymal transition
FAV	Fetal aortic valvuloplasty
FCNN	Fully connected neural network
FE	Finite element
FISH	Fluorescence In Situ Hybridization
FO	Foramen ovale

FSI	Fluid-structure interaction
GA	Gestational age
GPU	Graphics Processing Unit
HIF-1 α	Hypoxia-inducible factor-1 α
HLHC	Hypoplastic left heart complex
HLHS	Hypoplastic left heart syndrome
HR	Hazard Ratio
IFCIR	International Fetal Cardiac Intervention Registry
IPCCC	International Paediatric and Congenital Cardiac Code
IPTW	Inverse probability of treatment weighting
IVC	Inferior vena cava
IVPG	Intraventricular pressure gradients
LA	Left atrium
LPM	Lumped parameter model
LV	Left ventricle
MAPSE	Mitral annular plane systolic excursion
MPI	Myocardial Performance Index
MRI	Magnetic Resonance Imaging
MV	Mitral valve
NCARDS	National Congenital Anomaly and Rare Disease Registration Service
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
OSI	Oscillatory shear stress
PA	Pulmonary artery
PC-MRI	Phase-Contrast Magnetic Resonance Imaging
PINN	Physics-Informed Neural Network
RA	Right atrium
ROC	Receiver operating characteristic

RMSE	Root mean square error
RV	Right ventricle
<i>SiLU</i>	Sigmoid Linear Unit
SNR	Signal-to-noise ratio
SPH	Smoothed particle hydrodynamic
STIC	Spatio-temporal image correlation
TAWSS	Time-averaged wall shear stress
TDI	Tissue Doppler imaging
TGF β	Transforming Growth Factor-Beta
Time- & Surface- Averaged WSS	Time- and surface-averaged wall shear stress
TV	Tricuspid valve
UV	Univentricular
VFM	Intraventricular flow mapping
WSS	Wall shear stress
$\overrightarrow{WSS}$	Wall shear stress vector

Chapter 1

Introduction

1.1 Motivation and Rationale

Hypoplastic Left Heart Syndrome (HLHS) is a particularly severe form of congenital heart disease (CHD), characterized by the underdevelopment of the left side of the heart, including the left ventricle (LV), ascending aorta, and associated valves (1). This condition progresses in utero, results in poor postnatal cardiac function and necessitates complex surgical interventions for a chance of survival beyond the neonatal period. Prenatal diagnosis of HLHS can be made as early as the first trimester, where certain cardiac malformations are associated with a high risk of progression to HLHS at birth. One such malformation is critical aortic stenosis (CAS), along with abnormalities such as LV dysfunction, retrograde transverse aortic arch flow, left-to-right shunting of foramen ovale (FO) flow and monophasic mitral valve (MV) inflow (2, 3). This condition is usually referred to as critical aortic stenosis with evolving hypoplastic left heart syndrome (CAS-eHLHS) (4).

An in-utero catheter-based procedure, fetal aortic valvuloplasty (FAV), is performed to alleviate CAS in these CAS-eHLHS cases. Its motivation is to prevent progressive damage to the LV by increasing the flow through the LV so that these fetuses may achieve a postnatal biventricular (BV) circulation (5). The basis for FAV stems from the existing theory positing that flow-mediated factors are critical for the pathogenesis of HLHS (6), coined as the “no flow, no growth” hypothesis, supported by animal models (7-9). The evidence of FAV efficacy in preventing the in-utero progression to HLHS birth morphology has largely been demonstrated according to a few specialised paediatric cardiac care centres where the intervention is performed (4, 10-12). However, the evidence of the efficacy of FAV is inconsistent, where some report no significant difference in the BV

birthrate or transplantation-free survival between fetuses that undergone FAV compared to the natural history cohort (13, 14).

Despite these concerns, pre- and post-procedural examinations have shown that FAV can acutely alter the blood flow through the left heart, such as reversing systolic retrograde flow in the aortic arch to antegrade flow (5). These observations underscore the crucial role of haemodynamics in ventricular growth and remodelling. However, significant gaps remain in our understanding of the native in-vivo haemodynamics of CAS-eHLHS LVs and of the changes in the flow environment after FAV. The current understanding of the effects of FAV is primarily informed by echocardiographic anatomic measurements, such as Z-scores for LV long axis and MV diameter, valvular velocities, and qualitative physiological observations. These observations include the direction of flow in the aortic arch and FO, the MV inflow pattern and the inflow duration. Therefore, this thesis focuses on providing a complete characterisation of the altered haemodynamic environment in CAS-eHLHS LVs and the immediate changes observed two or three days after a technically successful FAV procedure.

Firstly, understanding the haemodynamics and flow environment is crucial for supporting future mechanobiological studies and enhancing our understanding of disease severity. Disease severity is particularly important in the context of CAS-eHLHS, where patients must meet the selection criteria for FAV. The selection criteria aim to identify patients with salvageable LV function—cases where the LV has not been irreversibly damaged or become severely hypoplastic and has the potential to grow and develop normally if the obstruction is relieved. Secondly, a thorough characterisation of the fluid mechanical forces can potentially provide biomechanical clues about potential causative factors that contribute to HLHS progression and malformation. Thirdly, understanding the fluid mechanical changes before and after FAV not only offers a more complete picture of the procedure's mechanical effects but can also be used to identify haemodynamic features between those who show strong responses to FAV and those who experience mild changes after the procedure. Lastly, refining the patient selection criteria for FAV is still necessary, as the current criteria may include fetuses with a high probability of BV outcome without the need for the procedure, therefore subjecting them

to unnecessary procedural risks. Hence, part of this thesis aims to perform patient-specific CFD models which can potentially address some of these concerns.

The remaining portion of the thesis addresses challenges faced in many image-based CFD approaches including those presented in this thesis in terms of calibrating computational models to match clinical measurements, obtaining reliable boundary conditions and realistic valve tissue mechanics in the case of fluid-structure interaction (FSI) models. Flow simulations can have discrepancies with clinical observations or flow velocities obtained from Doppler ultrasound, suggesting that model predictions may not be entirely representative of in-vivo flow haemodynamics. It is difficult to calibrate simulations to match with clinically observed flow velocities (15-17).

One of the aims of the thesis is to employ a deep learning framework using Physics-Informed Neural Networks (PINNs), which leverages 4D echocardiography images and colour Doppler flow imaging (CDFI) velocity data, to reconstruct high-quality in-vivo cardiac flow dynamics. PINNs have emerged as a promising way for semi-supervised deep learning reconstruction of biomedical flow fields (18). These networks enhance training by integrating the full governing laws of flow physics as constraints, allowing them to effectively leverage sparse data alongside physics constraints to reconstruct the solution field (19, 20), and derive realistic haemodynamic predictions even with real, noisy clinical data. This approach is particularly well-suited for clinical measurements of cardiac flow, where data is sparse and noisy, yet still enables the extraction of detailed fluid dynamics, including near-wall blood flow and wall shear stresses (WSS) (21). This is possible even without explicit specification of inlet and outlet boundary conditions, which avoids the problem of error propagation from boundary conditions to ventricular flow fields.

1.2 Specific Aims

The specific aims of this research are summarised as,

Specific Aim 1: Characterisation of the fluid mechanics in LVs of CAS-eHLHS fetuses at mid-gestation.

Specific Aim 2: Characterisation of the immediate effects of FAV on the LVs of CAS-eHLHS fetuses.

Specific Aim 3: Develop a robust semi-supervised PINN framework for 4D flow reconstruction using scarce and noisy synthetic CDFI velocity data generated from CFD models.

where the first two aims were achieved via 4D moving boundary CFD simulations for the LV using pre- and post-FAV ultrasound volume datasets acquired with Spatio-Temporal Image Correlation (STIC) mode. Due to the limited spatial resolution of the imaging, the valves and orifice sizes could not be obtained. Therefore, the sizes of the MV and aortic valve (AV) orifices were iteratively adjusted, and CFD was iteratively performed to match the fetal valvular velocities obtained from Spectral Doppler ultrasound. Additional calibration was done if MV regurgitation and AV regurgitation were present in certain cases. Parameters like vortex dynamics, WSS-derived metrics, energy dissipation and work done and blood turnover were quantified.

In the third specific aim, a time-marching approach to simulate transient intracardiac flows using PINNs was developed and demonstrated using CFD simulation data from healthy fetal LV models without valves and FSI simulation data of an adult LV model with valves. The accuracy of the predicted velocity and pressure fields was assessed, including some preliminary comparisons of haemodynamic quantities.

1.3 Thesis Outline

This thesis is structured into seven chapters, each dedicated to specific research aims explored in this study. Chapter 2 introduces the topic with Section 2.1 focusing on HLHS, providing an overview of the disease and its characteristics. It then outlines the key features of a healthy fetal circulatory system and discusses deviations from this baseline as seen in mid-gestation fetuses with a prenatal diagnosis of HLHS, including CAS-eHLHS. It examines the common pathophysiology of CAS-eHLHS, presents histopathological observations and concludes the subsection with a brief discussion of the prevailing theories about the HLHS aetiology. Section 2.2 provides a comprehensive overview of the FAV procedure, detailing its steps, efficacy as reported in medical literature, patient selection criteria, and the current understanding of its effects. Then

Section 2.3 provides a short overview of the prior work to elucidate fluid mechanics in fetal hearts.

Chapter 3 focuses on the first research aim, which is to investigate the fluid dynamics in the LV with CAS-eHLHS. This chapter outlines the methods used, including image acquisition, geometry preparation, cardiac motion tracking, and fluid dynamics simulation. It also presents a comparison of these results with those from healthy fetal LVs, discussing echocardiographic parameters, LV vortex dynamics, WSS metrics, and blood turnover.

Chapter 4 is dedicated to the second research aim, examining the changes in fluid mechanics in CAS-eHLHS LVs following a successful FAV procedure. The methods employed are similar to those presented in Chapter 3 with minor adjustments made to the CFD models to simulate AV regurgitation as a result of AV FAV dilation. Once again, changes in echocardiographic parameters, LV dynamics, WSS metrics, and blood turnover were quantified.

Chapter 5 addresses the third research aim, which involves reconstructing the 3D velocity and pressure fields in the LV using PINNs and synthetic CDFI generated from computational models. It introduces the challenges of simulating LV haemodynamics, explores alternative flow reconstruction methods, such as intraventricular flow mapping (VFM), and introduces PINN approach for flow reconstruction, including PINNs. This chapter details the development of the Coupled Sequential Frames Physics-Informed Neural Network (CSF-PINN) framework. The accuracy and robustness of the framework were evaluated using computational models of the LV as ground truth.

Chapter 6 provides a summary of the thesis findings, discusses their implications, and suggests directions for future research, including strategies to reduce PINN training times and potential clinical adoption of PINN-derived haemodynamics.

Chapter 7 outlines the original contributions made by the author, including peer-reviewed publications and conference presentations.

Chapter 2

Background

Recent data from the National Congenital Anomaly and Rare Disease Registration Service (NCARDRS) reported that CHDs are the most frequent type of congenital anomaly in England, manifesting in approximately 1 out of every 150 births (22). This rate contrasts with the global prevalence reported in 2013, which stood at around 1 in 100 births (23). CHDs encompass a range of structural heart defects that manifest before birth and can vary in severity. Within the spectrum of CHDs, severe conditions such as Tetralogy of Fallot and HLHS account for 20-44% of all cases (22, 24), typically requiring surgical intervention within the first year of life (25).

2.1 Hypoplastic Left Heart Syndrome

2.1.1 Definition and Overview

The general definition for HLHS was outlined in the 2021 International Paediatric and Congenital Cardiac Code (IPCCC) alongside the Eleventh Revision of the International Classification of Diseases (26):

“Hypoplastic Left Heart Syndrome is defined as a spectrum of congenital cardiovascular malformations with normally aligned great arteries without a common atrioventricular junction, characterized by underdevelopment of the left heart with significant hypoplasia of the left ventricle including atresia, stenosis or hypoplasia of the aortic or mitral valve, or both valves, and hypoplasia of the ascending aorta and aortic arch.”

The present classification of HLHS often leads to its subdivision into additional subtypes. However, a shared characteristic among these HLHS phenotypes is the underdeveloped LV, inadequate for systemic circulation due to its insufficient size or complications like MV atresia, severe MV stenosis, or severe mitral hypoplasia with concordant atrioventricular nodes and the proper alignment of the major arteries (27). The term HLHS is commonly used to refer to cardiac malformations at the severe end of

the disease spectrum. In contrast, Hypoplastic Left Heart Complex (HLHC) refers to the less severe end of the spectrum, distinguishing patients who do not have intrinsic valvar stenosis or atresia (28, 29). It is less severe because, although the aortic and mitral valves are hypoplastic, they still function normally, and the LV, while underdeveloped, remains adequately functional, allowing for the possibility of postnatal BV palliation (30, 31), which is not possible in classical HLHS.

In the United Kingdom, between 1998 and 2012, infants diagnosed with HLHS had survival rates of 84.4% at one week, 76.2% at one month, 63.5% at one year, 58.6% at five years, 54.6% at ten years, and 32.6% at 15 years post-diagnosis (32). The survival and prognosis for HLHS have notably improved following the introduction of the stage I Norwood procedure in the 1980s (33). Mortality at discharge after stage I was reported to be 2 – 23% (34-37). This procedure forms the first of a series of staged surgeries, including the Stage II (Glenn procedure) and Stage III (Fontan procedure) (38, 39), strategically designed to reconfigure the heart and circulatory system to allow a single ventricle to perform the work typically done by two. For various HLHS anatomical presentations, surgeons may employ alternative strategies such as the hybrid procedure (40, 41), thereby broadening the spectrum of viable interventions and enhancing the outlook for infants with HLHS. With a larger array of available surgical techniques, and greater technical experience in the recent era, survival rates have improved when comparing two cohorts of neonates: those born in the more recent era of 2006-2012 have shown better survival than those born in the earlier period of 1998-2005 (32).

To understand why HLHS prognosis is poor after birth, it is important to understand the morphological transition that takes place from fetal circulation and postnatal circulation, which is explained in the following section.

2.1.2 Fetal and Neonatal Circulation: Implications of HLHS

Fetal circulation is uniquely designed to support life in utero, with the placenta, not the lungs, being the main organ for oxygenation and nutrient exchange. The fetal circulation system is explained in Figure 1A, with comparison to the neonatal circulation

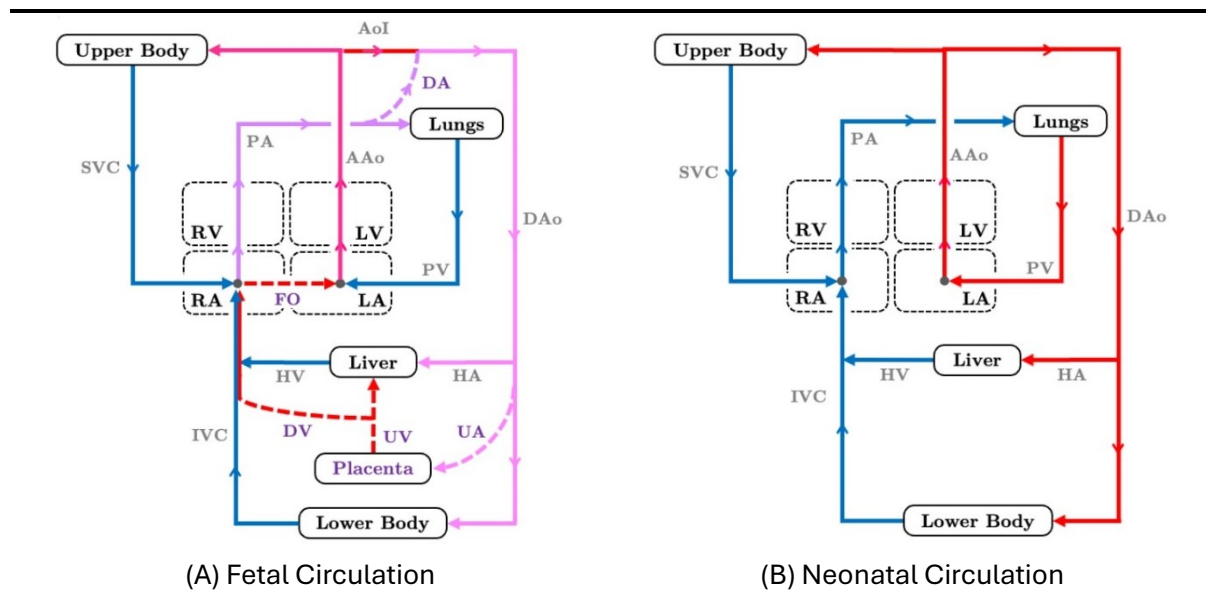


Figure 1: A simplified schematic representation of the fetal (A) and neonatal (B) circulatory systems, highlighting the shunts (dashed lines) unique to fetal circulation and the flow of oxygenated (red), deoxygenated (blue), and mixed (purple) blood in the fetus. In contrast, the neonatal system shows the complete circuit of oxygenation through the lungs after the closure or constriction of prenatal structures. AAO—ascending aorta, AOl—aortic isthmus, DAo—descending aorta, PV—pulmonary vein, SVC—superior vena cava, HV—hepatic vein, HA—hepatic artery, PA—pulmonary artery, DA—ductus arteriosus, IVC—inferior vena cava, UA—umbilical arteries, DV—ductus venosus, UV—umbilical vein, RA/LA—right/left atrium, RV/LV—right/left ventricle, FO—foramen ovale. Adapted from Zhang and Lindsey et al., 2023 (42).

system in Figure 1B. The five important vessels and shunts unique to prenatal life are the umbilical vein, umbilical artery, ductus venosus (DV), FO and ductus arteriosus (DA).

In the fetal circulation system, oxygen-rich blood from the placenta travels through the umbilical vein. This vein bifurcates, distributing 40% of blood between the liver and 60% to the inferior vena cava (IVC) via the DV (43). Blood rerouted to the liver returns into the IVC just before the right atrium (RA). Upon reaching the RA, the blood divides into two principal streams: one circulates through the right ventricle (RV) to the lungs and returns to the heart's left side, while the other bypasses the lungs and flows directly into systemic circulation through the DA. Additionally, blood with slightly higher oxygen saturation traverses from the RA to the left atrium (LA) via the FO. The LV is responsible for pumping blood to the upper body. Conversely, the lower body receives blood mixed from both ventricles. Venous blood from the body is either redirected to the placenta for oxygenation or returns to the heart, thus completing the circulatory loop.

After birth, the fetal vessels and shunts undergo significant changes to facilitate the transition from fetal to neonatal circulation. The initiation of breathing and increased blood oxygen levels reduces pulmonary vascular resistance (44), enhancing blood flow and venous return to the LA. Concurrently, clamping the umbilical cord reduces blood flow through DV, leading to its constriction and closure. The DV becomes the ligamentum venosum, detaching the neonate from the placental circulation and increasing systemic vascular resistance. This transition causes a rise in LA pressure due to enhanced pulmonary venous return and distal aortic pressure, while RA pressure falls due to decreased venous return via the IVC. This pressure gradient between LA and RA aids in the functional closure of the FO, which later solidifies into permanent closure through tissue proliferation.

The reduced pulmonary resistance also triggers a temporary reverse blood flow in the DA, enhancing pulmonary circulation (45, 46). Within the initial hour post-delivery, the output from the LV markedly rises, significantly improving blood oxygenation (47). This surge in oxygenation induces the smooth muscle cells in the DA to constrict, gradually reducing the size of the lumen and ultimately halting blood flow through the vessel (48). Initially, this closure is functional, but eventually, permanent closure is achieved through structural changes in the vessel's wall. The features, functions and postnatal transformation of these structures are summarised in Table 1.

TABLE 1: FETAL CIRCULATORY STRUCTURES AND THEIR POSTNATAL FATE

Structure	Features	Main function	Postnatal Fate
Umbilical vein	Approximately 80% oxygen saturation (49)	Carry oxygenated blood to the fetus from the placenta	Closes, become ligamentum teres
Umbilical arteries	40% oxygen saturation, resembling that of the IVC (50)	Carry deoxygenated blood from the fetus to the placenta	Contracts, become medial umbilical ligaments
Ductus venosus	(i) Blood enters the IVC close to the heart (ii) Oxygenated blood is “jet-streamed” into the medial aspect of IVC (51), overlaying deoxygenated blood from systemic regions	Shunts a portion of the blood flow from the umbilical vein directly into the IVC	Closes becomes ligamentum venosum
Foramen ovale	Allows blood to pass from RA to the LA, bypassing lungs	Facilitated by the Eustachian valve's position (52), oxygenated blood from the IVC is mostly directed through the FO to the LA	Closes becomes fossa ovalis
Ductus arteriosus	(i) Connects pulmonary artery to the aorta (ii) 40% of the combined cardiac output or 90% of the RV output passes through the vessel (53)	Diverts oxygenated blood away from pulmonary circulation, bypassing fluid-filled and underdeveloped fetal lungs	Constricts become ligamentum arteriosum

Note: IVC – Inferior vena cava, RA – right atrium, LA – left atrium, FO – foramen ovale, RV – right ventricle

In HLHS fetuses, the RV is primarily responsible for the combined cardiac output, perfusing the systemic circulation via the DA. The diminished flow from the undersized LV into the hypoplastic aorta leads to a reversal of flow through the transverse aortic arch. An atrial septal defect is sometimes observed, allowing left-to-right flow between the two atria, which is crucial for survival as the left side of the heart cannot effectively pump oxygenated blood. Figure 2 summarises these key morphological differences between a healthy and an HLHS neonatal heart.

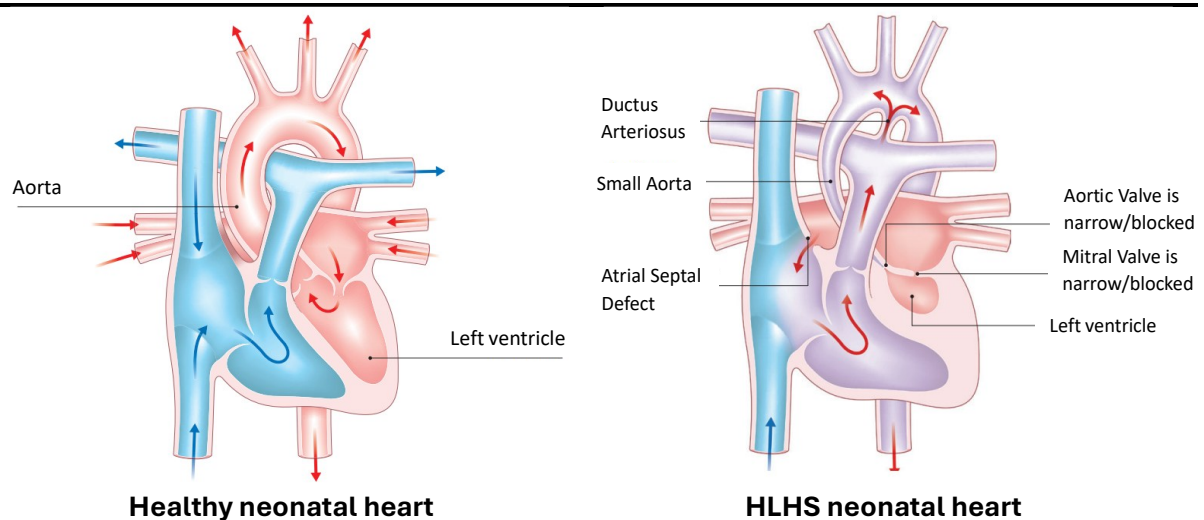


Figure 2: Comparison of healthy and Hypoplastic Left Heart Syndrome (HLHS) neonatal cardiac anatomy: The left panel illustrates a healthy heart with normal blood flow dynamics, while the right panel depicts the anatomical changes in HLHS, including a hypoplastic left ventricle and obstructions in the mitral and aortic valves, accompanied by an atrial septal defect and a hypoplastic small aorta. Adapted from the British Heart Foundation, "Understanding Your Child's Heart - Hypoplastic Left Heart Syndrome" (54).

Although this altered flow configuration is tolerable in utero, the post-birth constriction and closure of the DA lead to insufficient perfusion of the systemic circulation, making the condition fatal. Therefore, it is the basis on which the first staged Norwood is performed a few days after birth (55, 56). This involves reconstructing the aorta using a homograft patch, which is anastomosed to the main pulmonary arteries. This modification allows the RV to perfuse the systemic circulation. Since the main pulmonary artery (PA) is now part of the reconstructed aorta, a shunt is needed to ensure blood flow to the lungs for oxygenation. This can be achieved using either the Blalock-Taussig (BT) shunt, which connects the subclavian artery to the PA, or the Sano shunt (57), which directly connects the RV to the pulmonary arteries.

2.1.3 Critical Aortic Stenosis Between First and Second Trimester

The management of HLHS usually commences in early to mid-gestation, following a referral for comprehensive fetal echocardiographic anomaly screening if CHDs are suspected during pregnancy. In the United Kingdom and the Republic of Ireland, the antenatal detection rate of CHDs for the period 2017-2022 has been reported to range from 50.4% to 52.7% (58). For HLHS, the prenatal detection rates during this timeframe have been varying between 79.8% to 90.9% (58). Fetal echocardiography is vital in the early identification of complex cardiac conditions like HLHS, enabling timely and informed management decisions. A study on the natural history of fetal CHDs has shown that certain cardiac anomalies associated with the development of HLHS are detectable as early as 13-22 weeks of gestation (59). Congenital aortic stenosis (AS) accounts for approximately 20% of third-trimester CHD diagnoses (59), and has a prevalence of 0.2-0.5 per 1000 live births (60, 61), marking it as a prominent left heart obstructive lesion. Other left heart obstructive lesions include MV stenosis/atresia and/or coarctation of the aorta (62).

These left heart obstructive lesions can evolve through pregnancy. During early gestation, stenosis severity can be mild where the AV may appear mildly thickened or echogenic, with no obvious obstruction to LV outflow by Doppler interrogation. However, at late gestation, AV can become severely thickened and hypoplastic which opens slightly during systole (62). Early to mid-gestation fetal echocardiography screenings may miss the AS diagnosis, since the four-chamber view can appear relatively normal in some cases (63). In cases of CAS, besides the presence of a nearly immobile AV with minimal antegrade flow, it is also accompanied by retrograde systolic flow in the aortic arch and poor LV function (2).

2.1.4 Critical Aortic Stenosis and Evolving Hypoplastic Left Heart Syndrome

CAS that manifests earlier in gestation has a longer-lasting adverse effect on LV development and growth as compared to cases that manifest in later gestation. Progression to HLHS is frequently observed in these early or mid-gestation fetuses (62,

64, 65), hence the term CAS-eHLHS. LV hypoplasia not only precipitates earlier in gestation but exacerbates by a greater extent until birth (66), resulting in irreversible myocardial damage and impairment of LV growth, often before such issues can be identified through routine prenatal screenings. In contrast, fetuses with severe late-gestation AS have sufficiently preserved LV function and typically progress to CAS at birth with a BV heart morphology instead (67).

A BV outcome is characterized by the LV being the exclusive provider of systemic blood flow, with no intracardiac shunts apart from atrial communication (10). Conversely, patients requiring stage 1 or hybrid palliation are classified as having univentricular (UV) circulation. A BV outcome is preferable because it circumvents the need for the risky three-stage Norwood palliation or hybrid palliation; instead, patients may typically undergo postnatal AV balloon dilation to enlarge the LV outflow tract or the Ross-Konno procedure to replace the stenotic AV (68).

Table 2 provides a summary of abnormal echocardiographic parameters observed for mid-gestational fetuses with CAS-eHLHS, compiled from recent retrospective studies by the Boston Children's Hospital. This centre is recognised for its high volume of fetal CAS-eHLHS cases with experienced sonographers specialised in performing fetal echocardiograms for this condition for over 20 years. Z-scores for fetal cardiac structures are also established, using log-linear regression method described by Schneider et al. (69), which normalises cardiac dimensions to fetal size using biometric parameters like femur length (FL), biparietal diameter (BPD), or gestational age (GA), as elaborated in the Appendix.

In mid-gestational CAS-eHLHS fetuses, their diminished Z-scores for the AV and ascending aorta diameters lead to increased resistance to outflow from the LV (increased afterload). This causes significant LV dilation, demonstrated through increased LV size Z-scores and sphericity with signs of LV dysfunction. (64, 65, 70). Such fetuses usually exhibit MV regurgitation with a monophasic or fused MV inflow pattern (70, 71), in contrast to the biphasic inflow seen in healthy fetuses (72). The typical diastolic inflow profile of both the MV and tricuspid valve (TV) is characterised by a dual-peak Doppler waveform: an initial E-wave, resulting from passive atrial to ventricular flow, and the later A-wave, derived from atrial contraction, which facilitates late ventricular filling. This

TABLE 2: DEMOGRAPHIC CHARACTERISTICS AND BASELINE ECHOCARDIOGRAPHIC PARAMETERS OF MID-GESTATION FETUSES WITH CRITICAL AORTIC STENOSIS AND EVOLVING HYPOPLASTIC LEFT HEART SYNDROME.

Echocardiographic parameters	Boston Children's Hospital (n=118)
Demographic parameter	
Male fetal sex	89 (75.4%)
Gestational age, weeks	24.5 (22.9, 26.9)
Anatomic Measurements	
Aortic Valve Diameter, z-score	-2.67 (-3.23, -1.99)
Ascending aorta diameter, z-score	-0.26 (-1.41, 1.32)
Mitral Valve Diameter, z-score	-0.82 (-1.77, 0.03)
LV long axis dimension, z-score	0.50 (-0.47, 1.74)
LV end diastolic dimension, z-score	2.83 (0.98, 3.84)
LV end diastolic volume, z-score	1.95 (0.72, 3.11)
LV sphericity (Long Axis Length/Short Axis Length)	0.68 (0.59, 0.75) ^a
Endocardial Fibroelastosis grade (0-3)	2 (1-3) ^b
Endocardial Fibroelastosis ≥ moderate (n,%)	15 (75%) ^b
Physiologic Measurements	
LV ejection fraction, %	23.9 (17.6, 31.3)
Mitral valve inflow time, z-score	-3.50 (-4.37, -2.13)
Mitral regurgitation (>mild)	49 (53%) ^c
Mitral valve inflow index (msec/bpm)	0.75 (0.58, 1.06) ^d
Mitral Inflow / Tricuspid Inflow (%)	0.8 (0.6-1.0) ^b
LV systolic pressure (mmHg)	42.7 (32.9, 58.2) ^c
Aortic jet width index (jet width/annulus)	0.44 [0.35, 0.52] ^d
Mitral E (m/sec)	0.49 (0.33, 0.78) ^b
LV free wall E' (m/sec)	0.22 (0.18-0.66) ^b
LV free wall A' (m/sec)	0.35 (0.18-0.99) ^b
Septal E' (m/sec)	0.24 (0.16-0.56) ^b
Tricuspid E (m/sec)	0.40 (0.25 – 0.77) ^b
Tricuspid A (m/sec)	0.52 (0.36 – 0.72) ^b
Tricuspid Inflow Duration (ms)	146 (87 – 186) ^b
Haemodynamic Observations	
Mitral valve inflow pattern	

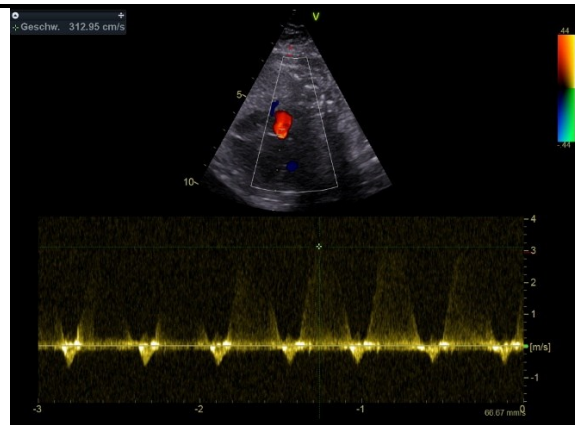
Biphasic	18 (15.5%)
Monophasic	81 (69.8%)
Partial fusion	17 (14.7%)
Patent Foramen Ovale flow	
Bidirectional or Right-to-Left	12 (10.8%)
Left-to-Right	99 (89.2%)
Antegrade arch flow	
Yes	0
No	118 (100%)

Note: Data is presented as either number of cases, n (%) for categorical variables or median (interquartile range) for continuous variables. Boston Children's Hospital (n=118) disease cohort data were obtained from Bradford et al. (73). Disease cohort data, (a) n = 88, (c) n = 92, (d) n = 82, (e) n = 75, were obtained from Prosnitz et al. (74). Disease cohort data, (b) n = 20, were obtained from Friedman et al. (70). Z-scores are derived using Schneider et al (69).

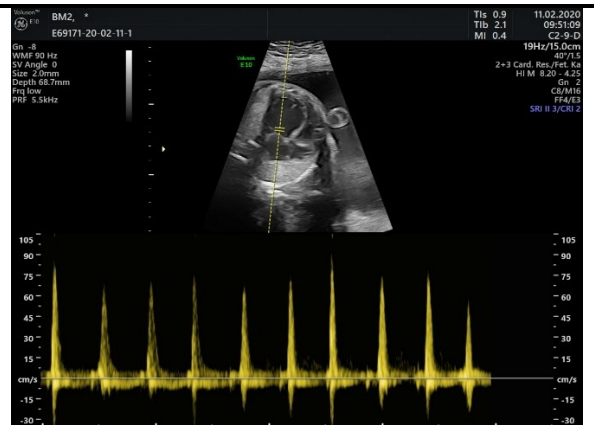
pattern reflects the sequential phases of diastolic filling in the heart. Figure 3 illustrates this difference between CAS-eHLHS and healthy fetuses at mid-gestation.

Moreover, the dilation of the MV annulus and the subsequent development of progressive MV regurgitation were observed in a proportion of CAS-eHLHS fetuses. Most of these fetuses demonstrate left-to-right patent FO flow with the RV assuming a greater role in managing the combined cardiac output due to the LV's compromised functionality (2). There is a discrepancy in MV and TV inflow times, where the MV inflow duration is notably shorter than the TV inflow duration, indicative of impaired LV filling and preload (70). LV dysfunction is further corroborated by Tissue Doppler Imaging (TDI) used to measure myocardial tissue motion. TDI-derived myocardial peak velocities reveal diminished contraction and relaxation in the LV free wall and septum (75). Higher LV end-diastolic volume and sphericity were linked to reduced diastolic function, evidenced by decreased LV myocardial peak velocities during early (E') and late (A') diastole (70, 76).

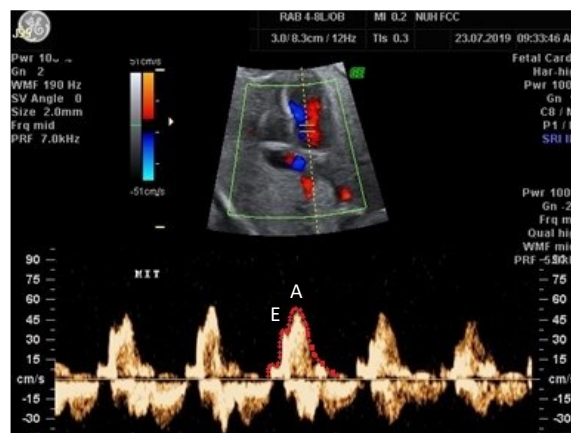
Furthermore, fetuses with CAS-eHLHS often present with endocardial fibroelastosis (EFE), a condition marked by the significant thickening of the endocardial layer from an overabundance of elastic and collagenous fibres (4, 10), shown in Figure 4. The severity of EFE is directly correlated with more pronounced diastolic dysfunction (70). EFE also restricts LV growth, affecting neonatal prognosis, frequently necessitating



(A) Abnormal Doppler with mitral regurgitation of CAS fetus with dilated LV (29 weeks)



(B) Abnormal Doppler with monophasic mitral valve inflow of fetus in (A) with different sample volume



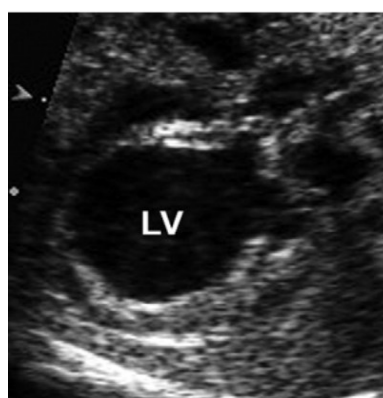
(C) Normal mitral valve Doppler of healthy fetus (31 weeks)

Figure 3: Comparison of mitral valve Doppler flow patterns between a fetus with critical aortic stenosis and evolving hypoplastic left heart syndrome (CAS-eHLHS) and a healthy fetus at mid-gestation.



Mild/Grade 1

(Presence of scattered echogenic spots within the LV)



Moderate/Grade 2

(Observation of non-contiguous echogenic patches distributed throughout the LV)



Severe /Grade 3

(Identification of contiguous echogenic lining encompassing the LV)

Figure 4: Echocardiographic images demonstrating the severity of endocardial fibroelastosis (EFE) in fetuses with CAS and dilated LV. Adapted from McElhinney et al., 2010 (77).

postnatal surgical resection of EFE in certain cases as part of the UV ventricle palliation strategy (77, 78). To summarise Section 2.1.4, there are several unifying features observed in all CAS-eHLHS cases but the natural history progression of CAS-eHLHS fetuses can vary significantly, depending on the occurrence of CAS and the irreversible damage incurred throughout gestation. This results in phenotypic variability among fetuses in the CAS-eHLHS cohort, which will be discussed in the following subsection.

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2.1.5 Variation in the Natural History of CAS-eHLHS

Following LV dilation and dysfunction, the LV undergoes progressive muscular hypertrophy with slow to no growth and development of left-sided cardiac structures (2, 62, 65). This poor growth is particularly evident in fetuses that become UV at birth (71). Fetuses with UV outcomes had significantly smaller MV Z-scores at mid-gestation and reduced MV and LV dimensions growth velocities throughout gestation (71).

Early gestational LV dilatation and dysfunction often lead to LV hypoplasia and HLHS at birth (64), while dilatation occurring later in gestation can result in a normal or enlarged LV size at birth (4). These differences in natural history are illustrated in Figure 5. However, determining postnatal circulation solely by gestational age (*GA*) is not feasible when considering CAS cases that develop at mid-gestation. Natural history studies demonstrate that 19-38% of fetuses with CAS-eHLHS diagnosis at mid-gestation are misdiagnosed as they evolve to a BV postnatal circulation (12, 14, 71).

In an earlier era (2000-2012), a low prenatal diagnosis rate of 8.5% among neonates with CAS and BV outcome was reported (67). Identifying these fetuses is challenging because the condition is either missed due to the relatively normal-looking four-chamber configuration at mid-gestation, or it can be misdiagnosed as CAS-eHLHS.

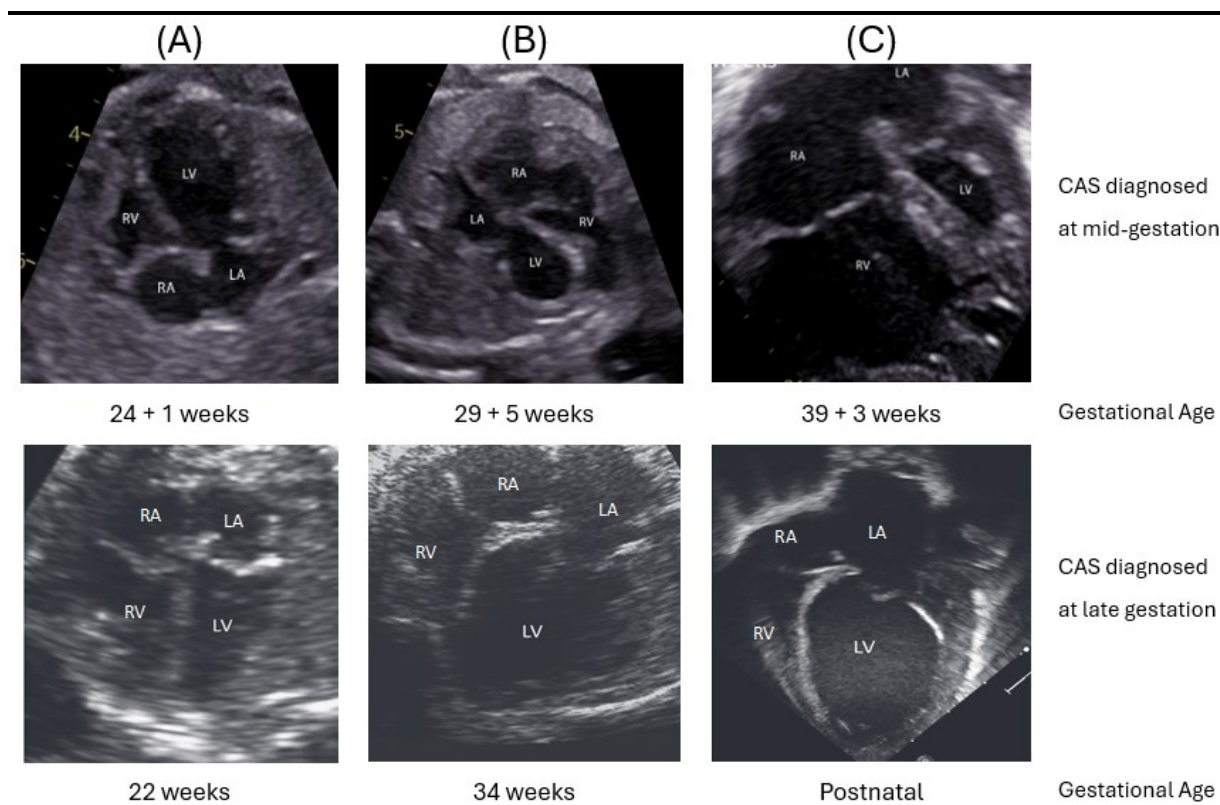


Figure 5: Series of ultrasound images that track the progression of two prenatal cases. One was diagnosed with critical aortic stenosis (CAS) during mid-gestation, adapted from Tulzer et al., 2022 (79), and another during late gestation, adapted from Freud et al., 2015 (67). In Panel A, the mid-gestation CAS case displays a dilated left ventricle, while the late-gestation CAS case appears normal during routine mid-gestation screening. Panel B shows both fetuses later in gestation, with the mid-gestation CAS case exhibiting signs of left ventricular hypoplasia and severe endocardial fibroelastosis, while the late-gestation CAS case displays late-stage left ventricular dilatation. Panel C presents the mid-gestation CAS fetus close to term with severe left ventricular hypoplasia characteristic of hypoplastic left heart syndrome, necessitating univentricular palliation, while the late-gestation CAS fetus achieves a biventricular outcome with significant left ventricular dilatation evident postnatally.

Currently, there is significant interest in identifying predictors of BV circulation, especially when considering patients for FAV and proper prenatal counselling.

2.1.5.1 Early-onset LV hypoplasia and diminutive LV

A subset of CAS-eHLHS fetuses would have already surpassed the initial dilation phase at mid-gestation and have early onset LV hypoplasia. These cases typically exhibit a ‘short, fat’ LV appearance, as depicted in Figure 6. These fetuses have Z-scores that are less than -2.0 for the MV, AV annulus and LV long-axis end-diastolic dimension (80), due to impaired growth from the presence of severe EFE (77, 78). Throughout pregnancy, there's a noticeable decrease in the Z-scores for left-heart echocardiographic

parameters, highlighting inadequate growth of the left-heart structures, ultimately leading to HLHS by birth (2, 81). Unlike mid-gestational CAS-eHLHS cases discussed in Section 2.1.4, these cases with early onset of LV hypoplasia have no detectable MV regurgitation, and flow through the left side of the heart is much poorer with redirection of flow across the FO, leading to enlarged RV to compensate for the combined cardiac output (62). CAS cases exhibiting this phenotype would universally develop HLHS (2).



Figure 6: Fetus with early left ventricular hypoplasia, non-apex forming left ventricle with critical aortic stenosis, with thickening of the heart walls from muscular hypertrophy. Evidence of endocardial fibroelastosis can be seen by the bright echogenic walls around the left ventricle. Adapted from A. Venardos et al., 2022 (80).

2.1.5.2 LV dilation and myocardial dysfunction, leading to congestive heart failure and hydrops

In some CAS-eHLHS cases, severe LV dilation can shift the septal wall, mechanically compressing the RV, as demonstrated in Figure 7A. This leads to reduced TV inflow duration and increased tricuspid E and A velocities, with a likelihood of E and A wave fusion in the TV inflow pattern, indicating RV dysfunction (70). Due to increased filling pressures, the RV is not able to support the combined cardiac output (82), resulting in congestive heart failure and elevated systemic venous pressure. This pressure causes fluid to leak into body tissues and cavities, leading to hydrops (83). In Figure 7, fluid accumulation in different fetal compartments, such as ascites (fluid in the abdomen), pericardial effusion (fluid around the heart), pleural effusion (fluid in the lungs), and skin oedema, were demonstrated. These complications can lead to intrauterine fetal demise

(84, 85). A retrospective study of CAS-eHLHS fetuses reported that hydrops were present in 15.8% (15/95) of their patient cohort (86).

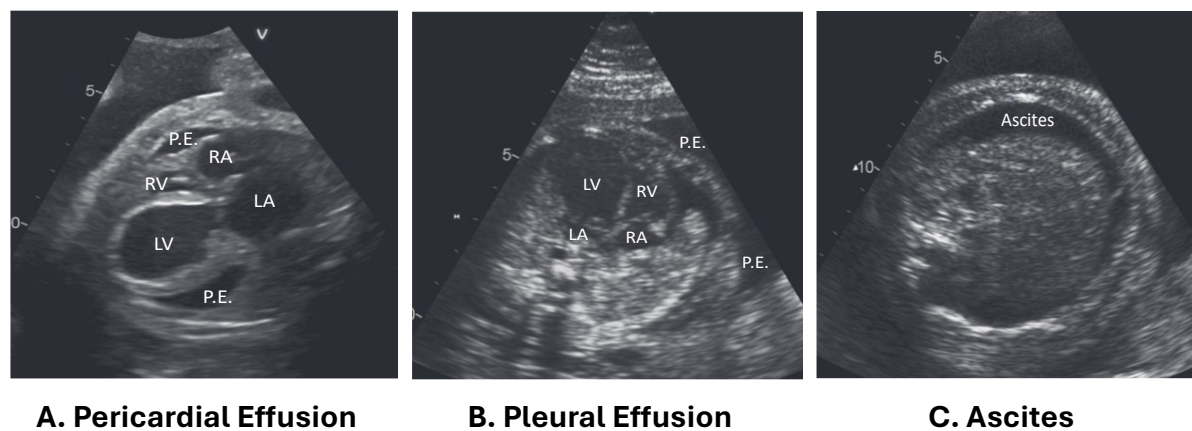


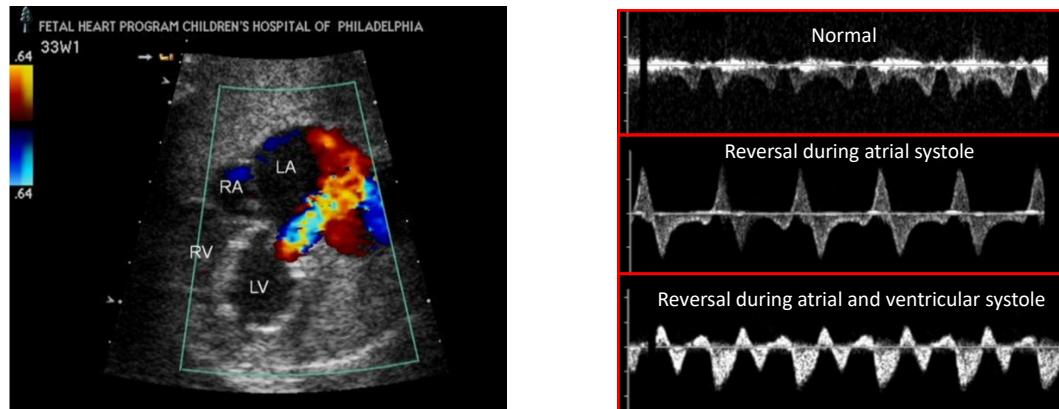
Figure 7: Ultrasound images depict fetuses with congestive heart failure and hydrops resulting from critical aortic stenosis, showcasing the presence of (A) pericardial effusions, (B) pleural effusion, (C) ascites and skin oedema (not shown). Adapted from Tulzer et al., 2021 (86).

2.1.5.3 LV dilation and myocardial dysfunction, severe MV regurgitation, giant LA

In some cases, MV regurgitation can become increasingly severe with the progression of LV dilation. This worsening can stem from primary MV issues, such as stenosis or atresia, or CAS-associated secondary factors, such as the dilation of the MV annulus, chordae tendineae shortening and immobility, papillary muscle displacement, and EFE presence (84). Such developments lead to elevated LA pressures, forcing premature narrowing or closure of the FO, and preventing LA depressurisation into the RA. This results in LA enlargement and pulmonary vein congestion, demonstrated in Figure 8, evidenced by abnormal pulmonary venous Doppler patterns showing reverse flow during atrial and ventricular systole (87), adversely affecting pulmonary vascular development (88).

The adverse effects of LV dilation on RV function are compounded by the presence of a giant LA, which also compresses the RA, further impeding RV filling (86). In fetuses with severe MV regurgitation and a giant LA, DV pulsatility index values were reported to be 1.11 (5th-95th percentile: 0.83-2.19) for gestational ages (GA) between 22 to 30 weeks (86), compared to a reference range of 0.88 (5th-95th percentile: 0.74 – 1.16) in healthy fetuses (89). This elevated DV pulsatility index indicates increased venous resistance to

blood flow and pressure, mirroring the severe pathological conditions discussed in Section 2.1.5.2, leading to hydrops and congestive heart failure (84).



(A) CAS-eHLHS fetuses with severe mitral regurgitation

(B) Pulmonary venous Doppler pattern

Figure 8: (A) An ultrasound examination of a 4-chamber view for a fetus with critical aortic stenosis and evolving hypoplastic left heart syndrome at 33 weeks, featuring colour Doppler imaging over the mitral valve, showing mitral valve regurgitation. The scan illustrates severe left atrial enlargement, with the atrial septum curving into the right atrium and possibly impeding the tricuspid valve. Additionally, the dilated left ventricle compresses the right ventricle. Adapted from Vogel et al., 2011 (84). (B) Comparison between the normal pulmonary venous Doppler pattern and potential alterations, including reversal during atrial systole or reversal during both atrial and ventricular systole.

2.1.6 The Diseased LV Myocardium

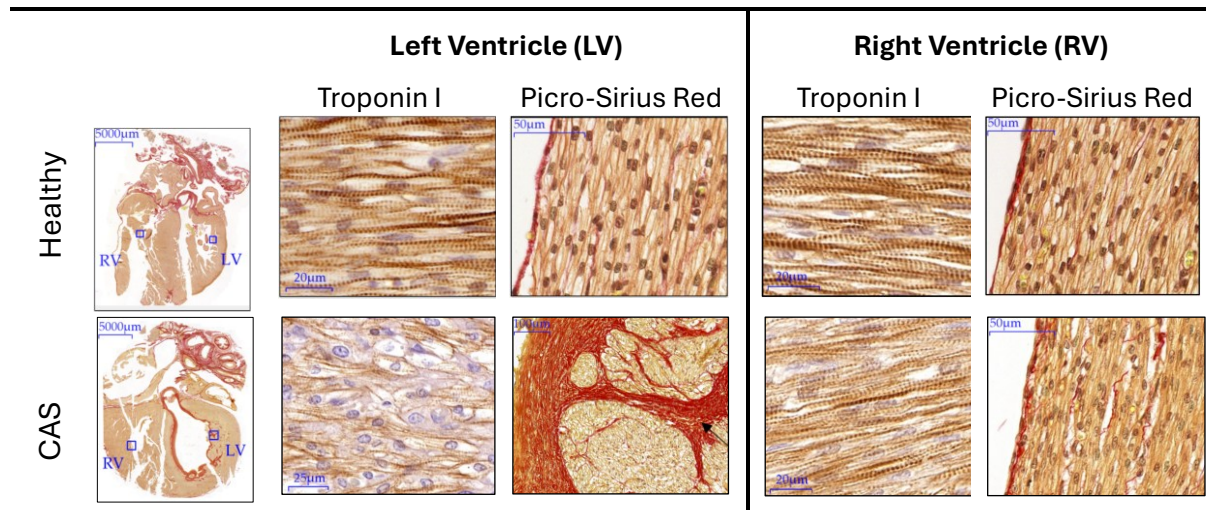


Figure 9: Comparative analysis of Troponin-I staining of left ventricle (LV) and right ventricle (RV) postmortem samples identifies organised cardiomyocyte network with clear banded pattern in the healthy control cases. Conversely, the representative critical aortic stenosis (CAS) fetus case had disturbed cardiomyocyte organisation in the LV but the RV was similar to healthy control cases. Picro-Sirius Red staining revealed a flat layer of endothelial cells and a homogenous pattern of interstitial fibroblast and collagen in the LV and RV. Whilst CAS fetus showed patchy fibrosis patterns in the subendocardium and myocardium (black arrow) from endocardial fibroelastosis (EFE) in the LV, the patterns in the RV were like healthy control cases. Adapted from Zwanenburg et al. (90).

The molecular cascades leading from CAS to LV hypoplasia and to HLHS at birth remain undefined, and there are likely variations in the exact cascades for different HLHS-associated cardiac malformations such as AV stenosis or atresia, and MV stenosis or atresia. Postmortem studies of CAS-eHLHS samples have identified specific structural abnormalities, compared to healthy controls (90), illustrated in Figure 9. Namely, disorganised cardiomyocyte arrays and notable endocardial thickening with patchy fibrosis that infiltrated the myocardium, due to hypoxia-driven or strain-driven endocardial-to-mesenchymal transition (EndMT) that cause EFE (91-93). There was also evidence of abnormal epicardial thickening with dilation of coronary vessels and subepicardial nerves (90).

Histopathological severity was linked to equivalent reductions in myocardial deformation of the LV, quantified by global or segmental longitudinal strain measurements using 2D speckle-tracking echocardiography of the ventricular wall (90). For the RV, global longitudinal strain values were lowered compared to healthy controls because of the septum's tethering effect, stemming from the fibrotic and dilated state of the LV that adversely impacted its contraction by encroaching into the RV space. In a larger cohort study of CAS-eHLHS fetuses, fetuses with BV postnatal outcomes had better 2D strain values than those with UV postnatal outcomes (94), corroborating the correlation with disease severity.

Patient-specific finite element (FE) models were previously performed and the implications of various CAS-eHLHS features such as AS, MV regurgitation, reduced LV contractility, and LV wall stiffness were modelled to investigate their biomechanical effects (95). Briefly, AS significantly increases LV peak systolic pressure and myocardial stress while reducing stroke volume and myocardial strain. MV regurgitation partially alleviated these effects, helping restore stroke volume and strain values. Increased wall stiffness to emulate EFE had minimal impact on LV function. Reduced LV contractility led to lower pressures, stroke volume, myocardial strain and stress (95).

In conclusion, these findings demonstrate the compromised state of the LV myocardium and how it has affected its ability to pump blood.

2.1.7 No flow, no growth hypothesis

The aetiology of CAS-eHLHS, and similarly HLHS, has not been fully elucidated. The “no flow, no growth” hypothesis has been used as a key explanation for the development of HLHS, where reduced flow through the left side of the heart is theorised to be the primary mechanism for LV hypoplasia and cardiac malformations. This theory posits that abnormal morphogenesis is mediated by altered mechanosensitive pathways, contributing to LV hypoplasia, EFE, and poor left heart growth (6).

Insights from small animal models like zebrafish provide supporting evidence that cardiac morphogenesis, valvulogenesis and trabeculae formation are mechanosensitive processes (96-98). Similarly, chick embryonic left heart hypoplasia models also provide unique benefits for investigating HLHS-associated malformations (99-101). However, zebrafish and chick embryonic models often struggle to fully uncover the aetiology of the disease, as these models do not capture the precise phenotypic features of HLHS, thus hindering studies aimed at understanding the molecular mechanisms underlying the condition. A mouse model for HLHS aims to address these shortcomings, offering several benefits compared to the chick embryonic model. Namely, flow restriction is done after septation, which more accurately mimics the developmental timing of human HLHS, and it provides higher phenotypic consistency and survival rates (9). By using an embolising agent in the fetal LA, it was able to recapitulate HLHS phenotypic features such as hypoplastic ascending aorta, retrograde aortic arch flow and LV hypoplasia.

Fetal lamb models for HLHS have also recently been proposed using coil implantation in the LA to induce LV inflow obstruction (102). This model effectively replicates left heart hypoplasia and pro-fibrotic tissue remodelling and has a longer gestation period, allowing for extended longitudinal studies. However, it is technically challenging, with a reported 56% mortality rate due to acute circulatory changes (102). Another notable fetal lamb model used supralvalvar aortic banding to create LV outflow obstruction to establish an LV hypoplasia model (103). Supralvalvar aortic banding led to a proportion of fetuses having LV dilatation and dysfunction with severe MV regurgitation and hydrops, similar to that seen in mid-gestation CAS-eHLHS fetuses, but none developed LV hypoplasia, no evidence of myocardial fibrosis and EFE was not observed.

Although these findings did not accomplish the original goal of developing an LV hypoplasia model, it does suggest that LV outflow obstruction alone may not be the direct mechanism driving LV hypoplasia in the CAS-eHLHS cohort.

Overall, these animal models highlight the significant role of reduced blood flow in the development of HLHS, reinforcing the rationale for performing FAV. The “no flow, no growth” hypothesis has been used as a key explanation for the development of HLHS, where reduced flow through the left side of the heart is theorised to be the primary mechanism for LV hypoplasia and cardiac malformations. Criticism remains, however, as the theory alone cannot fully account for the phenotypic variations observed in the HLHS spectrum, such as in the case of HLHC, where LV hypoplasia occurs in the absence of any valve stenosis or atresia. It has been used to challenge the “no flow, no growth” hypothesis and suggests other factors beyond haemodynamic flow are involved in the pathogenesis of HLHS (104).

2.1.8 Limitations of no flow, no growth hypothesis

One of the main limitations of the “no flow, no growth” hypothesis is that it does not account for genetic mutations that are recognised as critical contributors to the development of HLHS. Around 5% of HLHS cases are linked to chromosomal abnormalities, genetic syndromes or other genetic anomalies (105), including conditions like Turner syndrome, Jacobson syndrome or trisomies 13, 18 and 9 (106-111), identified with traditional genetic testing methods like chromosome analysis and Fluorescence In Situ Hybridization (FISH) analysis.

Alternatively, a genetic-based theory has been proposed and posits that primary intrinsic defects in myogenic programs—resulting from disrupted gene programs responsible for cell cycle regulation, autophagy, and cardiomyocyte maturation—are central to LV hypoplasia in HLHS (91, 104, 112). These defects impair cardiac differentiation and maturation, leading to myocardial disorganization and hypoplasia. However, given the genetic heterogeneity and phenotypic variability among fetuses, it also makes it difficult to identify a unifying genetic mechanism. The complexity of gene

interactions and the multifactorial nature of HLHS complicate the identification of specific genetic causes.

HLHS aetiology is likely to be heterogeneous, wherein a genetic predisposition to HLHS is inherently present (113), but the degree to which the genotype is phenotypically expressed may be significantly influenced by haemodynamic forces, resulting in a range of possible morphological features associated with HLHS (60). Whether flow haemodynamics plays a primary or secondary role in CAS-eHLHS malformations, it is nonetheless important to study their impact to better understand the progression of the disease.

2.2 Fetal Aortic Valvuloplasty to treat CAS-eHLHS

2.2.1 Procedure Description

Retrospective case series have shown that, in the natural history of CAS-eHLHS cases, BV circulation was only achieved in 19% to 38% (12, 14, 71). Achieving BV postnatal outcome is desirable, as it can prevent the need for postnatal UV staged palliation, which is known for high mortality and morbidity (114-116). As such, FAV has been proposed as a prenatal cardiac intervention procedure aimed at altering the natural history of fetuses with CAS-eHLHS (12), preventing progressive LV damage, and improving postnatal prognosis. The newborn would be subjected to fewer high-risk surgeries compared to those with a classic HLHS presentation at birth (3).

This procedure is illustrated in Figure 10. Under continuous monitoring by high-resolution ultrasound guidance and optimal fetal positioning (anteriorly oriented LV apex), a fine needle is inserted through the maternal abdomen, uterine wall and into the apex of the LV. A guidewire is passed through the needle and advanced across the stenotic AV, and a balloon catheter is threaded over the guidewire, positioned at the AV and inflated to dilate the AV (117, 118). A maximum inflated balloon size: AV annulus ratio of 1.1 ± 0.10 was reported (117, 119). Technical success of the procedure was defined as increased antegrade flow (wider Doppler jet) across the AV or the presence of new AV regurgitation.

Two high-volume centres for FAV report a learning curve associated with the procedure, with technical success rates improving from 75-78% in the earlier era (pre-2010) to 94-96% in the recent era (post-2010) (4, 117). With improved procedural experience in the recent era, there was also a reduction in procedural-related mortality from 11-12% to 4-6%. Other serious adverse events such as bradyarrhythmia, pericardial effusion, ventricular dysfunction or intracardiac thrombus are to be expected in the fetus (120).

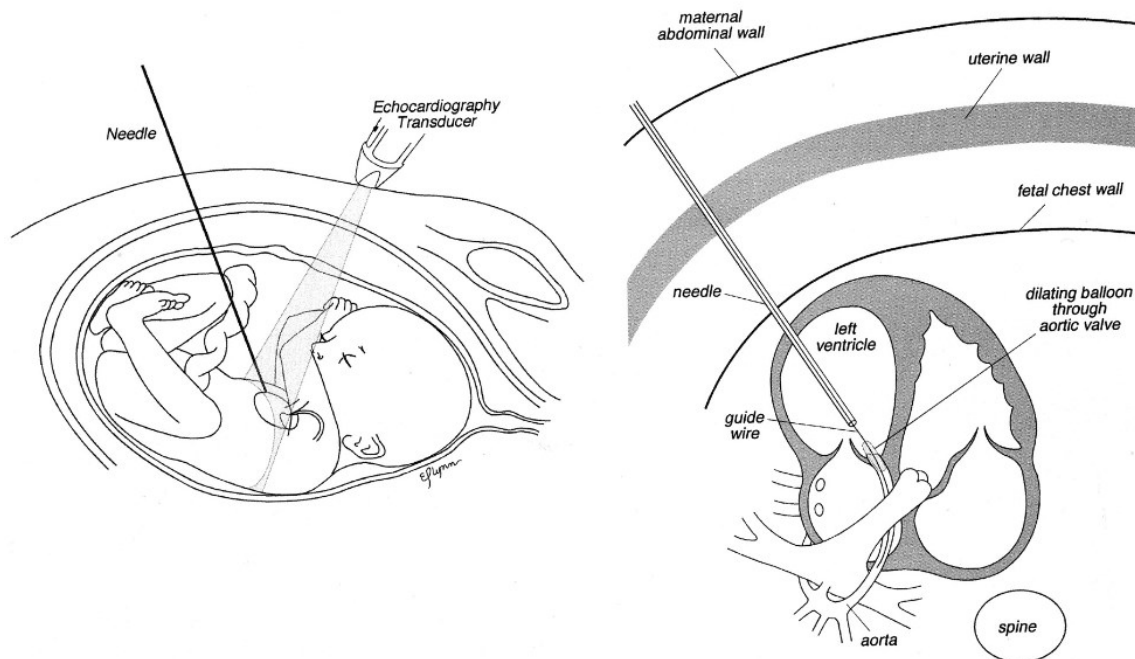


Figure 10: Left Image: The image illustrates the initial step of Fetal Aortic Valvuloplasty (FAV), where a needle is carefully inserted through the maternal abdominal wall and uterine wall into the fetal chest. The echocardiography transducer is used to guide the needle's placement into the left ventricle. Right Image: The image shows the needle's path through the fetal chest wall into the left ventricle. A guide wire is then advanced through the needle and across the stenotic aortic valve. A balloon catheter is subsequently inserted over the guide wire and inflated to dilate the stenotic valve, improving blood flow. The figure was adapted from Marshall et al. (118).

The first FAV was attempted in 1991 (121) and was later adopted and refined by the Boston Children's Hospital alongside Brigham and Women's Hospital in 2000 (122), with a total of 165 FAV attempts reported by this centre between 2000 and 2020 (117). A total of 125 FAV attempts were reported by another high-volume centre in Linz, Austria, approximately around the same period (4). It is a very uncommon procedure, as highlighted by the National Institute for Health and Care Excellence (NICE) guidance of FAV (123). For instance, during the 2015/2016 period, only six completed consultant episodes for 'Other specified therapeutic percutaneous operations on fetuses (R04.8)' were recorded within National Health Service (NHS) in England and the procedure is only performed within the context of research (123). This is due to the evidence of inconsistent efficacy reported. Furthermore, all reported studies have a retrospective study design with potential selection bias and institutional bias can lead to varying postnatal treatment strategies (124), affecting the perceived success of FAV. There is one ongoing prospective observational study ([NCT05386173](#)) by the Association for European

Paediatric and Congenital Cardiology (APEC) group, but no randomised controlled trials for FAV due to ethical challenges in randomisation for this patient population and the rarity of the disease.

2.2.2 FAV efficacy

FAV efficacy has been measured by the postnatal BV circulation rate (at the time of hospital discharge or 28 days after birth), reflecting the immediate success of the procedure in promoting adequate left heart function. The BV outcome rates from a few retrospective case series after technically successful FAV are summarised in Table 3. Most studies reported that live-born patients with technically successful FAV were more likely to achieve BV outcomes compared to the natural history cohort. However, the multicenter study by Kovacevic et al., which utilized propensity score matching and inverse probability of treatment weighting (IPTW), found that the proportion of BV outcomes was similar between the FAV cohort (36%) and the natural history cohort (38%), indicating no significant difference between the two groups (14). A systematic review and meta-analysis of 7 retrospective studies also concluded that FAV does not significantly improve the likelihood of achieving BV circulation, with only 46% of live-born patients achieving BV circulation, and this rate only slightly increasing to 52% in technically successful cases (13).

TABLE 3: BIVENTRICULAR OUTCOMES REPORTED IN RETROSPECTIVE CASE SERIES WITH MORE THAN 50 TECHNICAL SUCCESSFUL FETAL AORTIC VALVULOPLASTY (FAV) CASES

Study	Country	FAV cases (Successful)	BV outcome	BV outcome (live-born, excluding fetal deaths & TOP)
Corroenne et al. 2024 (125)	France	50	52% (26/50)	68% (26/38)
Bradford et al. 2022 (73)	USA	106	52% (55/106)	No data
Tulzer et al. 2021 (4)	Austria	90	63% (57/90)	71% (57/80)
Patel et al. 2020 (119)	IFCIR	108	31% (34/108)	42% (34/81)
Kovacevic et al. 2018 (14)	European Multicenter	59	32% (19/59)	40% (19/47) or 36% (13/36)*

* The cohort was filtered using propensity score matching and inverse probability of treatment weighting to enhance comparability between the FAV cohort and the natural history cohort. TOP: Termination of Pregnancy; IFCIR: International Fetal Cardiac Intervention Registry; BV: Biventricular; FAV: Fetal Aortic Valvuloplasty

FAV efficacy is also measured by the long-term transplantation-free survival rate, indicating the sustained impact of FAV on cardiac health, reducing the need for heart transplantation and improving overall survival prospects. The Boston group observed a 92% (95% CI, 83%-100%) transplantation-free survival to age 6 in patients who underwent FAV and achieved BV circulation, compared to 72% (95% CI, 58%-82%) in those with single-ventricle circulation (126). Procedural experience is an important factor in determining transplantation-free survival, and the Boston group only saw a significant improvement in outcomes in the FAV cohort after applying their analysis to cohorts from post-2009, reflecting current practices. A similar analysis applied to the IFCIR retrospective dataset, which included data accumulated from 15 fetal interventional centres averaging about 7-8 FAV procedures per centre, showed that FAV did not provide the same benefit. This was attributed to higher rates of fetal demise (18%) and lower technical success rates at centres with lower volumes of FAV procedures (126). Taking into account the technical success rate of FAV, fetal demise, and postnatal BV circulation, Pickard et al.'s decision model analysis projected that the probability of transplant-free survival to age 6 years with FAV was 82% (95% CI, 73%-89%) versus 72% (95% CI, 61%-82%) with expectant management (126).

Long-term efficacy was also studied by Kovacevic et al., where FAV was associated with a significant postnatal survival advantage over the natural history group after birth (14). IPTW Cox-regression analysis further confirmed this with a hazard ratio (HR) of 0.38 (95% CI, 0.23–0.64; $P = 0.0001$), after accounting for factors like postnatal circulation and different surgical treatment centres. However, when unsuccessful FAV cases were excluded from the analysis, long-term survival over a 10-year follow-up period was found to be similar between FAV and natural history cohorts (HR, 0.54; 95% CI, 0.14–2.08; $P = 0.37$) (14). This indicates that while FAV improves early postnatal survival, its long-term benefit, in terms of transplantation-free survival, might be less pronounced, highlighting the importance of technical procedural experience in relation to FAV efficacy.

Larger LV long-axis dimension z-scores and a higher ejection fraction (EF) are strong predictors of technical success. Conversely, smaller LV size—determined by the 5/6 area-length calculated volume—along with younger *GA* and longer procedure times

are more likely to experience serious adverse events (117). Such findings raise important questions regarding the selection criteria for patients undergoing the FAV procedure. If fetuses need to have a larger and healthier LV, capable of generating significant pressure and sufficient function for procedural technical success, do they need the procedure? Fetuses can be technically good candidates for FAV, but at what point might they not need the procedure to achieve BV outcome after birth? Identifying ideal candidates for FAV is still of great interest, given that only approximately half of the live-born fetuses with technically successful FAV have BV circulation. Why does the remaining half of the fetuses not respond to FAV?

2.2.3 Patient Selection Criteria

FAV is performed on selected CAS-eHLHS fetuses between 21 to 32 weeks gestation for several reasons. The 32-week cutoff effectively excludes those where CAS appears later in gestation, as these fetuses have a higher chance of achieving BV outcomes without intervention (67). This cutoff also excludes fetuses whose LV function may already be irreversibly compromised to such an extent that FAV would introduce unnecessary risk without likely benefit. In contrast, while it makes sense to perform FAV as early as possible to rescue LV function, younger fetuses encounter greater risks of meeting serious adverse effects (117). Therefore, performing FAV later in gestation, but not too late, is preferred but delaying FAV too long might result in missing the chance to prevent a UV outcome, as the window for effective intervention becomes smaller as the pregnancy advances.

In addition to *GA*, the inclusion criteria for the procedure have been refined over the years and with variations by different institutions (4, 10, 127). Refinement of the criteria has been driven by retrospective reviews of pre-procedural echocardiographic measurements and observations of historical FAV cases, segregating them according to BV and UV outcomes. Several studies have pointed out that larger LVs with better LV inflow, the ability to generate high LV pressures and larger ascending aorta were independent predictors of BV outcome (4, 10, 127, 128). In the ongoing prospective

observational trial by the APEC group, ideal candidates for FAV are selected using criteria defined in Table 4.

TABLE 4: PATIENT SELECTION CRITERIA FOR PROSPECTIVE OBSERVATIONAL TRIAL ON FETAL AORTIC VALVULOPLASTY (NCT05386173)

A. All of the following echocardiographic criteria need to be satisfied between 23 + 0 and 31 + 6 weeks	
1)	Presence of aortic valve stenosis with antegrade flow through the valve
2)	Presence of predominantly left-to-right shunt at the atrial level
3)	Presence of predominantly retrograde flow in the aortic arch between the first two brachiocephalic vessels.
4)	Qualitatively depressed left ventricular function
5)	LV end-diastolic diameter Z-score (short axis) > 0
6)	Left ventricular inlet length in diastole (long axis):
	a. Gestational age ≤ 24+6: Z-score > ±0
	b. 25+0 ≤ Gestational age ≤ 27+6: Z-score > -0.75
	c. Gestational age ≥ 28+0: Z-score > -1.50
7)	Mitral valve diameter in diastole Z-score > -2.0
B. The newborn infant will have a full range of postnatal treatment options available:	
1)	Surgical or catheter-based aortic valvotomy
2)	Ross-Konno surgery
3)	Norwood or hybrid stage-one surgery

Note: LV – left ventricle. Z-scores are derived using Schneider et al (69)

This set of criteria is slightly different compared to the criteria used by the Boston group and that used by the Linz group. The Boston group's criteria incorporate a detailed decision tree analysis, known as Classification and Regression Tree (CART) analysis. It is a predictive modelling technique that identifies key parameters and their interactions by dividing the dataset into subsets based on these parameters. These parameters include additional mid-gestation echocardiographic parameters, derived from retrospective reviews of pre-FAV scans around 25 weeks, that help differentiate between BV and UV outcomes, such as LV pressure (determined by AS or MV regurgitation velocity), MV inflow pattern, and ascending aortic diameter (73). The CART analysis helps in systematically evaluating and establishing cut-off values for these parameters to predict neonatal BV outcomes more accurately (127). In Pickard et al.'s decision-making model and probabilistic sensitivity analyses, FAV is preferable unless the risk of fetal demise exceeds 12% or the probability of BV circulation is less than 26% (126).

In contrast, the Linz group proposes utilising a combination of the right-to-left ventricular (RV/LV) length ratio and LV pressure measured by the MV regurgitation velocity. They also employed CART analysis to refine their criteria, where the analysis of

their criteria showed a 97% sensitivity and 94% specificity in predicting postnatal BV circulation at 1 year of age (4).

Aside from echocardiographic-derived measurements, strain measurements have become particularly of interest as potential predictors of postnatal outcomes. As mentioned in section 2.1.6, strains correlated with disease severity, 2D strains were significantly higher in CAS-eHLHS fetuses that had postnatal BV outcomes (94). Recognising the potential of biomechanical parameters, Green et al. proposed myocardial stress as a superior predictor of postnatal outcomes (129). Although limited with sample size, image-based FE biomechanics modelling of the LV myocardium in fetuses with CAS-eHLHS demonstrated that higher peak systolic myofiber stress was significantly associated with BV outcomes. A simplified empirical equation was proposed to estimate myocardial stress from routine echocardiography measurements by applying principal component analysis to echocardiographic measurements such as valvular velocities, LV dimensions and stroke volume and then followed by regression analysis. The equation was then validated on a retrospective CAS-eHLHS cohort (n=37) by comparing the predicted myocardial stress values with actual postnatal BV or UV outcomes, determined through clinical follow-up. The high predictive capability of the myocardial stress values was demonstrated through receiver operating characteristic (ROC) analysis, showing a strong correlation with BV outcomes (129).

While the criteria for identifying fetuses with CAS that will progress to HLHS have improved after 20 years of experience, the criteria to exclude fetuses that would naturally progress to a BV outcome remain less well-defined. This refinement is crucial to avoid unnecessary interventions, reducing the risk of fetal demise or adverse events associated with the procedure. An optimised criteria will ensure that only those who truly can benefit from FAV should undergo the procedure, thereby optimizing outcomes and minimizing risks.

In summary, efforts to evaluate and consolidate current published criteria, including those from Boston, Linz and newer alternative metrics such as myocardial stress, are warranted in the future to improve on the current reported standard of FAV efficacy and ensure that the evidence supporting the procedure is robust. Current efforts to improve patient selection criteria are focused on identifying fetuses that are at

significant risk of progressing to HLHS and exhibit characteristics that indicate potential for LV recovery after FAV. This is still crucial given that a substantial proportion of FAV patients still do not respond to the procedure, which leads to scepticism about its effectiveness and what the procedure is accomplishing. Therefore, one of the worthy aims of this thesis is to quantify acute haemodynamic changes a few days after the procedure. Does FAV drastically alter the haemodynamic environment of the LV?

2.2.4 Changes after successful FAV

TABLE 5: PAIRED DATASETS OF EARLY FETAL HAEMODYNAMIC CHANGES AFTER FAV

Echocardiographic parameters	Pre-FAV	Post-FAV	Change
Presence of antegrade systolic transverse aortic arch flow	0	65%	65%
Bidirectional Foramen Ovale flow	11%	27%	16%
Change in Mitral Inflow Pattern			
Worsened to monophasic or partially fused, or unchanged monophasic	-	-	21%
Unchanged partially fused or biphasic	-	-	-5%
Improved to partially fused or biphasic	-	-	-25%
MV inflow time, msec	105 [81, 150]	155 [133, 190]	32 [8, 78]
MV inflow time z-score	-3.4 [-4.4, -1.8]	-1.5 [-2.5, -0.2]	1.2 [0.3, 3.0]
MV inflow index (msec/bpm)	0.75 [0.58, 1.06]	1.06 [0.89, 1.36]	0.26 [0.03, 0.53]
Mitral regurgitation (> mild)	49 (53%)	37 (40%)	-13%
LV end-diastolic volume (mL)	2.23 [1.53, 3.73]	2.22 [1.35, 3.21]	-0.06 [-0.19, 0.06]
LV end-diastolic volume z-score	1.78 [0.72, 3.04]	1.26 [0.25, 2.94]	-0.26 [-1.3, 0.97]
LV ejection fraction (%)	23 [18, 32]	28 [22, 41]	6.9 [-0.4, 14.7]
LV systolic pressure (mmHg)	45.9 [35, 60.3]	40.1 [32.1, 55]	-3.6 [-10.9, 3.2]
Aortic jet width (mm)	1.3 [1, 1.5]	2.8 [2.4, 3.1]	1.5 [1.1, 1.9]
Aortic jet width index (jet width/annulus)	0.44 [0.35, 0.52]	0.88 [0.75, 0.97]	0.41 [0.31, 0.54]
Aortic regurgitation (< mild)	0 (0%)	31 (34%)	34%

Note: Data is presented as either number of subjects (% of total) for categorical variables or median, [25th percentile, 75th percentile] for continuous variables. Z-scores are derived using Schneider et al (69). MV, mitral valve; LV, left ventricle. End-diastolic volume was estimated using five-sixths (5/6) plane method. Data extracted from Prosnitz et al. 2018 (74).

Current understanding of the effects of FAV is primarily gleaned from pre- and post-FAV echo. Table 5 summarizes the early fetal hemodynamic changes following FAV, highlighting the significant changes observed. It induces acute changes in

haemodynamics such as improving antegrade AV flow, reducing pressure load on the LV, allowing for better LV preload and increasing flow through the LV (5, 74).

According to the Boston group (74), these acute changes are evidenced by significant improvements in parameters like the presence of antegrade systolic transverse aortic arch flow, which increased from 0% to 65%, and the proportion with bidirectional FO flow, increased from 11% to 27%. There were notable improvements in MV inflow time, reversion to biphasic or partially fused MV inflow pattern, and increased LV EF. The LV end-diastolic volume also decreases as a sign of pressure decompression and a greater antegrade AV flow is observed.

One of the implications of FAV is that it also introduces AV regurgitation, which is a sign of technical success for the procedure, with significant severity occurring in 34% of cases. However, AV regurgitation is reported to be well tolerated by most fetuses and tends to improve as the pregnancy progresses (118).

TABLE 6: TISSUE DOPPLER IMAGING PARAMETERS IN BIVENTRICULAR AND UNIVENTRICULAR OUTCOMES BEFORE AND AFTER FETAL AORTIC VALVULOPLASTY (FAV)

Echocardiographic parameters	Biventricular (n=14)		Univentricular (n=9)	
	Pre-FAV	Post-FAV	Pre-FAV	Post-FAV
Mitral				
MAPSE (mm) *	3.0 ± 0.9	3.6 ± 0.9	2.8 ± 0.6	2.9 ± 0.3
TDI-E' (cm/s) *	3.06 ± 0.82	4.15 ± 1.53	2.31 ± 0.33	2.68 ± 0.61
TDI-S' (cm/s) *	3.07 ± 1.10	3.86 ± 1.40	2.83 ± 1.70	3.14 ± 1.10
E'/A' *	0.65 ± 0.18	0.82 ± 0.19	0.62 ± 0.13	0.63 ± 0.18
E/E' *	18.91 ± 5.30	13.71 ± 5.37	21.98 ± 13.25	22.94 ± 17.67
MPI' *	1.20 ± 0.27	0.77 ± 0.24	1.34 ± 0.30	1.06 ± 0.31
Tricuspid				
TDI-A' (cm/s) *	9.15 ± 1.87	7.63 ± 1.34	8.56 ± 1.98	8.93 ± 1.78
TDI-S' (cm/s) *	4.82 ± 1.40	5.72 ± 1.42	5.19 ± 1.01	5.24 ± 0.74
MPI' *	0.80 ± 0.18	0.60 ± 0.11	0.74 ± 0.10	0.72 ± 0.22

Note: Data presented as mean and standard deviation. * denote parameters with statistical significance ($P < 0.05$) for changes before and after FAV in the biventricular cohort. No statistical significance was observed in any of the parameters in the univentricular cohort. E/A, ventricular diastolic filling ratio; MAPSE, mitral annular plane systolic excursion; TDI, tissue Doppler imaging; E', early diastolic myocardial peak velocity; A', late diastolic with atrial contraction myocardial peak velocity; S', Systolic myocardial peak velocity; MPI', myocardial performance index; TAPSE, tricuspid annular plane systolic excursion.

Additionally, Wohlmuth et al. and Friedman et al. both used TDI to detect significant changes in ventricular function and myocardial performance (70, 76). Wohlmuth et al. demonstrated that pre- and post-FAV changes in TDI-derived cardiac parameters were only significant for fetuses that responded to FAV and achieved a BV outcome, compared to those who did not respond and progressed to HLHS at birth (76). Table 6 compares TDI-derived echocardiographic parameters before and after FAV, categorized by their postnatal outcomes: BV or UV.

These key parameters reflect various aspects of heart function. TDI-E', which relates to ventricular relaxation and filling, shows higher values post-FAV, suggesting improved relaxation. TDI-S', reflecting systolic function, increases post-FAV, indicating better systolic performance. Mitral annular plane systolic excursion (MAPSE) measures MV movement during systole, and higher values post-FAV reflect improved LV function. The E'/A' ratio, showing early to late diastolic velocities, increases post-FAV, indicating better diastolic function. The E/E' ratio decreases post-FAV, suggesting reduced LV filling pressures. Myocardial performance index (MPI), a combined measure of systolic and diastolic function, decreases post-FAV, indicating better overall heart performance in the BV cohort, but not in the UV cohort. Additionally, on the right side, significant changes were observed in TDI-A', TDI-S' and MPI, likely due to the shared septum between the LV and the RV, meaning that improvements in the LV function can positively affect RV performance.

2D strains increased after FAV in the BV cohort but not the UV cohort, which further corroborates the notion of improved diastolic function and myocardial deformation in the BV cohort (94). Furthermore, in an FE analysis of patient-specific CAS-eHLHS LVs, FAV partially restored physiological features towards healthy levels, by increasing stroke volume and myocardial strains, reduced AV and MV regurgitation velocities, LV pressure, LA pressure and peak myofiber stress (130). It was also shown that AV regurgitation can compromise LV and LA depressurization during diastole as it interferes with ventricular filling and atrial ejection but can also further increase stroke volume and introduce greater myocardial deformation stimuli.

Late gestation post-FAV echocardiographic findings were also studied (5, 14, 131). Kovacevic et al. demonstrated significantly better growth, less deterioration and

improved haemodynamics in the left heart later in gestation for fetuses that underwent successful FAV compared to both unsuccessful FAV and natural history cases (14). Specifically, the growth of the MV and overall haemodynamics was significantly better in fetuses with technically successful FAV compared to unsuccessful FAV. Growth of the AV, MV, LV long and short axis was significantly better in the technically successful cohort compared to the natural history cohort.

TABLE 7: COMPARISON OF PRE-FAV ECHOCARDIOGRAPHIC PARAMETERS ASSOCIATED WITH BIVENTRICULAR AND UNIVENTRICULAR OUTCOMES: ANATOMIC AND PHYSIOLOGIC ASSESSMENT IN FETUSES WITH AORTIC STENOSIS

Echocardiographic parameters	Biventricular (n = 50)	Univentricular (n = 46)
Anatomic Parameters		
AV diameter z-score *	-2.82 (-3.09, -1.94)	-3.42 (-4.20, -2.70)
Ascending Aorta diameter z-score *	0.71 (-0.63, 1.91)	-1.02 (-1.65, 0.02)
MV diameter z-score *	-1.84 (-2.93, -0.86)	-3.14 (-3.81, -2.13)
LV long axis z-score *	-0.80 (-1.48, -0.44)	-2.42 (-3.13, -1.16)
LV short axis z-score *	-0.05 (-1.84, 1.26)	-1.23 (-2.35, -0.14)
LV EDV z-score *	-0.70 (-2.05, 0.84)	-2.92 (-3.54, -1.13)
Physiologic Parameters		
LV ejection fraction *	48.6 (36.8, 57.6)	36.8 (26.1, 44.8)
LV dysfunction (≥moderate) *	24 (48%)	39 (86.7%)
MV inflow pattern *		
Biphasic	40 (88.9%)	28 (73.7%)
Monophasic	5 (11.1%)	5 (13.2%)
Partial fusion	0 (0%)	5 (13.2%)
Aortic jet width (mm) *	3.8 (3.5, 4.5)	3.4 (2.6, 4.0)
LV pressure estimate (mmHg) *	70.78 (54.71, 86.71)	59.81 (39.64, 74.50)
Antegrade aortic arch flow *	26 (52.0%)	7 (15.6%)
PFO flow (bidirectional or right-to-left) *	25 (59.5%)	7 (17.1%)

Note: *denote parameters with statistical significance ($P < 0.05$) comparing between biventricular and univentricular cohorts. AV, aortic valve; MV, mitral valve; LV, left ventricle; EDV, end-diastolic volume using five-sixths (5/6) plane method; PFO, patent foramen ovale. Z-scores are derived using Schneider et al (69).

Beattie et al. and Bradford et al. expanded on Kovacevic's findings and found some late gestation anatomic and physiologic parameters associated with circulation outcome after FAV (73, 131), these are summarised in Table 7. BV cases exhibited less reduction in AV diameter and ascending aorta diameter Z-scores, indicating better valve and aortic growth. MV diameter and LV long-axis z-scores were also better in BV cases.

Additionally, LV EF was higher, and LV dysfunction was less prevalent in BV cases, demonstrating better cardiac contractile function. BV outcomes had a higher incidence of biphasic MV inflow patterns and antegrade aortic arch flow, indicating more efficient blood flow dynamics. Lastly, BV cases had a greater likelihood of bidirectional or right-to-left flow through the patent FO, suggesting better overall haemodynamics.

2.2.5 Summary

Overall, FAV holds the potential to change the prognosis of CAS-eHLHS fetuses, as demonstrated by two prominent high-volume centres. The success and evidence of its efficacy depend heavily on proper case selection, technical experience, and comprehensive postnatal management. There is also a need for further refinement of selection criteria to ensure that FAV is not performed on fetuses that could achieve BV circulation naturally, thereby avoiding unnecessary risks associated with the procedure, including fetal and maternal morbidity and mortality.

There is a great emphasis on flow haemodynamic features in the context of CAS-eHLHS, where features such as retrograde systolic aortic arch flow, monophasic MV inflow and left-to-right FO flow are regarded as strong predictors for evolution to HLHS. The absence of any haemodynamic improvements and poorer growth in those with UV postnatal outcomes compared to those with BV outcomes corroborate the notion that HLHS aetiology is influenced by haemodynamic forces.

2.3 Fluid Mechanics and its Biological Significance in Fetal LV Development

Characterisation of fluid mechanics in LVs has been studied extensively in adults, using advanced imaging modalities such as computed tomography (CT), Magnetic Resonance Imaging (MRI) and transthoracic echocardiography, often supplemented by computational techniques like CFD, FSI, and electrophysiological simulations (132). However, such studies in the developing fetal heart are relatively limited. The difficulty in capturing high-resolution images of fetal cardiac flow in utero and the limited availability of advanced simulation techniques pose significant challenges.

Nonetheless, recent studies have begun to fill this gap. Fetal LV haemodynamics were previously quantified by Lai et al. and Wiputra et al. (133, 134), where patient-specific 4D ultrasound studies showed that differences in vortex formation between fetal and adult LVs are due to lower E/A wave velocity ratios, shorter diastolic duration, and higher heart rate in fetal hearts. In fetal hearts, quicker vortex formation and dissipation result in less stable blood flow patterns compared to adults. Wiputra et al. reported a peak diastolic Reynolds number of 250-350 inside LVs of mid-gestation (20 weeks of gestation) and also investigated the potential haemodynamic environments at earlier stages of development (135). This was done by applying length scaling to simulate the smaller size of the LV typical of earlier *GAs*. Through this process, they identified a critical transition point around 11 weeks, where the fetal LV shifts from a low Reynolds number environment dominated by viscous forces to a higher Reynolds number regime. As the heart grows, this shift allows inertial forces to become more prominent, leading to the formation of diastolic vortex rings and a significant increase in WSS. This increase in shear stress is thought to provide biomechanical stimuli that are essential for promoting normal heart growth and development.

While such haemodynamic features are increasingly well described, their biological relevance to myocardial development warrants closer attention. In early gestation, fetal LV growth is driven predominantly by cardiomyocyte hyperplasia, a process regulated by both genetic programs and mechanical stimuli. For example, mechanical stretch (as described by Laplace's Law) and preload are known to influence

intracellular pathways like IGF-1 and Hippo-YAP/TAZ, which regulate proliferation, differentiation and myocardial patterning (136). While these stretch-mediated responses are elucidated, it is still possible that flow-derived forces, particularly shear stress and pressure gradients, may also serve as upstream regulators of myocardial growth. This hypothesis is supported by valvular development studies, where WSS has been shown to modulate endothelial behaviour, extracellular matrix remodelling, and leaflet morphogenesis (137).

However, for these growth processes to be sustained, the myocardium must also meet rising metabolic demands. Cardiomyocytes rely on mitochondrial ATP production, which in turn depends on adequate oxygen delivery. In the setting of CAS-eHLHS, high afterload imposes a major energetic constraint. According to the Tension-Time index (TTI) — a physiological measure that approximates myocardial oxygen consumption — energy demand is not solely determined by cardiac output (i.e., stroke work), but by the product of systolic pressure and contraction duration (138). In other words, TTI captures the total "tension time" that the myocardium experiences during each beat. This means that even when the net forward flow is low, as in CAS-eHLHS, the energy cost of sustaining high pressures remains substantial.

In CAS-eHLHS, the fetal LV must generate abnormally high systolic pressures to overcome outflow obstruction, yet often achieves only minimal stroke volume. As per the TTI model, this results in disproportionately high oxygen consumption for relatively little mechanical output, effectively diverting ATP away from biosynthetic pathways essential for hyperplastic growth.

This mismatch between pressure generation and effective output is further illustrated by the pump-function curves in Figure 11, which describe the heart's ability to generate output at a given pressure. In Figure 11A, a healthy LV operates on a pump-function curve where a moderate pressure rise yields a proportional increase in output. In contrast, the curve in CAS-eHLHS is likely shifted upward and leftward due to early muscular hypertrophy (shown in Figure 11B), meaning the LV generates high pressures but very little output. In this regime, the LV acts more like a pressure source than a flow generator (shown in Figure 11C). This not only signals inefficient cardiac mechanics but also exacerbates energy scarcity for tissue growth. Over time, if afterload remains

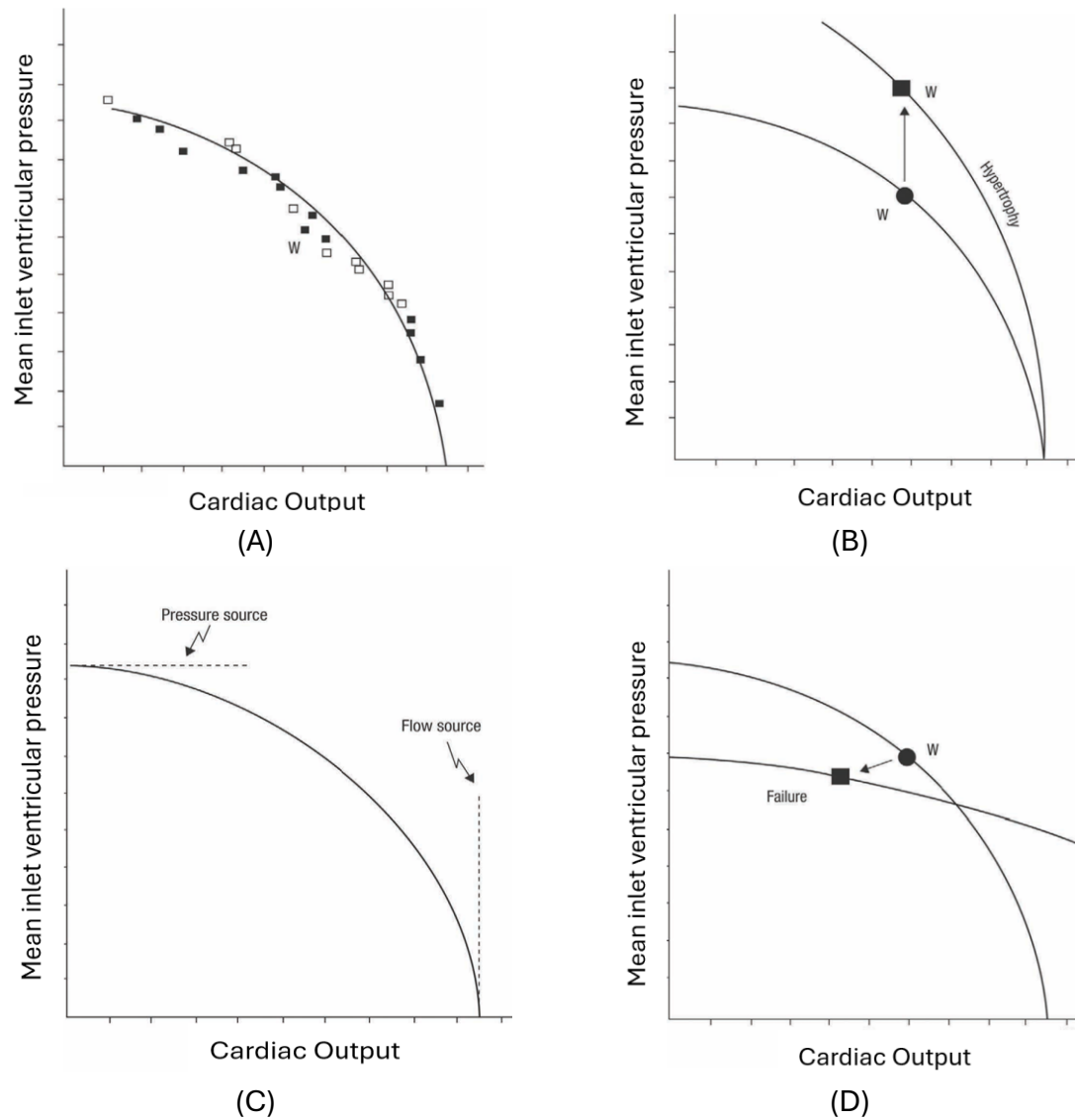


Figure 11: Conceptual pump-function curves illustrating LV performance in CAS-eHLHS. (A) Baseline LV function: inverse relation between pressure and output. (B) CAS-eHLHS: chronic pressure overload leads to hypertrophy and an upward-shifted curve, with higher pressure but limited flow. (C) The LV acts as a pressure source, requiring large pressure increases for small flow gains. (D) Prolonged overload flattens the curve, indicating pump failure where further pressure yields minimal output. Figure adapted from Nichols et al., McDonald's Blood Flow in Arteries, 7th ed., CRC Press, 2022 (138).

unresolved, the LV may move toward the flat portion of the curve (Figure 11D), where further pressure increases fail to improve output. This is a hallmark of mechanical failure and a trajectory consistent with HLHS progression.

Taken together, these insights underscore the importance of fluid dynamic parameters, not just as diagnostic or descriptive tools, but as biomechanical determinants of cardiac development. There is a clear need for more image-based, patient-specific simulations in CAS-eHLHS to deepen our understanding of flow dynamics and their biological implications. Such work may help identify new

mechanistic links between flow and growth, support better risk stratification, and ultimately guide a more rational selection of candidates for FAV.

Chapter 3

Aim 1 - Investigating Fluid Mechanics in LV of CAS-eHLHS

The objective of this chapter is to elucidate the detailed fluid dynamics within CAS-eHLHS LVs before FAV is performed with a comparison to healthy fetal LVs. The results of this chapter will further be used to analyse the fluid mechanical environment after FAV, presented in Chapter 4. Understanding the detailed fluid mechanics of CAS-eHLHS is crucial because current knowledge is limited. The ability to accurately simulate and characterize these flow patterns can provide insights into disease progression, mechanical forces influencing heart development, and the effects of FAV. Insights from fluid mechanics can potentially be used to improve intervention outcomes and patient selection for FAV.

To achieve this objective, image-based patient-specific CFD simulations are employed to quantify flow patterns, vortex dynamics, and WSS in CAS-eHLHS LVs. There was high variability across CAS-eHLHS cases, but also the following unifying features. Firstly, in CAS-eHLHS, the diastole MV inflow manifested as a narrowed, high-velocity jet impinging on the apical region, in contrast to the broad, gentle inflow seen in healthy LVs. This was likely due to a malformed MV with impaired opening dynamics. This altered inflow increased vorticity dynamics and elevated WSS at the apical zone rather than mid-ventricle and the overall WSS magnitude averaged over the entire LV was reduced. Secondly, CAS-eHLHS LVs also featured elevated fluid-mechanical energy loss, intraventricular pressure gradients (IVPGs) and vortex formation numbers, suggesting inefficient haemodynamic energy transfer to flow and additional ventricular workload. Thirdly, CAS-eHLHS LVs had poor blood turnover, suggesting a hypoxic environment, which could be associated with EFE that is often observed in these patients. The results from this chapter have been published in the Annals of Biomedical Engineering with minor modifications to results to reflect a larger patient cohort size (139).

3.1 Methods

The methodology for creating patient-specific CFD models of the LVs is illustrated in Figure 12. The image-based CFD pipeline can be broken down into three sub-steps:

- 1) Patient Data Collection
- 2) Motion Tracking, Segmentation and LV reconstruction
- 3) CFD modelling with ANSYS Fluent

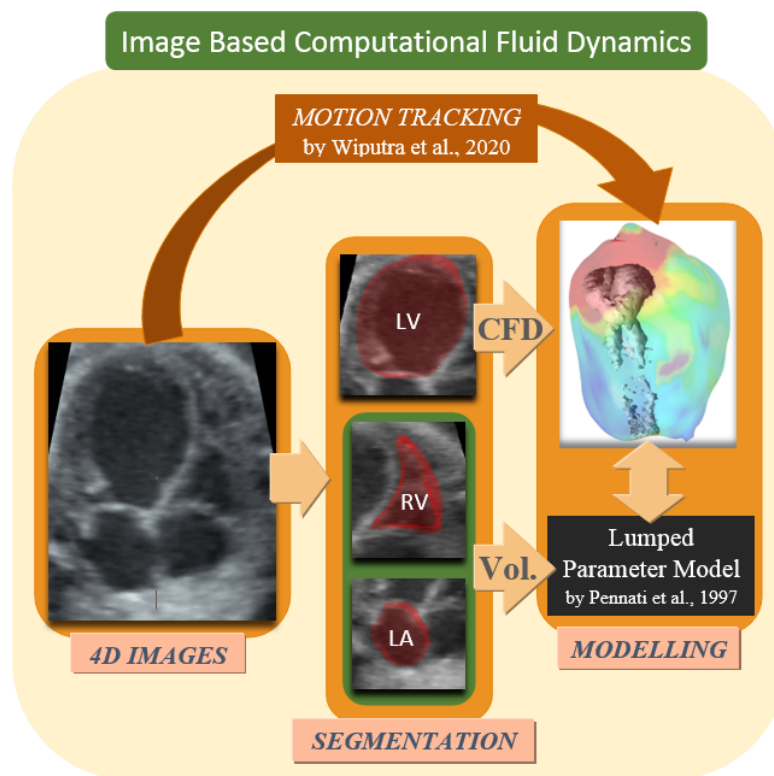


Figure 12: Procedures for generating patient-specific computational fluid dynamics models from 4D echocardiographic images. LV-left ventricle, RV-right ventricle and LA – left atrium.

3.1.1 Patient Data Collection

Patients with CAS-eHLHS underwent pre-FAV echocardiographic examinations, conducted by a pediatric cardiologist at Kepler University Hospital in Linz, Austria. These procedures were performed under the ethics approval granted by the Institutional Review Board, protocol number 1009/2017, with written consent obtained from all participants. Additionally, echocardiographic data from five healthy fetuses, aged between 21 and 32 weeks, were collected at the National University Hospital in Singapore. This was also

done with ethics approval from the Domain Specific Review Board of the National Healthcare Group (Singapore), protocol number 2014/00056.

For the CAS-eHLHS fetal hearts, 4D B-mode ultrasound images of the four-chamber view were acquired using the STIC technique with an RM6C transducer (frequency range 2.0–6.0 MHz) and the Vivid-7®, Vivid E9®, or Vivid E95® ultrasound systems (GE Healthcare, Zipf, Austria). Meanwhile, images of the healthy fetal hearts were obtained using a RAB 4–8L transducer (frequency range 4.0–8.0 MHz) in conjunction with the Voluson 730 ultrasound system (GE Healthcare, Singapore). An imaging sweep over 10–15 seconds, with an image capture rate of 70–90 frames per second, giving approximately 30–40 image volumes per cardiac cycle.

Pulsed-wave Doppler spectral tracings were also obtained at the MV and AV regions to measure peak diastolic filling velocities, MV regurgitation (if present), and AV outflow velocities and waveforms. The characteristics of CAS-eHLHS fetuses are listed in Table 8.

TABLE 8. CHARACTERISTICS OF CRITICAL AORTIC STENOSIS WITH EVOLVING HYPOPLASTIC LEFT HEART SYNDROME (CAS-eHLHS) FETUSES BEFORE FETAL AORTIC VALVULOPLASTY, POSTNATAL OUTCOMES AND POSTNATAL PROCEDURES ARE UNDERTAKEN.

Case	GA at scan, (week + day)	Bradycardia	LV thrombus	Pericardial effusion	Hydrops	Postnatal Circulation	Postnatal procedures
CAS-eHLHS – 1	22 + 1	Y	N	N	N	UV	NW, UV-BV conv., RK
CAS-eHLHS – 2	22 + 4	N	Y	N	N	UV	NW
CAS-eHLHS – 3	29 + 1	N	N	N	Y	BV	RK
CAS-eHLHS – 4	29	N	N	N	Y	BV	RK
CAS-eHLHS – 5	24 + 6	Y	N	N	N	BV	AV dilation
CAS-eHLHS – 6	30 + 1	N	N	N	N	BV	AV dilation
CAS-eHLHS – 7	23 + 3	Y	N	N	N	UV	Hybrid

Note: CAS-eHLHS – critical aortic stenosis and evolving hypoplastic left heart syndrome, AV- aortic valve; GA - gestational age; LV - left ventricle; BV - biventricular; UV - univentricular; RK - Ross-Konno procedure; UV-BV conv. - univentricular to biventricular conversion; NW - Norwood procedure; Hybrid – Hybrid procedure; Y - yes; N - no.

3.1.2 Segmentation, LV Reconstruction and Motion Tracking

Stacks of 2D cine images were exported for each time frame, with 0.5mm spacing between consecutive planes, using 4DView software (GE) for processing. The chamber

of the LV, RV and LA for the first time point were segmented into binary images using a custom-written semi-automatic lazy-snapping algorithm (140). It's important to note that when referring to the chamber, both the chamber cavity and the endocardium are included, the myocardial wall is excluded. The .stl files of the 3D geometries were reconstructed via the Vascular Modelling Toolkit software (141), and the reconstructed geometry was smoothed with Geomagic Studio (Geomagic Inc., Morrisville, NC, USA). To touch briefly upon how the geometries were used, the patient-specific LV was used for CFD analysis, while the volume information of the RV and LA were used to calibrate the fetal lumped parameter model (LPM).

Over the cardiac cycle, the movement of the segmented chamber and its wall boundary was estimated using a validated motion-tracking algorithm that has been previously published (142). The motion of the segmented model was estimated using pairwise image registration across consecutive time frames. The displacement fields were quantified through an open-source Python module, known as SimpleElastix (143). The algorithm uses B-spline transformations to capture and quantify the displacement vector field using a grid of control points. Variations in deformation across the control points are smoothed so that the changes are gradual and not overly complex. After estimating the displacement fields using B-spline transformations, the temporal deformation of the segmented chamber is captured using the Fourier series, which ensures that the periodic nature of the cardiac cycle is accurately represented. The Fourier coefficients are computed to describe the cyclic variations in the displacement fields over time. This approach not only enforces temporal smoothness but also respects the inherent cyclic nature of cardiac motion, allowing for a more consistent and physiologically accurate representation of the heart's dynamics throughout the cycle.

The motion model, referred to as the B-spline Fourier (BSF) model, is described as follows:

$$\vec{X}_{BSF}^t = \sum_{f=0}^N \vec{F}_f \cos\left(\frac{2\pi f t}{T}\right) + \vec{F}_{f+N+1} \sin\left(\frac{2\pi f t}{T}\right) \quad (1)$$

where $\vec{X}_{BSF}^t$ represents the Cartesian coordinate locations at the current time frame t , f is the frequency mode, N is the number of Fourier frequency modes, $\vec{F}_f$ are the Fourier

coefficients and T is the period of the cardiac cycle. Briefly, spatial variations of $\vec{F}_f$ are described by a weighted sum of cubic b-spline coefficients at control points. The BSF model is optimised to fit the image-registration displacement fields. The motion tracking algorithm includes the option for additional pairwise image registration inputs, enabling the prioritisation of specific time frames during the optimisation process. This feature was employed to highlight images captured at end-systole and end-diastole, representing the smallest and largest states of the heart chambers, respectively. By doing so, the image-tracking process aims to approximate the stroke volume of the chamber contraction more accurately. Figure 13 demonstrates the motion tracking of the LV for one representative healthy case and one CAS-eHLHS case over the cardiac cycle.

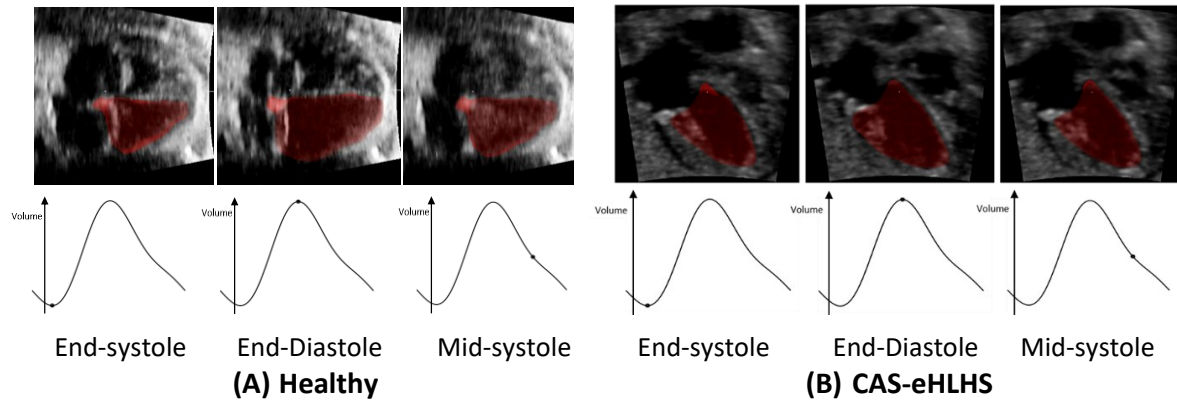


Figure 13: Left ventricle (LV) segmentation and motion tracking of healthy and critical aortic stenosis and evolving hypoplastic left heart syndrome (CAS-eHLHS) LVs. The red regions represent the segmented LV volume after motion tracking, superimposed onto 2D slices extracted from 4D image stacks. These images illustrate the temporal evolution of the LV volume at three distinct time points during the cardiac cycle: end-systole, end-diastole, and mid-systole.

3.1.3 Computational Fluid Dynamics Modelling with ANSYS Fluent

Following the motion tracking process, CFD simulations for the LV were carried out using ANSYS FLUENT software (Ansys Inc., Canonsburg, PA, USA). The LV chamber was modelled with unstructured mesh with at least 1 million tetrahedral elements. This is in line with previously reported mesh convergence studies of fetal ventricles (134). Dynamic mesh simulations were conducted with node locations being updated at each time step using the BSF model from the motion tracking algorithm, facilitated by an ANSYS user-defined function. The built-in solver was used to solve the Navier-Stokes

equations with 200-400 timesteps per cardiac cycle. 3D incompressible Navier-Stokes and continuity equations that ignore gravitational forces are modelled in simulations:

$$\frac{\partial \vec{u}}{\partial t} + (\vec{u} \cdot \nabla) \vec{u} = -\frac{1}{\rho} \nabla p + \frac{\mu}{\rho} \nabla^2 \vec{u} \quad (2)$$

$$\nabla \cdot \vec{u} = 0 \quad (3)$$

where ρ denotes the fluid density, μ is the dynamic viscosity, p is the fluid pressure, $\vec{u}$ is the velocity field vector. Each time step iteration adhered to convergence criteria set at less than 10^{-4} for all residuals. To minimize artefacts stemming from a static initial condition, a minimum of three cardiac cycles were simulated. Blood density was modelled with a constant density, 1060 kg/m^3 , and blood viscosity, μ , was modelled with a Carreau-Yasuda model with the following equation:

$$\mu = \mu_{\infty} + (\mu_0 - \mu_{\infty}) [1 + (\lambda \dot{\gamma})^2]^{\frac{n-1}{2}} \quad (4)$$

where μ_{∞} is the high shear viscosity limit, 0.0035 Pa s , μ_0 is the zero shear viscosity limit, 0.056 Pa s , λ and n are constants set as 3.313 s and 0.3568 , respectively (133, 144). These constants were derived by curve-fitting to experimental blood viscosity data. While non-Newtonian behaviour has limited impact under high-flow conditions ($\text{Re} > 400$), it becomes increasingly relevant in low-shear regions and recirculation zones ($\text{Re} = 100$ and below), where viscosity varies significantly with shear rate.

Due to the limited resolution of fetal echo images, the detailed morphology and dynamics of the valves could not be accurately captured. Consequently, the CFD models did not include valve leaflets. To simulate the flow in and out of the LV, the opening and closing of valves were simplified. This simplification involved instantaneously switching between a zero-pressure boundary and a closed-wall boundary condition. For example, during diastole, the AV orifice remained closed, and the MV orifice had zero-pressure boundary conditions. Conversely, these valve conditions were reversed during systole. These boundary conditions were used for CFD models of healthy fetal LVs. This approach was used in simulations of healthy fetal LVs and is illustrated in Figure 14.

To more closely mimic physiological valve behaviour, a dynamic switching mechanism was also implemented. At each time step, the solver assessed the direction of flow through each orifice. If backflow was ever detected, such as retrograde flow into

Valve conditions

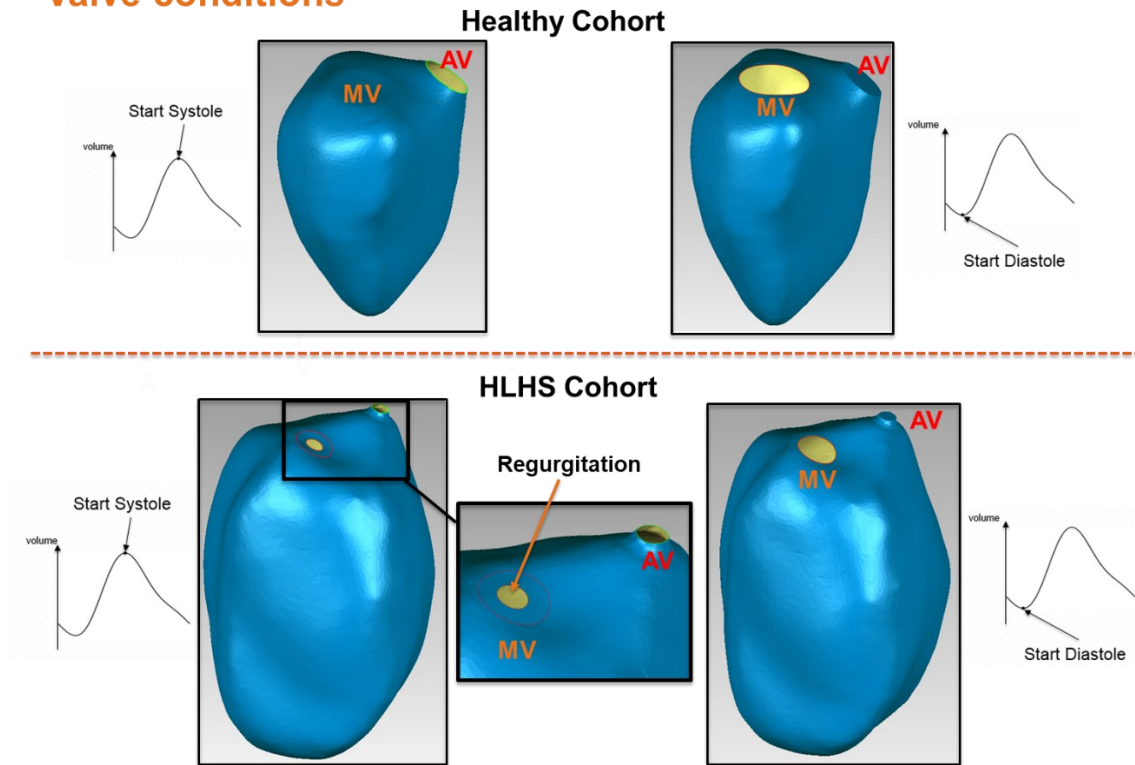


Figure 14: Valve boundary conditions during the cardiac cycle. Schematic showing CFD inlet and outlet regions applied to the mitral valve (MV) and aortic valve (AV) surfaces at systolic and diastolic phases. In healthy conditions (top row), inflow occurs through the MV at the start of diastole, and outflow through the AV at the start of systole. In CAS-eHLHS cases (bottom row), additional mitral regurgitation flow during systole is observed. To model this, a secondary regurgitant orifice was introduced at the center of the MV region, active only during systole. Volume-time curves alongside each panel indicate the timing of each phase.

the LV through the AV at the end of systole, the simulation re-evaluated the time step with the AV closed and the MV open, thereby reflecting physiologic valve closure in response to flow reversal. A similar adjustment was applied at the MV to prevent unrealistic outflow into the LA during diastole.

In the CAS-eHLHS cohort, MV regurgitation is also observed. To capture this phenomenon, an additional regurgitation orifice was introduced within the presumed centre of the MV orifice zone. Consequently, zero-pressure boundary conditions were replaced with prescribed pressure calculated from a LPM, which will be discussed in the following section.

Since it was not possible to directly determine the sizes of the orifices, iterative adjustments to the orifice sizes and repeated CFD simulations were required. The inlet and outlet orifice sizes were progressively modified until the velocities simulated by the CFD closely matched the patient-specific Doppler velocities. Similarly, the size of the

IMPLEMENTATION

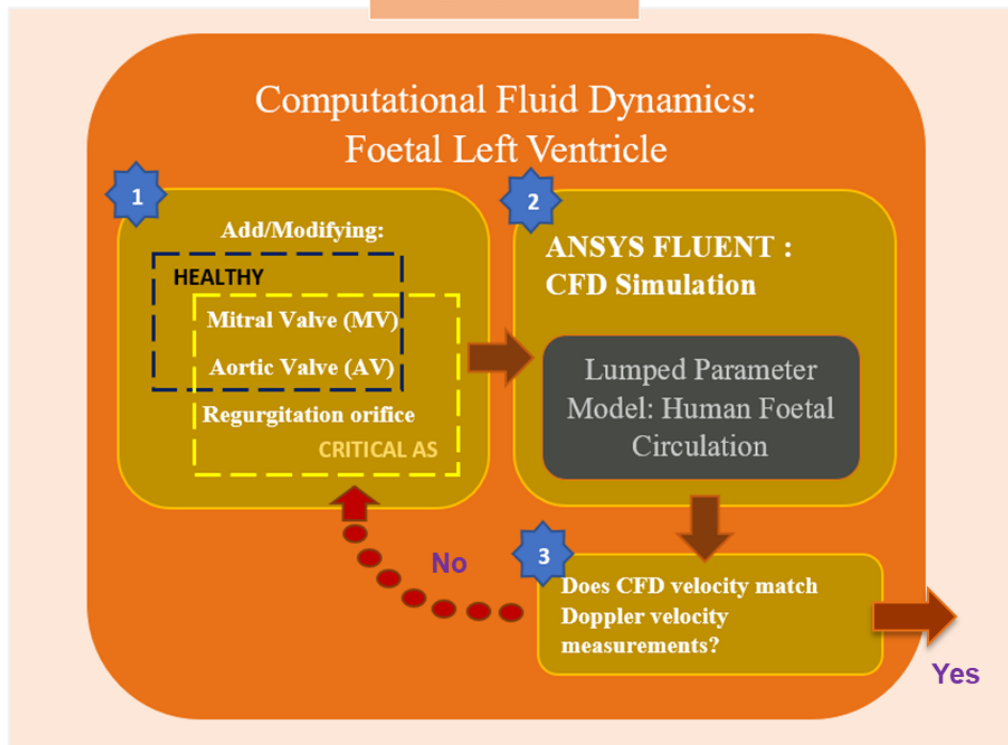


Figure 15: Implementation and tuning pipeline for CFD simulation of fetal left ventricular flow. This schematic outlines the iterative workflow used to simulate intraventricular haemodynamics using ANSYS Fluent, coupled with a lumped parameter model (LPM) of the fetal circulation. Valve boundary conditions (mitral, aortic, and regurgitant orifices) are defined based on whether the case is healthy or critical aortic stenosis (CAS-eHLHS) case. The regurgitation orifice is added specifically for CAS-eHLHS cases to account for observed mitral regurgitation during systole. Simulated velocities are compared against Doppler echocardiography measurements. If the CFD results do not match Doppler-derived peak velocities, valve orifice sizes are iteratively adjusted, and the simulation is repeated until agreement is achieved.

regurgitation orifice was adjusted in a stepwise manner to align with the peak MV regurgitation observed in the patient. Simulations are coupled to the lumped parameter which will be described in the following section. This tuning pipeline is illustrated in Figure 15.

3.1.4 Lumped Parameter Model

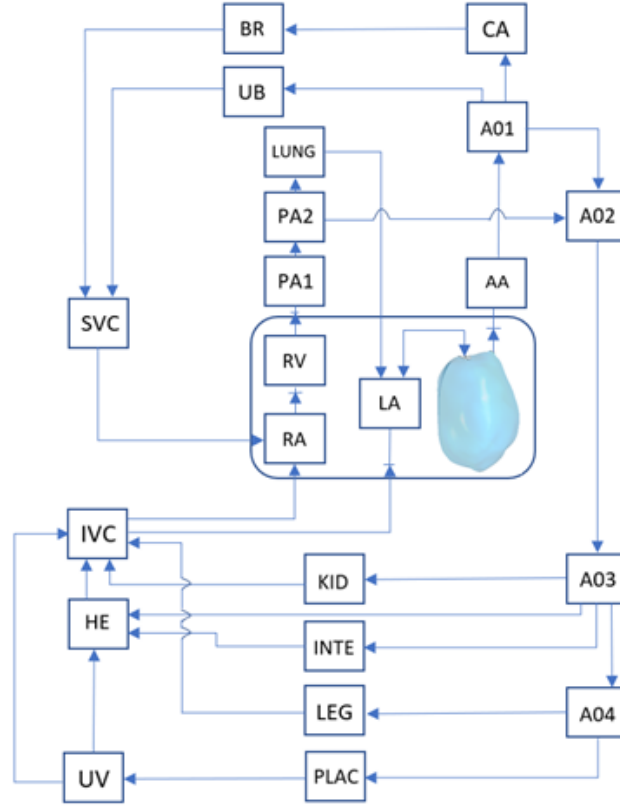


Figure 16: The schematic representation of the lumped parameter model for the human fetal circulation coupled with the CFD model of the left ventricle, adapted from the work of Pennati et al (145). AA: Ascending aorta, A01: aortic arch, A02: thoracic descending aorta, A03: abdominal descending aorta, A04: femoral descending aorta, BR: brain, CA: cerebral arteries, HE: liver, INTE: intestinal circulation, IVC: inferior vena cava, KID: kidney, LA: left atrium, LEG: lower limbs, LUNG: lungs, PA1: main pulmonary artery, PA2: pulmonary arteries, PLAC: placenta, RA: right atrium, RV: right ventricle, SVC: superior vena cava, UB: upper body, UV: umbilical vein.

LPM of the fetal circulation was coupled with the CFD simulations in the CAS-eHLHS cohort, as illustrated in Figure 16. The model was originally proposed by Pennati et al. (145), and can be scaled to different GA s, spanning between 22 to 38 weeks of gestation (146). The methodology for allometric scaling of the LPM from 38 weeks of gestation to other GA was described as,

$$R_{GA} = R_{38} \left(\frac{W_{GA}}{W_{38}} \right)^{-1} \quad (5)$$

$$C_{GA} = C_{38} \left(\frac{W_{GA}}{W_{38}} \right)^{1.33} \quad (6)$$

$$L_{GA} = L_{38} \left(\frac{W_{GA}}{W_{38}} \right)^{-0.33} \quad (7)$$

$$K_{GA} = K_{38} \left(\frac{W_{GA}}{W_{38}} \right)^{-1.33} \quad (8)$$

$$\log_{10} W_{GA} = 0.2508 + 0.1458 GA - 0.0016 GA^2 \quad (9)$$

where R_{GA} , C_{GA} , L_{GA} and K_{GA} are the resistance, compliance, inertances, and valvular dissipative coefficient, respectively, of an element in the LPM at the gestational age of GA .

The LPM was originally calibrated to fit fetal lamb measurements (145), but in the current implementation, it was modified to fit with invasive clinical measurements of the human fetal systolic and diastolic LV and abdominal aortic pulse pressures across different GA s (147, 148). To do this, all resistances were scaled by an age-dependent factor, while all compliances were scaled by another. The original value of the inertances and valvular dissipative coefficients were retained from Pennati et al (145). The age-scaling factors for resistance, R_{scale} and capacitance, C_{scale} are expressed by the following equations:

$$R_{scale} = A_r * GA^6 + B_r * GA^5 + C_r * GA^4 + D_r * GA^3 + E_r * GA^2 + F_r * GA + G_r \quad (10)$$

$$C_{scale} = B_c * GA^5 + C_c * GA^4 + D_c * GA^3 + E_c * GA^2 + F_c * GA + G_c \quad (11)$$

where the coefficients for the higher-order polynomials are in Table 9.

TABLE 9: COEFFICIENTS FOR EQUATION (10) AND EQUATION (11) USED FOR THE POLYNOMIAL RESISTANCE AND CAPACITANCE SCALING FACTORS.

Coefficients	R_{scale}	C_{scale} (below 27wk)	C_{scale} (after 27wk)
$A, mmHg s ml^{-1} wk^{-6}$	$-7.316 * 10^{-7}$	—	—
$B, mmHg s ml^{-1} wk^{-5}$	$1.336 * 10^{-4}$	$5.259 * 10^{-7}$	$2.183 * 10^{-4}$
$C, mmHg s ml^{-1} wk^{-4}$	$1.009 * 10^{-2}$	$9.465 * 10^{-5}$	-0.0261
$D, mmHg s ml^{-1} wk^{-3}$	0.403	$6.85 * 10^{-3}$	1.247
$E, mmHg s ml^{-1} wk^{-2}$	8.978	0.2489	-29.74
$F, mmHg s ml^{-1} wk^{-1}$	105.7	4.529	354.4
$G, mmHg s ml^{-1}$	513.9	-32.79	-1688

The expression for the age-scaling factors was derived by iteratively adjusting the resistance and capacitance factors at various GA s between 22 and 38 weeks, ensuring that the abdominal aortic pulse pressures and fetal LV systolic and diastolic pressures

predicted by the LPM corresponded to the values reported in the literature ages (147, 148). The input to the LPM model was an idealised sinusoidal volume-time waveform, based on LV and RV end-systolic and end-diastolic volumes from fetal nomograms (149). Subsequently, a polynomial curve-fitting tool was used to fit a polynomial across the age-scaling factors, allowing them to vary smoothly across the GA range.

10 cardiac cycles were simulated to ensure that a steady and periodic state was achieved. Figure 17 illustrates a realistic physiological pressure-volume loop for a test case at 38 weeks of gestation (A), alignment with abdominal aortic pulse pressure (B) and LV invasive pressure measurements (C). The minimum diastolic LV pressure was interpreted to be 5 mmHg regardless of age, rather than following the age-dependent increase proposed by Johnson et al. (147), as applying their model would result in excessively high diastolic pressure when extrapolated to a near-term fetus.

The LPM calibrated for healthy fetuses was directly applied to age-matched CAS-eHLHS cases with several key modifications. First, patient-specific RV and LV volume change over time obtained from motion tracking were used as inputs to the LPM. Secondly, MV regurgitation was present in all the examined CAS-eHLHS cases which would cause some pressure elevation in the LA. This elevation was modelled in the LPM through the introduction of a reference ground pressure specific to the LA, denoted as $P_{GND,LA}$, and computed using:

$$P_{GND,LA} = \frac{EDV_{LA}}{C_{LA}} \quad (12)$$

where EDV_{LA} is the segmented end-diastolic LA volume and C_{LA} is the LA compliance. This reference pressure acts as a baseline to which dynamic atrial pressure from atrial contraction—generated by atrial elastance, U_{LA_0} , and an activation function, $A(t)$, is added during the simulation.

This was similarly done for the RA, but the end-diastolic volume of the RA was estimated using healthy fetal atrial length, width and the ellipsoid method (150). After the LPM simulation reached a steady and periodic state, the flow rate predictions for each vascular connection and the pressure predictions for each vascular block from the LPM served as starting conditions for when the CFD simulation was conducted.

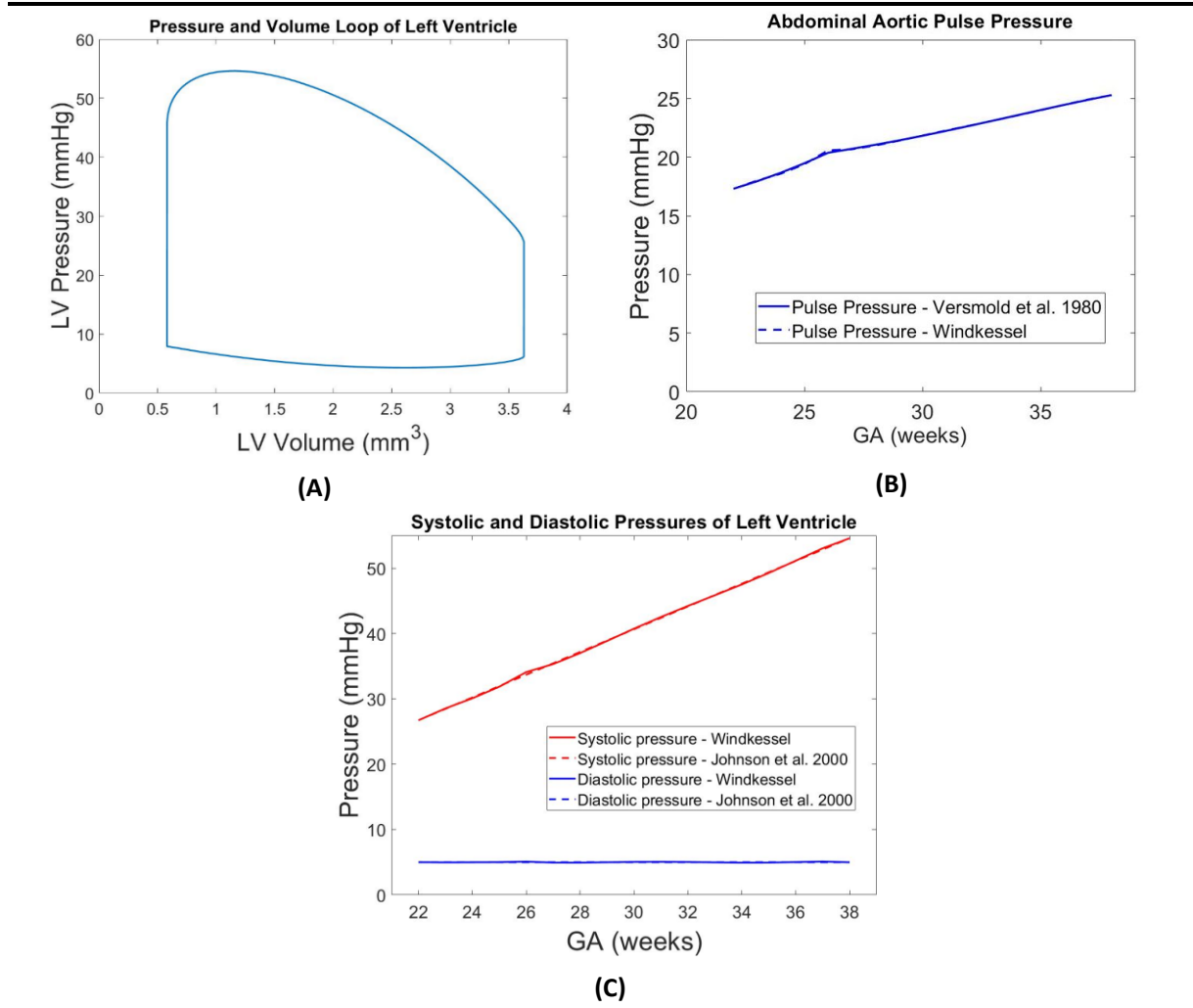
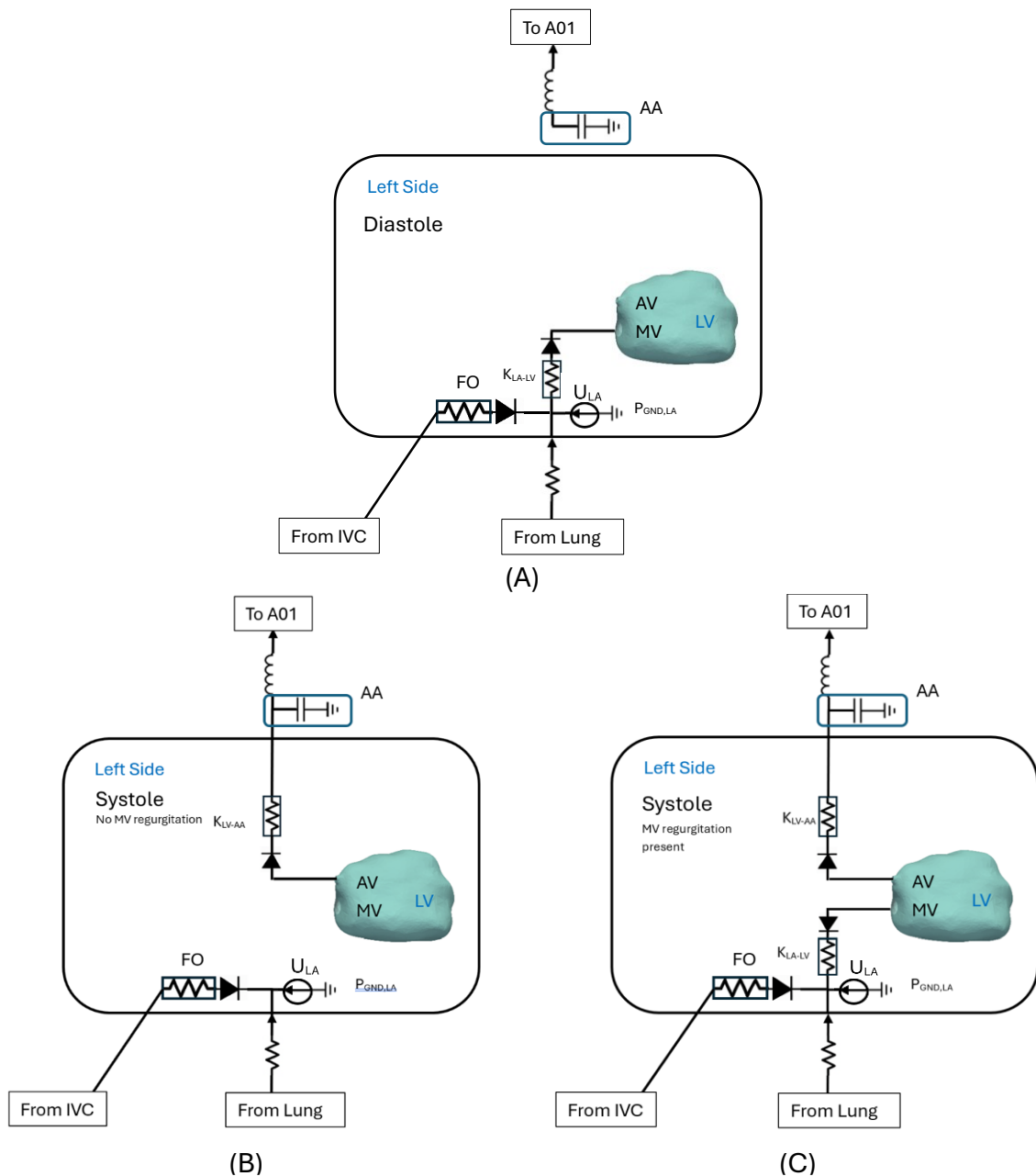


Figure 17: Results from lumped parameter model showcase the physiological (A) pressure-volume loop for the left ventricle (LV), presented as a test case for 38 weeks of gestation. Additionally, (B) illustrates trends in the abdominal aortic pulse pressure across gestational age, and (C) demonstrates variations in systolic and diastolic pressures of the LV across different gestational ages. In comparison, literature values representing clinically measured abdominal aortic pulse pressure by Versmold et al. (148), and LV pressures by Johnson et al. are plotted as well (147), affirming the strong concordance between the lumped parameter model and these literature values.

Two-way coupling between the CFD model and the LPM was established by incorporating the LPM as an ANSYS user-defined function. At each time step, flow was simulated in the LV and the flow rates at the open valve orifices were used by the LPM to predict pressures at the ascending aorta and the LA. These pressures then served as surface-averaged pressure boundary conditions at the valve orifices in the CFD model at the next time step.

The configuration of the LPM around the LV CFD model is altered according to systole and diastole of the cardiac cycle. The LPM circuit around the LV CFD model is



described further in Figure 18. During diastole (Figure 18A), where flow moves from the LA into the LV, the LV-AA connection is removed to simulate aortic valve closure. Likewise, during systole (Figure 18B), where flow moves from the LV into the ascending aorta (AA), the LA-LV connection is removed to simulate MV closure. However, in CAS-eHLHS cases

with MV regurgitation (Figure 18C, both the LA-LV and LV-AA connections remain active during systole, allowing a portion of the LV outflow to regurgitate into the LA. In this case, the valvular dissipative coefficient between the LA and the LV, K_{LA-LV} , remains active to model the energy loss associated with regurgitant flow across the MV.

3.1.5 Quantifying Energy Dynamics and Wall Shear Stress Parameters

Energy Dynamics of the LV was investigated in the form of the normalised haemodynamic energy loss across time, ϵ . This was calculated as:

$$\epsilon = \int_{t_{start}}^{t_{end}} \frac{Wd(t) + KE_{bulk} + KE_{MV} + KE_{AV} (+KE_{MV Regurgitation})}{SV} dt \quad (13)$$

where t_{start} is the starting time and t_{end} is the end time under consideration. In the expression, the work performed by the ventricle wall on the blood is denoted as $Wd(t)$, is defined by the expression:

$$Wd(t) = \int_{CS(t)} P (\vec{v} \cdot \hat{n}) dA \quad (14)$$

where P is the fluid pressure, $(\vec{v} \cdot \hat{n})$ is the dot product of the velocity vector with the surface normal, $CS(t)$ is the moving control surface (endocardium) and A is the surface area of the control surface. KE_{bulk} is the change in kinetic energy of the fluid in the ventricle, expressed as:

$$KE_{bulk} = \frac{d}{dt} \left(\int_{CV(t)} \frac{1}{2} \rho |\vec{v}(t)|^2 dV \right) \quad (15)$$

with CV representing the control volume, ρ is the blood density, $|\vec{v}(t)|$ is the absolute value of the velocity vector and V is the control volume.

Moreover, the kinetic energy associated with the flow through the MV, AV or MV regurgitation orifice (if applicable) is expressed as:

$$KE_x = \int_{CS(t)} \frac{1}{2} \rho |\vec{v}(t)|^2 (\vec{v} \cdot \hat{n}) dA \quad , \quad x \in \{MV, AV, MV regurgitation\} \quad (16)$$

where this formulation characterises the kinetic energy for each specific component, and x takes values from the set $x \in \{MV, AV, MV regurgitation\}$.

Wall shear stress vector ($\overrightarrow{WSS}$) was calculated as:

$$\tau = \mu(\nabla \vec{v} + (\nabla \vec{v})^T) \quad (17)$$

$$\overrightarrow{WSS} = \tau - (\tau \cdot \hat{n})\hat{n} \quad (18)$$

where τ is the viscous stress tensor at the wall, $(\nabla \vec{v})$ is the gradient velocity tensor and $\hat{n}$ is the unit surface normal vector. A few wall WSS metrics were derived, namely the time- and surface-averaged wall shear stress (Time- & Surface-Averaged WSS), and maps of the time-averaged wall shear stress (TAWSS) and oscillatory shear index (OSI) were generated (151), given as:

$$Time \& Surface - Ave. WSS = \left(\frac{1}{t_{end} - t_{start}} \right) \left(\frac{1}{A_{wall}} \right) \int_{t_{start}}^{t_{end}} \int_{wall} \overrightarrow{WSS} dA dt \quad (19)$$

$$TAWSS = \frac{1}{T} \int_0^T |\overrightarrow{WSS}| dt \quad (20)$$

$$OSI = \frac{1}{2} \left(1 - \frac{\left| \int_0^T \overrightarrow{WSS} dt \right|}{\int_0^T |\overrightarrow{WSS}| dt} \right) \quad (21)$$

where T is the period of the cardiac cycle and A_{wall} is the area of the wall surface boundary.

The λ_2 vortex criterion was used to identify regions where vortices are likely to occur within the LV using the velocity gradient tensor. λ_2 is defined by:

$$\lambda_2 = \mathbf{S}^2 + \mathbf{\Omega}^2 \quad (19)$$

$$\mathbf{S} = \frac{\nabla v + (\nabla v)^T}{2} \quad (20)$$

$$\mathbf{\Omega} = \frac{\nabla v - (\nabla v)^T}{2} \quad (21)$$

where $\mathbf{S}$ and $\mathbf{\Omega}$ are the symmetric and antisymmetric strain rates, respectively. At each point in the velocity field, three eigenvalues, λ_1, λ_2 and λ_3 of $\mathbf{S}^2 + \mathbf{\Omega}^2$ were calculated and ordered in a way such that $\lambda_1 \geq \lambda_2 \geq \lambda_3$. Points with a negative λ_2 value is used as part of a vortex core.

3.1.6 Blood Turnover and Washout Efficiency

To assess blood turnover dynamics within the LV chamber, simulations utilising two distinct fluids: the pre-existing blood within the chamber and fresh blood introduced through the mitral inflow, were conducted. The introduction of fresh blood was marked using a dye in mass-fraction simulations, providing a comprehensive evaluation of blood turnover within the LV.

In these simulations, the dye used to identify fresh blood was characterised by a low diffusivity coefficient of $10^{-8} \text{ m}^2/\text{s}$. The decision to use a low diffusivity coefficient for the dye was intended to match the anticipated convection-dominated transport conditions within the LV chamber. The simulations served as a means to assess the turnover of blood and, consequently, oxygen within the LV chamber.

3.1.7 Diastolic Vortex Formation Time

To further characterize diastolic vorticity, the formation time (τ^*) of the mitral inflow vortex rings was calculated as,

$$\tau^* = \overline{U/D} * t \quad (22)$$

where U was the mitral inflow velocity, D was the jet diameter, t was the duration of either the E or A wave, whichever was higher, or the entire diastolic duration for monophasic flow, and $\overline{U/D}$ was the time-averaged ratio between U and D (152).

3.1.8 Statistical Analysis

The normality of continuous variables was assessed using the Anderson-Darling test. Subsequently, a paired one-tailed t-test was applied for comparing continuous data between the healthy fetal cohort and the CAS-eHLHS cohort, using a t-test for independent samples when the distribution was normal, while the Mann-Whitney U-test was used otherwise. Statistical significance was determined at $P < 0.05$.

3.2 Results: Comparisons between CAS-eHLHS and Healthy LVs

3.2.1 Physiological Features

A comparison between CFD-simulated peak velocities and Doppler-derived velocity tracings is demonstrated for a healthy case and a CAS-eHLHS case in Figure 19. The peak local valve velocities obtained from the simulations were effectively calibrated to closely match the Doppler velocity measurements at the valves. This is highlighted in Tables 10 and 11. The velocity discrepancies were kept as low as possible, with an absolute difference of 0.050 ± 0.046 m/s, translating to a percentage error of $5.7\% \pm 4.7\%$. Any errors could be due to ultrasound noise affecting motion tracking.

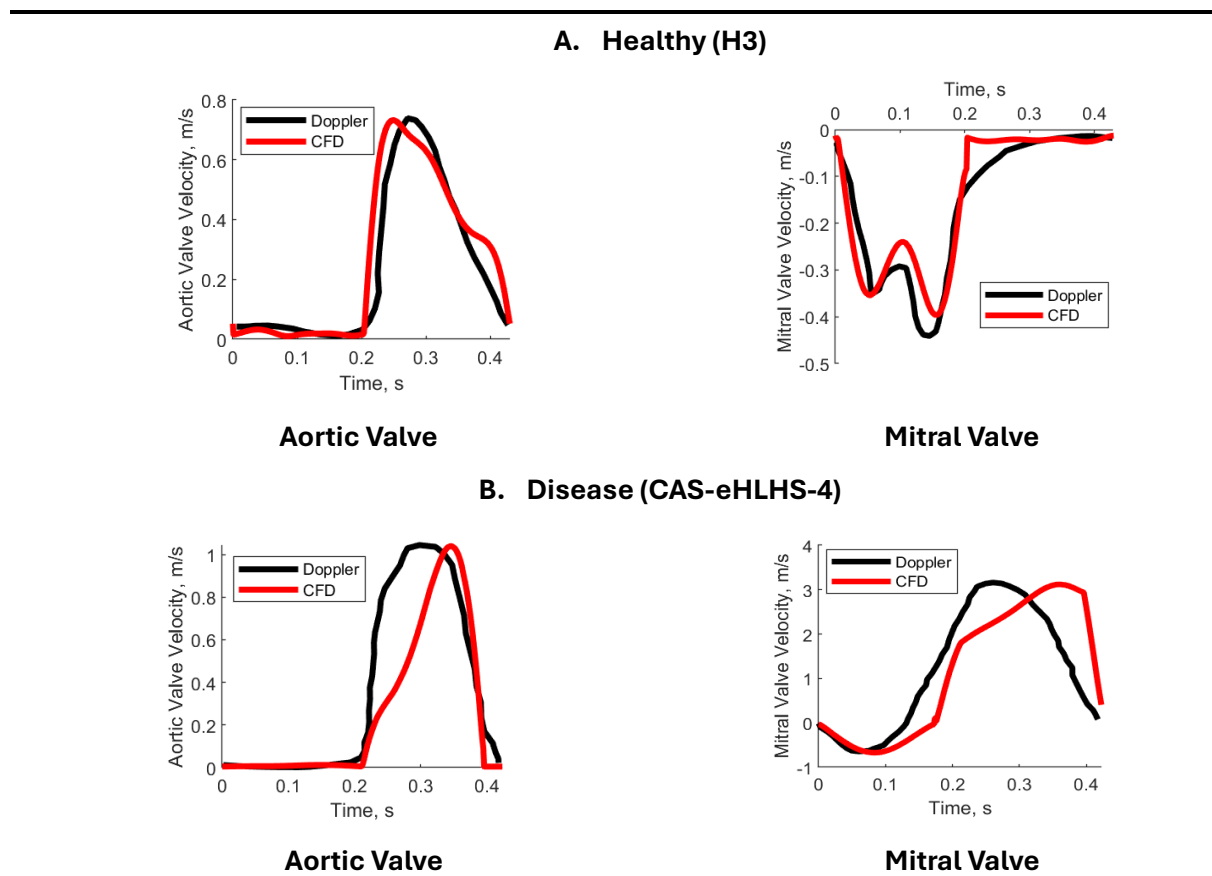


Figure 19: Comparison of CFD-simulated maximum valve velocities with Doppler-derived velocities at the aortic valve (left) and mitral valve (right) across the cardiac cycle. Comparisons are shown for a (A) healthy case, H3 and a (B) disease case, CAS-eHLHS-4. Positive velocities represent outflow from the ventricle, while negative velocities reflect flow into the ventricle.

TABLE 10: CASE-SPECIFIC COMPARISON BETWEEN COMPUTATIONAL FLUID DYNAMICS SIMULATION RESULT AND CLINICAL MEASUREMENTS OF VALVE VELOCITIES FOR HEALTHY COHORT.

Parameter	Healthy Cohort									
	H1 (21 wk)		H2 (31 wk)		H3 (28 wk)		H4 (31 wk)		H5 (21 wk)	
	CFD	Dop.	CFD	Dop.	CFD	Dop.	CFD	Dop.	CFD	Dop.
Peak AV, m/s	0.65	0.68	0.82	0.80	0.77	0.80	0.91	0.82	0.65	0.65
Diastolic E, m/s	-	-	0.40	0.40	0.46	0.40	0.59	0.60	0.30	0.30
Diastolic A, m/s	0.41	0.45	0.46	0.55	0.47	0.45	0.35	0.38	0.48	0.45

Note: AV – aortic valve, CFD – Computational fluid dynamics, Dop. - Doppler

TABLE 11: CASE-SPECIFIC COMPARISON BETWEEN COMPUTATIONAL FLUID DYNAMICS SIMULATION RESULT AND CLINICAL MEASUREMENTS OF VALVE VELOCITIES FOR CAS-EHLHS COHORT.

Parameter	Disease Cohort													
	CAS-eHLHS-1 (22.14wk)		CAS-eHLHS-2 (22.57wk)		CAS-eHLHS-3 (29.14wk)		CAS-eHLHS-4 (29wk)		CAS-eHLHS-5 (24.86wk)		CAS-eHLHS-6 (30.14wk)		CAS-eHLHS-7 (23.43wk)	
	CFD	Dop.	CFD	Dop.	CFD	Dop.	CFD	Dop.	CFD	Dop.	CFD	Dop.	CFD	Dop.
Peak AV, m/s	1.55	1.65	1.80	1.84	2.38	2.44	1.16	1.10	3.44	3.50	1.27	1.33	1.33	1.35
Diastolic E, m/s	0.77	0.83	-	-	-	-	-	-	-	-	-	-	0.43	0.50
Diastolic A, m/s	0.49	0.53	0.38	0.35	1.05	1.11	0.70	0.80	0.71	0.83	0.55	0.63	0.58	0.60
MV Regurgitation, m/s	2.70	2.60	3.04	3.00	3.15	3.20	3.12	3.10	4.22	4.20	3.76	3.50	2.39	2.30

Note: AV – aortic valve, MV – mitral valve, CFD – Computational fluid dynamics, Dop. – Doppler, CAS-eHLHS – Critical aortic stenosis and evolving hypoplastic left heart syndrome

When comparing the left heart parameters between the CAS-eHLHS cases and the healthy control group (shown in Table 12), significant differences were observed. In the CAS-eHLHS cohort, AV velocities were notably elevated, surpassing the 95th percentile of normal LVs, with some cases exhibiting velocities more than double this threshold. Similarly, diastolic E-or A-wave velocities were above the 95th percentile for normal LVs, except in the case of CAS-eHLHS-2. Notably, all but one CAS-eHLHS case exhibited monophasic MV inflow, resulting in the absence of recorded E-wave velocities. Severe MV regurgitation was evident, with peak regurgitation velocities at 3.13 ± 0.62 m/s.

These elevated velocities led to significantly increased transmitral pressure gradients, ranging from 21 to 71 mmHg, as calculated using MV regurgitation velocity via the simplified Bernoulli's equation. The elevated pressure likely contributed to the enlargement in LV end-diastolic volume. LV stroke volume was reduced to varying degrees, and given the enlarged LV, the EF was significantly low. The RV of a few CAS-eHLHS cases had moderate enlargement and elevated stroke volumes when compared

TABLE 12. COMPARISON OF CARDIAC PARAMETERS BETWEEN HEALTHY FETUSES AND CRITICAL AORTIC STENOSIS AND EVOLVING HYPOPLASTIC LEFT HEART SYNDROME FETUSES

<i>Parameter</i>	<i>Healthy Cohort</i>			<i>Disease Cohort</i>							<i>Overall Mean</i>	<i>P value</i>
	21-22 wk (n = 6)	28-31 wk (n = 6)	Overall Mean	CAS- eHLHS-1 22.14wk	CAS- eHLHS-2 22.57wk	CAS- eHLHS-3 29.14wk	CAS- eHLHS-4 29wk	CAS- eHLHS-5 24.86wk	CAS- eHLHS-6 30.14wk	CAS- eHLHS-7 23.43wk		
Peak AV, m/s	0.66 ± 0.09 [0.57, 0.75]	0.83 ± 0.12 [0.71, 0.96]	0.74 ± 0.14	1.65	1.84	2.44	1.10	3.50	1.33	1.35	1.89 ± 0.84	0.005*
Diastolic E, m/s	0.31 ± 0.08 [0.21, 0.40]	0.40 ± 0.09 [0.31, 0.49]	0.33 ± 0.14	0.83	-	-	-	-	-	-	-	-
Diastolic A, m/s	0.37 ± 0.06 [0.31, 0.44]	0.45 ± 0.09 [0.35, 0.55]	0.41 ± 0.09	0.53	0.35	1.11	0.80	0.83	0.63	0.60	0.69 ± 0.25	0.02*
Diastolic E after intervention, m/s	-	-	-	0.60	-	0.80	0.50	0.60	0.51	No Inflow	0.60 ± 0.12	0.17
Diastolic A after intervention, m/s	-	-	-	0.30	0.55	1.50	0.60	0.87	0.55	No Inflow	0.73 ± 0.42	0.09
MV Regurgitation, m/s	-	-	-	2.60	3.00	3.20	3.10	4.20	3.50	2.30	3.13 ± 0.62	-
RV SV, ml	0.59 ± 0.12 [0.46, 0.71]	1.67 ± 0.58 [1.07, 2.27]	1.13 ± 0.57	0.73	1.09	1.56	2.30	0.82	2.34	0.73	1.37 ± 0.71	0.19
LV SV, ml	0.53 ± 0.16 [0.37, 0.70]	1.92 ± 0.84 [0.88, 2.96]	1.16 ± 0.90	0.17	0.14	1.23	0.68	0.64	0.84	0.11	0.54 ± 0.42	0.05
LV SV after intervention, ml	-	-	-	0.50	0.16	1.94	1.22	0.94	1.48	0.11	0.91 ± 0.70	0.35
RV EDV, ml	1.02 ± 0.19 [0.82, 1.22]	3.92 ± 1.14 [2.72, 5.11]	2.47 ± 1.29	1.30	2.91	3.16	5.29	1.84	6.13	2.20	3.26 ± 1.80	0.002*
LV EDV, ml	0.90 ± 0.21 [0.68, 1.13]	3.62 ± 1.14 [2.20, 5.03]	2.14 ± 0.16	2.46	2.99	17.42	11.09	2.23	6.68	2.40	6.47 ± 5.83	0.009*
LV EF, %	59 ± 10 [49, 69]	53 ± 15 [37, 69]	56.2 ± 12.0	6.80	4.63	7.06	6.25	28.7	12.6	5.0	10.1 ± 8.6	0.001*

***P<0.05 comparing CAS-eHLHS cases to healthy cases. AV - aortic valve; MV – mitral valve; RV - right ventricle; SV - stroke volume; LV - left ventricle; EDV - end-diastolic volume; EF - ejection fraction; Regurg. - regurgitation. Data are presented as mean ± SD and [5th percentile, 95th percentile]. For a healthy cohort, data from the current study were combined with those from Hadi et al. (134).**

to the appropriate age-matched controls. Overall, there is substantial variability in the characteristics of each CAS-eHLHS case owing to different severity, but ultimately shows compromised LV physiology and functionality, similar to previous clinical reports.

3.2.2 Diastolic Flow Patterns

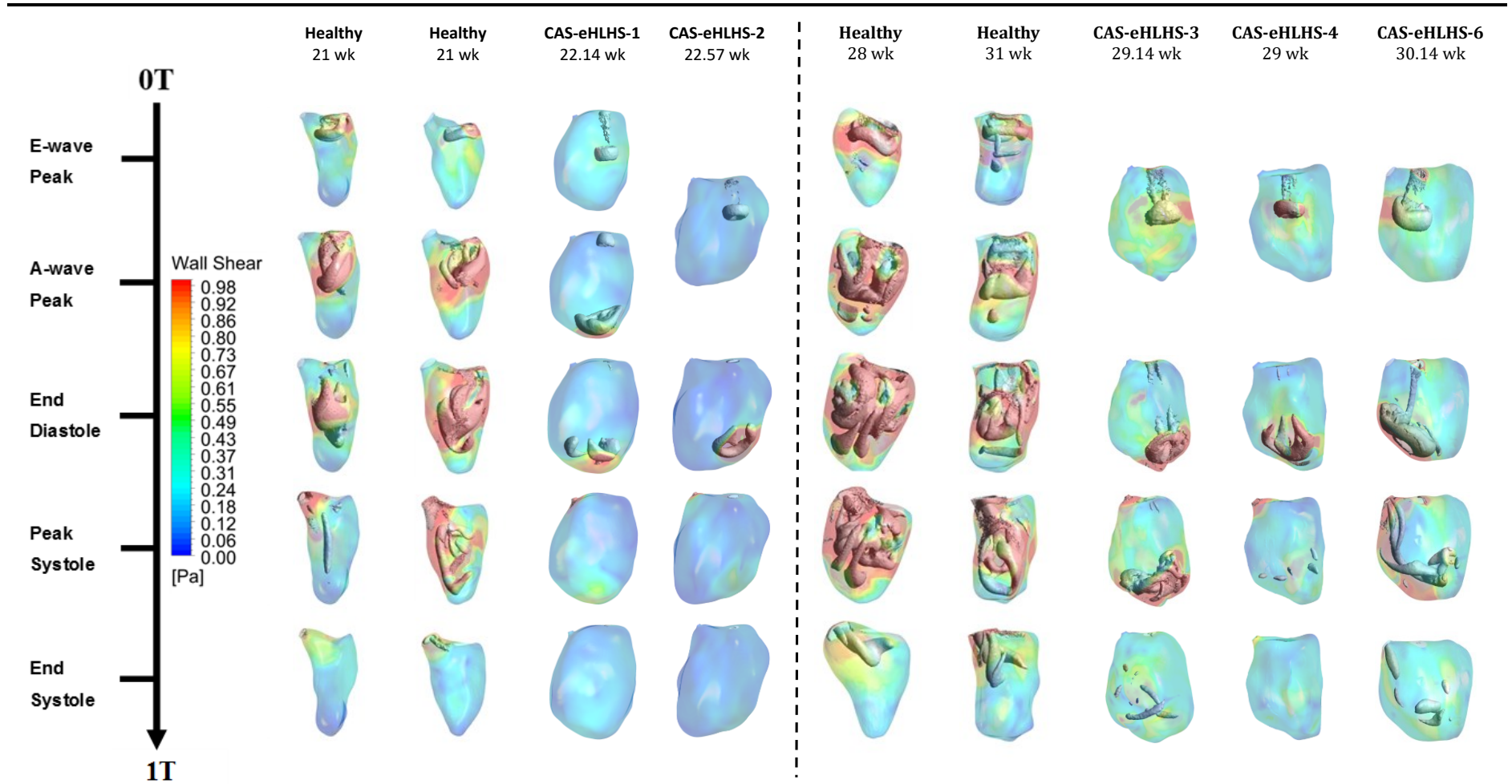


Figure 20: Wall shear stress (WSS) colour maps and λ_2 iso-velocity surfaces of representative healthy fetal LVs and age-matched CAS-eHLHS LVs.

The flow patterns within CAS-eHLHS fetal LVs, as generated from the CFD simulations, are illustrated in Figure A1 in the Appendix with age-matched comparisons in Figure 20. The vortex dynamics observed in healthy fetal LVs were consistent with prior descriptions (133), where broad diastolic vortex rings associated with E- and A-wave flows were seen propagating from the MV towards the apex. These vortex rings spanned the entire LV width and advanced to varying degrees towards the apex. Along their path, the rings interacted with the endocardial walls, generating elevated WSS and forming complex secondary vorticity structures, which persisted and were ejected during the subsequent systolic phase. All simulated healthy cases are presented in Figure A2.

In contrast, flow patterns in CAS-eHLHS cases were markedly different. The MV inflow in these cases was characterized by a narrow, high-velocity jet that generated a small-diameter vortex ring, quickly propagating towards and impacting the apex. The simulations necessitated a narrowed mitral inlet orifice to match the elevated MV inflow velocities observed in Doppler measurements, suggesting mild MV stenosis. Despite the narrow inflow, the MV annulus dimensions were not reduced (Table 13), indicating that the MV itself was not hypoplastic, but that the stenosis likely resulted from an impaired ability of the valve to open fully. After FAV, while stroke volume increased, peak MV inflow velocities remained elevated in some cases, exceeding the 95th percentile for normal hearts (as presented in Table 12). This persistent elevation in MV inflow velocity implies that the stenosis may be due to a structurally abnormal valve rather than solely due to LV congestion impeding proper valve motion.

TABLE 13: CASE-SPECIFIC MITRAL VALVE PARAMETERS OF HEALTHY LVs AND CAS-eHLHS LVs

Parameter	Healthy	Disease							Overall Mean	P-value
	Overall Mean (n = 5)	CAS-eHLHS-1 22.14 wk	CAS-eHLHS-2 22.57 wk	CAS-eHLHS-3 29.14 wk	CAS-eHLHS-4 29 wk	CAS-eHLHS-5 24.86 wk	CAS-eHLHS-6 30.14 wk	CAS-eHLHS-7 23.43 wk		
MV annulus (mm)	8.2 ± 1.8	7.8	5.7	13.5	9.5	8.1	11.4	7.5	10.0 ± 2.8	0.26
MV annulus (z-score)	0.37 ± 0.50	1.48	-0.96	3.35	0.67	0.79	1.67	0.74	1.1 ± 1.3	0.10
MV orifice area (mm ²)	32.9 ± 21.0	3.6	2.3	16.6	9.8	7.4	20.4	1.6	8.8 ± 7.3	0.03*
MV orifice area/MV annulus (mm)	4.14 ± 1.70	0.46	0.40	1.23	1.03	0.91	1.79	0.21	0.86 ± 0.55	<0.01*

Note: **P*<0.05 comparing all healthy cases to diseased cases. MV - mitral valve, CAS-eHLHS – critical aortic stenosis and evolving hypoplastic left heart syndrome. Z-scores are derived from Schneider et al. (69). The overall mean is presented as mean ± SD.

In CAS-eHLHS LVs, the narrow inflow jet and chamber dilation resulted in minimal interaction between the MV inflow vortex rings and the endocardial walls until they impinged on the apex, generating secondary vortices and elevated WSS at the site of impingement. The strength and complexity of these vortices, along with WSS magnitude, were influenced by both EF and inflow velocity. From Figure A1, CAS-eHLHS-1, -2, and -7, with lower EFs, displayed weaker vortices, while CAS-eHLHS-5, with a higher EF, exhibited chaotic vortices and elevated WSS across a larger area of the LV. This chaotic flow in CAS-eHLHS-5 was largely due to its stronger EF and relatively normal LV end-diastolic volume, which allowed for greater interaction between the vortex ring and the walls. Additionally, higher inflow velocities intensified vortex interactions, as seen in CAS-eHLHS-3, where despite having a similar EF to CAS-eHLHS-1, the greater velocity led to stronger and more persistent vortical structures that persisted through systole.

Unlike healthy fetal hearts, which typically exhibit biphasic inflow, the CAS-eHLHS LVs generally displayed monophasic inflow patterns, a common feature in this condition (2). Only CAS-HLHS-1 and -7 exhibited a clear biphasic mitral inflow profile, which typically suggests better diastolic ventricular function. This biphasic pattern resulted in two sequential inflow vortex rings. However, despite this difference, these rings did not lead to any significant large-scale secondary vortices, making their flow patterns resemble those with a monophasic inflow profile. Nonetheless, this variation in inflow pattern contributed to the differences in vorticity complexity observed among the diseased subjects. Overall, while CAS-eHLHS LVs exhibited distinct flow characteristics compared to healthy LVs, significant variability was also observed within the diseased group.

3.2.3 LV Energy Dynamics

The results for flow stresses and energy dynamics from the CFD simulations are presented in Table 14. The systolic IVPG was defined as the maximum pressure difference between the apical region of the LV and the aortic outflow boundary during systole. Similarly, the diastolic IVPG was calculated as the pressure difference between the apical region and the mitral inlet during diastole. As expected, the systolic IVPG was

TABLE 14: COMPARISON BETWEEN HEALTHY LVs AND CAS-EHLHS LVs DERIVED FROM CFD SIMULATIONS

Parameter	Healthy			Disease								P - value
	21-22 wk (n = 6)	28-31wk (n = 6)	Overall Mean	CAS- eHLHS-1 22.14wk	CAS- eHLHS-2 22.57wk	CAS- eHLHS-3 29.14wk	CAS- eHLHS-4 29wk	CAS- eHLHS-5 24.86wk	CAS- eHLHS-6 30.14wk	CAS- eHLHS-7 23.43wk	Overall Mean	
Systolic IVPG (Pa)	194 ± 58 [133, 255]	292 ± 91 [197, 387]	242 ± 89	1211	1607	2936	697	5884	831	932	2014 ± 1868	0.003*
Diastolic IVPG (Pa)	32 ± 11 [21, 44]	51 ± 32 [17, 85]	41 ± 25	91	22	211	105	194	305	42	139 ± 102	0.20
Normalised Systolic Ejection Work Done (J/m ³)	123 ± 33 [88, 157]	204 ± 74 [126, 282]	164 ± 69	2255	2846	2765	2534	4952	1800	1920	2725 ± 1060	<0.001 *
Normalised Systolic Energy Loss (J/m ³)	22 ± 6 [15, 29]	26 ± 7 [19, 32]	22 ± 8	132	163	214	161	256	119	138	169 ± 49	<0.001 *
Normalised Diastolic Energy Loss (J/m ³)	16 ± 9 [6, 26]	22 ± 10 [12, 32]	17 ± 10	114	34	167	98	156	78	100	107 ± 45	0.003*
Time- & Surface-Ave WSS (Pa)	0.68 ± 0.19 [0.48, 0.87]	0.91 ± 0.27 [0.63, 1.19]	0.77 ± 0.26	0.25	0.15	0.56	0.39	1.02	0.52	0.19	0.44 ± 0.30	0.01*
Diastolic Peak Surface-Ave WSS (Pa)	0.81 ± 0.29 [0.50, 1.12]	1.11 ± 0.39 [0.71, 1.51]	0.90 ± 0.38	0.51	0.18	0.90	0.56	2.17	1.00	0.33	0.81 ± 0.67	0.19
Diastolic Time- & Surface-Ave WSS (Pa)	0.54 ± 0.16 [0.38, 0.71]	0.81 ± 0.25 [0.55, 1.07]	0.51 ± 0.36	0.27	0.14	0.46	0.38	1.21	0.42	0.20	0.44 ± 0.36	0.04*
Systolic Peak Surface-ave WSS (Pa)	1.41 ± 0.31 [1.08, 1.74]	1.69 ± 0.29 [1.39, 2.00]	1.52 ± 0.36	0.31	0.23	1.03	0.49	1.37	1.00	0.24	0.67 ± 0.46	0.007*
Systolic Time- & Surface-Ave WSS (Pa)	0.85 ± 0.17 [0.67, 1.03]	1.05 ± 0.20 [0.84, 1.26]	0.93 ± 0.22	0.23	0.17	0.62	0.40	0.83	0.62	0.17	0.43 ± 0.26	0.002*

Note: *P<0.05 comparing all healthy to all diseased cases. IVPG indicates intraventricular pressure gradient; Ave, averaged; WSS, wall shear stress. Data are presented as mean ± SD and [5th percentile, 95th percentile]. ‡ Data from healthy patient cases combined with Hadi et al. (134).

significantly elevated in CAS-eHLHS LVs compared to normal hearts, primarily due to the outflow obstruction. Diastolic IVPG was also higher in all cases except for CAS-eHLHS-2, which had a lower inflow IVPG due to relatively normal MV inflow velocity.

Additionally, there was a marked increase in both systolic and diastolic haemodynamic energy losses, as well as in systolic fluid-mechanical work done, across all CAS-eHLHS cases. The presence of AV stenosis led to increased AV velocities and a narrowed outflow channel, resulting in heightened fluid stresses and significant energy losses within the flow convergence zone at the outlet. As a result, a significant portion of the pressure generated by the LV was lost within the fluid domain, rather than being effectively transmitted to the systemic circulation. Diastolic energy losses were also elevated, likely driven by increased inflow velocities and the concentration of fluid forces at the point of impingement, leading to enhanced viscous dissipation. Overall, the CAS-

eHLHS cohort exhibited significantly elevated pressure gradients, haemodynamic energy losses, and ventricular workload on the blood.

3.2.4 LV Wall Shear Stress

Figure 21 compares the spatial distributions of TAWSS and OSI for the healthy LVs with age-matched CAS-eHLHS cases. In healthy LVs, WSS was typically elevated in the mid-ventricular region and near the outlet, with lower levels near the apex, consistent with previous findings (133, 134). The increase in WSS at the mid-ventricle likely resulted from the significant interaction between diastolic vortex rings and the endocardial walls in this area, while the outlet region saw elevated WSS due to systolic flow convergence, where velocities were heightened within the narrowed flow channel. These regions of high WSS were also characterized by low OSI, indicating a consistent flow direction at these walls.

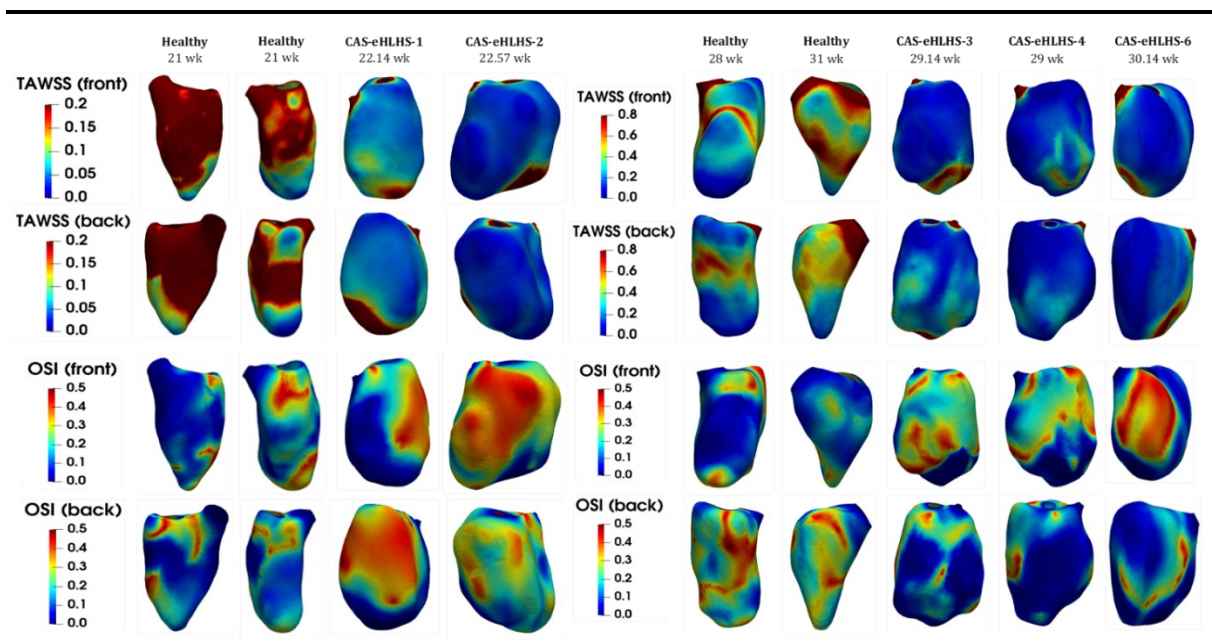


Figure 21: Comparison of Time-Averaged Wall Shear Stress (TAWSS) and Oscillatory Shear Stress (OSI) distribution on the left ventricle endocardial surface between healthy representative cases and age-matched critical aortic stenosis and evolving hypoplastic left heart syndrome (CAS-eHLHS) cases. The rows depict the front and back views of TAWSS and OSI.

In the CAS-eHLHS cohort, similar patterns of high WSS and low OSI were noted at the outlet, likely due to systolic flow convergence. However, unlike in healthy LVs, WSS was generally elevated not in the mid-ventricular region, but at the site of diastolic inflow jet impingement and its vicinity, near the apex. While OSI remained low at the impingement site, it was generally higher in other regions of the LV. The WSS parameters in Table 14 demonstrate significant differences

between healthy LVs and CAS-eHLHS LVs. The diseased cohort generally shows reduced WSS across most parameters, because of narrower-spreading vortex rings compared to healthy LVs.

The results in Table 14 show that the CAS-eHLHS cohort generally exhibits lower WSS values compared to the healthy cohort across various parameters, including time- and surface-averaged WSS during both diastole and systole. Systolic peak WSS was notably reduced in the CAS-eHLHS cases, though this trend was not as evident for diastolic peak WSS. Higher LV EFs and MV inflow velocities appear to correlate with increased WSS. In particular, CAS-eHLHS-5 exhibited relatively normal, if not elevated, WSS levels compared to healthy cases, likely due to its relatively better LV EF. Additionally, a comparison between CAS-eHLHS-1 and CAS-eHLHS-3 reveals that although they have similar EFs, CAS-eHLHS-3 has significantly higher WSS metrics—more than double for some metrics—due to the MV inflow velocity. Overall, there is notable WSS variability within the disease cohort, reflecting the heterogeneity of the condition.

3.2.5 Turnover of Fluid Within LV

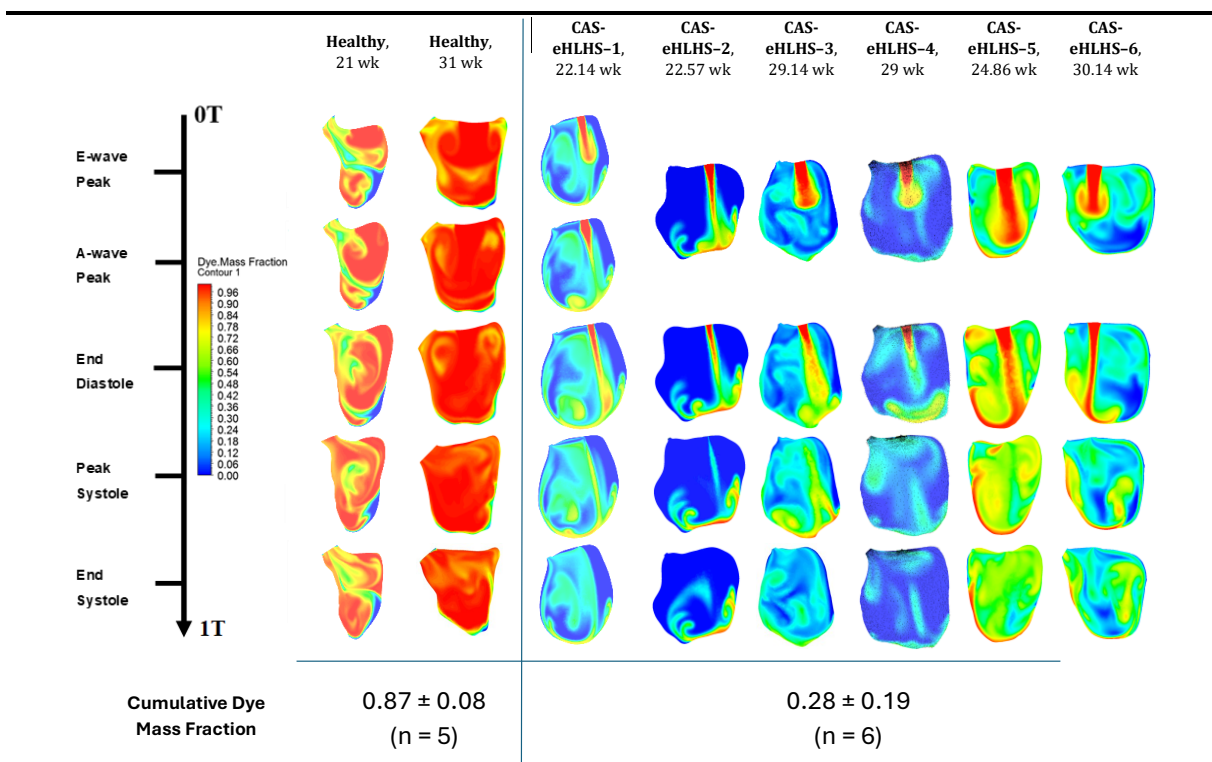


Figure 22: In-plane contour maps of passive dye mass fraction across the cardiac cycle after three cardiac cycles for two representative healthy left ventricles at different ages and six critical aortic stenosis and evolving hypoplastic left heart syndrome (CAS-eHLHS) LVs. A mass fraction of 1 (red) indicates the presence of ‘fresh’ blood entering through the mitral valve, while a mass fraction of 0 (blue) represents ‘old’ blood initially present in the ventricle.

Figure 22 presents the colour contours of the blood turnover mass fraction, representing the ratio of newly introduced blood from the MV to the blood initially present in the LV, after three cardiac cycles. Areas in red indicate a high proportion of fresh blood, while blue areas indicate a low proportion. The turnover of blood in the LVs of CAS-eHLHS cases was significantly lower than in healthy LVs, with the volume-averaged cumulative dye mass fraction in healthy LVs being nearly three times greater than that in CAS-eHLHS LVs. In healthy LVs, turnover was lowest at the apex, whereas, in CAS-eHLHS LVs, the highest turnover occurred at the apex, driven by the high-velocity inflow jet that rapidly directed incoming fluid towards the apex. Additionally, turnover appeared to be influenced by EF, as shown in the regression analysis in Figure 23.

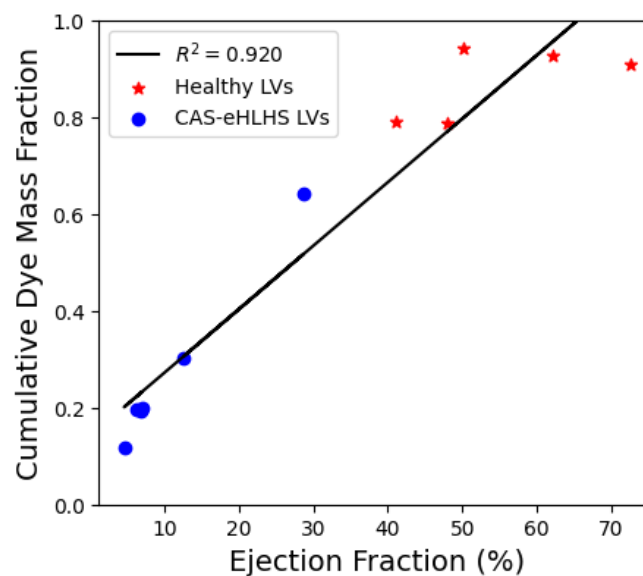


Figure 23: Scatterplots evaluating the volume-averaged cumulative dye mass fraction after three cardiac cycles versus left ventricle ejection fraction (%).

3.2.6 Diastolic Vortex Formation Time

TABLE 15: CASE-SPECIFIC VORTEX FORMATION TIMES OBTAINED FROM CFD SIMULATIONS

Parameter	Healthy					Disease							P - value
	H1 21 wk	H2 31 wk	H3 28 wk	H4 31 wk	H5 21 wk	CAS- eHLHS-1 22.14 wk	CAS- eHLHS-2 22.57 wk	CAS- eHLHS-3 29.14 wk	CAS- eHLHS-4 29 wk	CAS- eHLHS-5 24.86 wk	CAS- eHLHS-6 30.14 wk	CAS- eHLHS-7 23.43 wk	
Formation time	9.2	4.9	3.5	3.8	3.5	39.6	25.9	18.8	22.0	34.7	13.0	45.9	<0.001

Table 15 shows the vortex formation time for all simulation cases, where vortex formation time is significantly higher compared to the healthy cohort, owing to higher MV

inflow velocities and smaller MV orifice sizes. H1 healthy case had slightly higher vortex formation times due to its monophasic inflow, which is typically not seen in healthy fetuses.

3.3 Discussion

Image-based flow simulations were conducted in the LVs of fetuses with CAS-eHLHS to explore key differences in fluid dynamics when compared to healthy LVs. The study's disease cohort consisted of patients who met the criteria for FAV and subsequently underwent the procedure. Within this patient cohort, variability in birth outcomes following FAV was observed, with CAS-eHLHS-1, -2, and -7 having UV circulation. Despite being subjected to the procedural risks of FAV, these fetuses did not respond as hoped.

The analysis demonstrated considerable variability in flow patterns and forces within the disease cohort, though certain consistent features were observed. A notable characteristic in the dilated CAS-eHLHS LVs was the presence of a narrow, high-velocity inflow jet that moved from the MV to the apical region, resulting in heightened vortex dynamics at the apex while the basal region remained relatively undisturbed. Conversely, in healthy LVs, the MV inflow vortices were slower, enveloping across the width of the LV as it propagated towards the apex. This led to greater interaction with the mid-ventricular endocardial walls, resulting in elevated vorticity dynamics at the mid-ventricular and basal regions, with more stable flow conditions at the apex.

The elevated MV inflow velocity was unexpected, especially considering that the high LV pressure in the CAS-eHLHS cohort would typically hinder MV inflow, and the reduced EF would generally be associated with lower velocities. The analysis suggests that this elevated inflow velocity was not due to a hypoplastic MV annulus but likely due to compromised MV opening dynamics, as evidenced by a small inflow jet diameter despite a normal-sized MV annulus. Despite undergoing FAV, these velocities did not significantly improve, indicating that the MV was likely structurally abnormal which is not unusual when considering previous reports of EFE associated with CAS-eHLHS cohort (87). Post-mortem examinations revealed thickening and stiffening of MV leaflets, which limits their ability to open effectively (87). Signs of mild to severe EFE were observed in a

few of the CAS-eHLHS cases. This was particularly notable in CAS-eHLHS-3, where the highest diastolic inflow velocities were also observed.

Given the observed abnormalities in MV inflow, the formation time of diastolic inflow vortex rings was calculated (Table 15), as this has been proposed to be capable of classifying abnormal LV fluid dynamics (153). The vortex formation times in healthy LVs were similar to those reported in healthy adult hearts, which typically range between 2 and 7 (154). This finding suggests that vortex characteristics are consistent between pre- and post-natal LVs. In contrast, CAS-eHLHS cases exhibited significantly longer formation times, indicating that diastolic vortex generation in CAS-eHLHS LVs was less energy-efficient, aligning with the observed higher diastolic energy losses.

Another notable characteristic of CAS-eHLHS LVs was the presence of elevated IVPGs and substantial haemodynamic energy losses across both the mitral inflow and aortic outflow tracts. The energy losses associated with aortic outflow, calculated per unit volume of ejected blood, were nearly an order of magnitude higher than normal. This is consistent with the severity of the outflow tract obstruction. From a fluid-mechanical standpoint, this reflects a pressure-dominant regime in which substantial energy is required to overcome the valvular obstruction, but only a small portion is converted into effective forward flow. The remainder is dissipated through elevated viscous losses.

Another notable characteristic of CAS-eHLHS LVs was the presence of elevated IVPGs and substantial haemodynamic energy losses across both the mitral inflow and aortic outflow tracts. The energy losses associated with aortic outflow, calculated per unit volume of ejected blood, were nearly an order of magnitude higher than normal. This is consistent with the severity of the outflow tract obstruction. From a fluid-mechanical standpoint, this reflects a pressure-dominant regime in which considerable energy is required to overcome the valvular obstruction, but only a small portion is converted into effective forward flow. The remainder is dissipated through elevated viscous losses. While these metrics strictly characterise energy transfer from the LV to the blood and not myocardial oxygen consumption, they nevertheless signal an inefficient hydraulic environment that likely imposes additional mechanical burden on the LV and may indirectly affect perfusion and growth capacity.

The third notable feature in CAS-eHLHS LVs was a significant reduction in blood turnover, with the renewal of blood within the LV occurring approximately 60% more slowly on average. This reflects impaired washout, prolonged blood residence and inefficient intracavitary flow. When considered in conjunction with the elevated myocardial energy demands described earlier, such stagnation may limit coronary perfusion and exacerbate the mismatch between oxygen supply and demand in the myocardium. Although oxygen content remains unchanged as blood transits the LV, inefficient forward flow may reduce perfusion pressure in the coronary circulation. Evidence of compromised oxygen delivery has already been observed in downstream tissues, such as the fetal brain. McElhinney et al. (2010) reported that CAS-eHLHS fetuses exhibit abnormal cerebral blood flow profiles, including reduced pulsatility and resistive indices in the middle cerebral artery (MCA) (155). These findings are consistent with chronic cerebral vasodilation, a compensatory response to fetal hypoxia.

This potential for myocardial hypoxia is particularly concerning given its role in activating pathological signalling cascades. Hypoxia-driven signalling has been implicated in several maladaptive processes in the fetal heart, including the activation of hypoxia-inducible factor-1 α (HIF-1 α), which is associated with myocardial injury and cardiac fibrosis (91). Additionally, hypoxia-driven EndMT has been proposed as a critical mechanism in the development of EFE (92, 156). The activation of Transforming Growth Factor-Beta (TGF β) signalling and suppression of the protective effects of Bone Morphogenetic Proteins (BMPs) further promote EndMT and the development of EFE (91, 92).

EFE has been associated with diastolic dysfunction in CAS-eHLHS, where LVs with significant EFE often exhibit worse diastolic function and greater limitations in LV growth following FAV (70, 77). Previous research has demonstrated that flow disturbances, such as stagnant intracavity flow and left ventricular distention can also contribute to EFE development (157). In CAS-eHLHS LVs, vortex dynamics appeared relatively quiescent, WSS patterns were altered, and there was a general trend toward reduced time- and spatially-averaged WSS magnitude, though with variability across cases. While the direct role of WSS in stimulating myocardial growth remains uncertain, these findings raise the possibility that altered intracavitary flow and WSS characteristics

may contribute to an environment less conducive to normal myocardial development. Although evidence is currently limited, it is plausible that WSS-mediated mechanobiological pathways such as those implicated in valvular development could also play a role in influencing myocardial patterning or tissue remodelling, including processes such as EndMT (137).

Further investigation seems warranted to fully understand the mechanistic link between mechanical stimuli and the pathophysiology of CAS-eHLHS. The diminished fluid stresses on the myocardium and valves, and the findings by Laura et al., which highlighted diminished myocardial deformation and increased myocardial stress (130), are likely to closely relate to the lack of growth of left-sided cardiac structures.

Additionally, some of the key differences between CAS-eHLHS and healthy LVs appeared to be mitigated following FAV. In this study, post-FAV data showed that a significant number of fetuses developed a biphasic MV inflow profile and experienced improved stroke volumes, as shown in Table 12. It is uncertain how these changes would alter fluid patterns, whether they would alleviate hypoxic and hypertensive stresses or reduce the heart's workload. Additionally, the occurrence of AV regurgitation in post-FAV LVs likely further altered fluid dynamics, making them quite different from pre-FAV CAS-eHLHS LVs and healthy LVs, warranting further detailed investigation.

This chapter establishes a baseline characterization of the CAS-eHLHS disease, enabling us to compare pre-and post-FAV LV fluid dynamics in the following chapter. This approach can also be applied to assess the impact of growth and remodelling on cardiac fluid dynamics, such as changes resulting from re-stenosis after the intervention or a decline in cardiac contractility. Additionally, it allows us to explore the relationship between fluid mechanical stimuli and cardiac growth during gestation. These insights could enhance our understanding of the intervention's effects and the influence of mechanical stimuli on outcomes, ultimately guiding clinical decisions regarding patient suitability for the intervention, optimal timing, and the potential benefits of novel medical devices like fetal transcatheter AV replacement (158) or maternal hyperoxygenation therapy (159, 160).

3.4 Limitations

The findings in this chapter have certain limitations that need to be acknowledged. Firstly, the simulations conducted did not incorporate fluid-structure interactions between the fluid and the MV. Instead, the modelling of the MV is simplified to be an effective inlet orifice area. This simplification was necessary due to the poor resolution and high noise levels in obstetrics imaging, which made it challenging to accurately observe the valve morphology. This likely affected the resulting flow patterns, as the presence of the MV would typically enhance vortex penetration, WSS and viscous dissipation (161). Despite this, the primary flow phenomena, including the inflow jet vortex ring and its interaction with the ventricular walls, are expected to have been reasonably well represented. Secondly, the locations of the valve orifices were approximated because the valve annulus locations can be hard to discern in the images, which may have introduced errors into the model. Lastly, the limitations in imaging resolution and the presence of noise likely contributed to inaccuracies in segmentation and cardiac motion estimation. These factors may have affected the precision of the simulation results. Despite these challenges, it is believed that the overall trends and key flow dynamics were preserved, providing valuable insights into the fluid mechanics of the cardiac system.

3.5 Conclusion

In conclusion, patient-specific 4D flow simulations were conducted for both healthy and CAS-eHLHS LVs, revealing significant differences between the two. Although there was considerable variability among the CAS-eHLHS cases, certain unifying characteristics were identified. Firstly, CAS-eHLHS LVs exhibited an abnormally fast and narrow diastolic MV inflow jet, likely due to a malformed MV with limited opening capability and LA hypertension. This inflow jet impinged on the apex, leading to increased vorticity dynamics and elevated WSS localised at the apex region. However, despite this localised elevation, the overall spatially averaged WSS magnitude experienced by the entire LV was considerably lower than that in healthy cases. Secondly, CAS-eHLHS LVs displayed elevated IVPG, along with higher systolic and diastolic energy losses, which

were attributed to both AV and MV stenosis. Thirdly, these LVs exhibited reduced blood turnover, indicative of impaired washout and prolonged blood residence. When considered alongside potentially higher elevated myocardial energy demands, such stagnation may compromise coronary perfusion and contribute to hypoxic stress in the myocardium. This along with diminished WSS stimuli, may be crucial in understanding the development of EFE and poor LV growth.

Chapter 4

Aim 2 – Changes in Fluid Mechanics in LV after Successful FAV

This chapter builds upon the analysis conducted in Chapter 3 by focusing on the haemodynamics of CAS-eHLHS LVs after FAV. The objective is to compare pre- and post-FAV haemodynamic changes within a short window (2-3 days) after technically successful FAV to better understand the effects of the intervention on fetal LV fluid mechanics. The efficacy of FAV in improving postnatal prognosis for CAS-eHLHS fetuses is inconsistent and the acute haemodynamic effects of the intervention are primarily based on physiological observations, such as improved antegrade aortic flow and changes in aortic jet width. Detailed fluid dynamics, such as the impact of FAV on flow patterns, WSS, and energy losses, have not been thoroughly explored. Addressing these gaps is crucial for understanding the full impact of FAV and how mechanical stimuli can affect cardiac growth for the remainder of gestation. Furthermore, this investigation provides insight into whether FAV truly enhances LV function or if alternative innovations, like fetal transcatheter AV replacement, are warranted.

To achieve this objective, pre- and post-procedural CFD simulations were conducted using patient-specific scans after technically successful FAV. These simulations aimed to quantify key hemodynamic parameters, including LV stroke volume, EF, vortex dynamics, WSS, energy losses, and blood turnover, providing a detailed comparison of LV function before and after FAV. Findings from this chapter indicate that while FAV significantly rescues LV stroke volume and EF, it also introduces highly chaotic flow patterns due to AV regurgitation, leading to increased energy losses within the LV. Despite the intervention's success in improving certain physiological metrics, such as stroke volume, the overall flow within the LV becomes more chaotic in some cases, contributing to inefficiency.

4.1 Methods

Post-FAV scans of the CAS-eHLHS cases analysed in Chapter 3 were reviewed and patient-specific CFD simulations were generated from 4D fetal echocardiography, using the proposed method in Chapter 3. Please refer to section 3.1 for more details. CAS-eHLHS-1 was excluded from modelling due to missing valve Doppler velocity data, which hindered patient-specific modelling. The characteristics of the cohort are re-listed in Table 16, excluding CAS-eHLHS-1.

TABLE 16. CHARACTERISTICS OF CRITICAL AORTIC STENOSIS WITH EVOLVING HYPOPLASTIC LEFT HEART SYNDROME (CAS-eHLHS) FETUSES, POSTNATAL OUTCOMES AND POSTNATAL PROCEDURES ARE UNDERTAKEN.

<i>Case</i>	<i>Pre-FAV GA at scan, (week + day)</i>	<i>Bradycardia</i>	<i>LV thrombus</i>	<i>Pericardial effusion</i>	<i>Hydrops</i>	<i>Postnatal Circulation</i>	<i>Postnatal procedures</i>
CAS-eHLHS – 2	22 + 4	N	Y	N	N	UV	NW
CAS-eHLHS – 3	29 + 1	N	N	N	Y	BV	RK
CAS-eHLHS – 4	29	N	N	N	Y	BV	RK
CAS-eHLHS – 5	24 + 6	Y	N	N	N	BV	AV dilation
CAS-eHLHS – 6	30 + 1	N	N	N	N	BV	AV dilation
CAS-eHLHS – 7	23 + 3	Y	N	N	N	UV	Hybrid

Note: *GA* - gestational age; *LV* - left ventricle; *BV* - biventricular; *UV* - univentricular; *RK* - Ross-Konno procedure; *NW* - Norwood procedure; *Hybrid* – Hybrid procedure; *AV* – aortic valve; *Y* - yes; *N* - no.

A key modification to the existing method involved modelling AV regurgitation as an additional orifice zone in the CFD models of post-FAV LVs. The sizes of the MV inlet, AV outlet, MV regurgitation and AV regurgitation were iteratively adjusted in these cases to achieve a close match between the CFD-simulated valve velocities and patient-specific Doppler velocities, demonstrated in Figure 24. The case-specific breakdowns are demonstrated in Table 17.

In this chapter, the Anderson-Darling test was performed to assess the normality of all continuous variables. Subsequently, a paired one-tailed t-test was adopted to compare continuous data for pre- and post-FAV when the data had a normal distribution; otherwise, the non-parametric Wilcoxon signed-rank test was utilised. Additionally, t-tests for independent samples were performed for comparisons between sub-groups

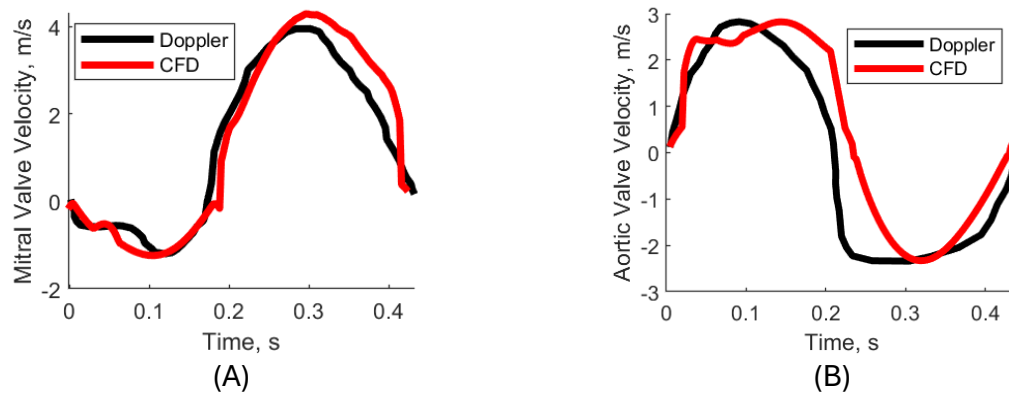


Figure 24. Comparison of CFD-simulated (red) and Doppler-derived (black) velocity profiles at the mitral valve (left) and aortic valve (right) orifices for a representative CAS-eHLHS case following fetal aortic valvuloplasty. The velocity traces are plotted at the respective valve locations, showing the temporal evolution of inflow and outflow. Negative velocities represent inflow (into the left ventricle through the MV or regurgitant AV jets), while positive velocities represent outflow (through the AV or MV regurgitant jets).

TABLE 17. CASE-SPECIFIC COMPARISON BETWEEN COMPUTATIONAL FLUID DYNAMICS SIMULATION RESULT AND CLINICAL MEASUREMENTS OF VALVE VELOCITIES FOR THE CAS-eHLHS COHORT

Parameter	Post-FAV Cohort											
	CAS-eHLHS-2 (22.57 wk)		CAS-eHLHS-3 (29.14 wk)		CAS-eHLHS-4 (29 wk)		CAS-eHLHS-5 (24.86 wk)		CAS-eHLHS-6 (30.14 wk)		CAS-eHLHS-7 (23.43 wk)	
	CFD	Dop.	CFD	CFD	CFD	Dop.	CFD	Dop.	CFD	Dop.	CFD	Dop.
Peak AV, m/s	0.79	0.75	2.59	2.48	2.06	2.10	2.23	2.25	1.58	1.60	0.81	0.85
Diastolic E, m/s	-	-	0.77	0.80	0.52	0.50	0.66	0.60	0.60	0.51	-	-
Diastolic A, m/s	0.70	0.55	1.60	1.50	0.60	0.60	0.86	0.87	0.60	0.55	-	-
MV Regurgitation, m/s	2.80	2.80	4.29	4.00	3.62	3.60	3.35	3.30	3.64	3.75	1.64	1.40
AV Regurgitation, m/s	1.92	1.87	2.83	2.78	2.55	2.50	2.26	2.25	2.61	2.50	1.98	2.00

Note: CAS-eHLHS – Critical aortic stenosis and evolving hypoplastic left heart syndrome; AV – aortic valve; MV – mitral valve, CFD – Computational fluid dynamics, Dop. – Doppler.

(healthy vs. pre-FAV, healthy vs. post-FAV) if they followed a normal distribution, or the Mann-Whitney U test was used for non-normally distributed data. A P value of <0.05 was considered statistically significant for all tests.

4.2 Results: Comparisons between Pre- and Post-FAV LVs

4.2.1 Physiological Features

TABLE 18. ECHOCARDIOGRAPHIC-DERIVED CARDIAC MEASUREMENTS BEFORE AND AFTER SUCCESSFUL FETAL AORTIC VALVULOPLASTY (FAV) OF FETUSES WITH AORTIC STENOSIS AND EVOLVING HYPOPLASTIC LEFT HEART SYNDROME (CAS-EHLHS)

<i>Parameter</i>	<i>Pre – FAV</i>						<i>Post – FAV</i>						<i>P-value</i>
	CAS-eHLHS-2 (22.57wk)	CAS-eHLHS-3 (29.14wk)	CAS-eHLHS-4 (29 wk)	CAS-eHLHS-5 (24.86 wk)	CAS-eHLHS-6 (30.14wk)	CAS-eHLHS-7 (23.43wk)	CAS-eHLHS-2 (22.85wk)	CAS-eHLHS-3 (29.43wk)	CAS-eHLHS-4 (29.29wk)	CAS-eHLHS-5 (25.14 wk)	CAS-eHLHS-6 (30.43wk)	CAS-eHLHS-7 (23.71wk)	
LV long axis Z-score	1.77	3.72	1.89	1.08	0.49	0.18	1.60	3.45	1.74	1.10	0.56	-0.20	0.04*
RV/LV ratio	1.10	0.83	0.82	1.06	1.05	1.13	1.07	0.84	0.82	1.09	1.10	1.17	0.11
LV filling time index to cycle time, ms	0.14	0.33	0.41	0.40	0.43	0.31	0.30	0.33	0.43	0.46	0.40	0.58	0.06
Peak AV, m/s	1.84	2.44	1.10	3.50	1.33	1.35	1.87	2.78	2.50	2.25	2.50	2.00	0.18
Diastolic E, m/s	-	-	-	-	-	0.50	-	0.80	0.50	0.60	0.51	-	0.39
Diastolic A, m/s	0.35	1.11	0.80	0.83	0.63	0.60	0.55	1.50	0.60	0.87	0.55	-	0.27
MV regurgitation, m/s	3.00	3.20	3.10	4.20	3.50	2.30	2.80	4.00	3.60	3.30	3.75	1.40	0.40
MV annulus z-score	-0.96	3.35	0.67	0.79	1.67	0.74	-1.13	3.04	0.7	0.16	1.98	0.77	0.20

Note: *P<0.05 paired comparison between pre-FAV and post-FAV cases. LV – left ventricle; AV - aortic valve; MV – mitral valve; RV - right ventricle; SV - stroke volume; MR – Mitral regurgitation, MI – Mitral inflow. Z-scores derived from Schneider et al. (69).

Echocardiographic-derived cardiac parameters of CAS-eHLHS cohort before and after successful FAV are detailed in Table 18. In Chapter 3, it was reported that CAS-eHLHS LVs were distended, likely due to the elevated pressure. Similarly, the pre-FAV LVs had LV long axis Z-scores that were significantly larger than the mean for their GA. Following FAV, LV dimensions significantly decreased, likely due to the reduction in ventricular afterload following the relief of stenosis. The LV filling times showed a trend towards an increase after FAV, likely due to the decreased LV pressure allowing for greater inflow, although this change was not statistically significant (P = 0.06), due to sample size.

TABLE 19. COMPARISON OF CARDIAC PARAMETERS BETWEEN CAS-eHLHS BEFORE AND AFTER FAV, WITH HEALTHY COMPARISON

<i>Parameter</i>	<i>Healthy Cohort</i>	<i>Disease Cohort</i>	
	<i>Overall Mean</i>	<i>Pre-FAV (n = 7)</i>	<i>Post-FAV (n = 6)</i>
Gestational age, wk	26.4 ± 5.1	25.9 ± 3.4	26.8 ± 3.3
Peak AV, m/s	0.74 ± 0.14	1.89 ± 0.83 †	1.67 ± 0.73 †
Diastolic E, m/s	0.33 ± 0.14	-	0.63 ± 0.21 † (e.c.)
Diastolic A, m/s	0.41 ± 0.09	0.69 ± 0.25 †	0.87 ± 0.42 † (e.c.)
MV regurgitation, m/s	-	3.13 ± 0.62	3.14 ± 0.95
AV regurgitation, m/s	-	-	2.32 ± 0.34
* MV flow orifice area, mm ²	33.97 ± 21.06	9.44 ± 7.01 †	12.01 ± 7.41 † (e.c.)
* AV flow orifice area, mm ²	20.54 ± 14.39	1.53 ± 2.55 †	2.73 ± 2.41 † ‡
RV SV, ml	1.13 ± 0.57	1.37 ± 0.71	1.77 ± 1.06
LV SV, ml	1.16 ± 0.90	0.54 ± 0.42	0.96 ± 0.71 ‡
RV EDV, ml	2.47 ± 1.29	3.26 ± 1.80 †	4.60 ± 2.24 †
LV EDV, ml	2.14 ± 0.16	6.47 ± 5.83 †	6.64 ± 4.26 †
LV EF, %	56.2 ± 12.0	10.1 ± 8.6 †	18.7 ± 11.5 † ‡
AR volume: LV SV, %	-	-	28.1 ± 15.3 (e.c.)
MV Annulus z-score	0.37 ± 0.50	1.1 ± 1.3	0.92 ± 1.45

AV - aortic valve; MV – mitral valve; RV - right ventricle; SV - stroke volume; LV - left ventricle; EDV - end-diastolic volume; EF - ejection fraction; Disease cohort data from the current study were combined with pre-FAV and healthy cohort data from Chapter 3. All data are presented as mean ± standard deviation. (e.c.) - mean and standard deviation excluded CAS-eHLHS-7 due to no MV inflow.

† $P < 0.05$ comparing diseased pre-FAV to healthy and post-FAV to healthy; ‡ $P < 0.05$ comparing diseased pre-FAV and corresponding post-FAV cases.

*** Valve orifice areas were backcomputed from CFD, by seeking orifice areas that enabled CFD valve velocities to best match Doppler measurements.**

Table 19 presents a comparison of physiological and anatomical parameters between pre-FAV and post-FAV CAS-eHLHS LVs, as well as with healthy fetal LVs. It presents Doppler-measured valve velocities and valve orifice areas estimated through simulations to align with Doppler data. Before FAV, the AV orifices were markedly smaller compared to healthy hearts, reflecting critical aortic stenosis. Post-FAV, the AV orifice sizes doubled from their pre-FAV measurements. However, these sizes were still notably smaller than those found in healthy hearts. In Chapter 3, pre-FAV LVs were reported to exhibit significantly higher AV velocities due to stenosis. Post-FAV, there was minimal change in peak AV velocities despite the alleviation of the stenosis. While the post-FAV AV orifice area was larger, the alleviation of the stenosis likely increased flow rates and consequently velocities, counteracting the expected reduction. AV regurgitation was observed post-FAV in all cases, with an average velocity of 2.32 ± 0.34 m/s, indicating that technically successful FAV may also cause damage to the AV.

In Chapter 3, it was found that the MV orifice area was considerably smaller and that diastolic MV inflow velocities were higher. Unsurprisingly, this elevation remained as so after FAV but a biphasic or partially fused MV inflow pattern developed in most cases after FAV. FAV did not allow for a significant change in MV inflow as reflected in the MV orifice sizes, as estimated through simulations. However, the LV EF has increased and there was a noticeable increase in LV stroke volume after FAV with simulations estimating a $28.1 \pm 15.3\%$ of the stroke volume was attributed to the AV regurgitation. This estimation excluded one case, CAS-eHLHS-7, where clinicians detected no MV inflow in their echocardiographic scans, and simulations used AV regurgitation as the sole proponent of flow into the LV.

Although LV long-axis z-scores showed a significant decrease after FAV (Table 18), no significant difference was observed when considering the entire cavity volume between the pre- and post-FAV end-diastolic volumes (Table 19). There was notable decompression in most cases with some remaining unchanged or slight increase. Please refer to Table A1 and A2 in the Appendix for a comprehensive case-specific breakdown.

Pre-FAV RVs were larger than those of healthy controls and exhibited higher stroke volumes, though these differences did not reach statistical significance due to the high variability in the data, partially attributed to differences in *GAs*. This enlargement is consistent with prior observations about the compensatory response to the reduced pumping efficiency of the LV caused by CAS (62). However, two days following FAV, the RV size and function generally remained unchanged, indicating that the intervention did not immediately impact these measures.

4.2.2 Diastolic Flow Patterns

Representative results of LV flow vortex patterns throughout the cardiac cycle are shown in Figure 25, with case-specific breakdowns available in Figure A1 and A2. As reported in Chapter 3, most subjects in the pre-FAV cohort exhibited a monophasic diastolic inflow pattern. This inflow jet typically impinged on the apex, leading to localized elevation in WSS at the point of impact. While peak MV inflow velocities were relatively high in some cases, the mitral orifice area was significantly reduced compared to healthy

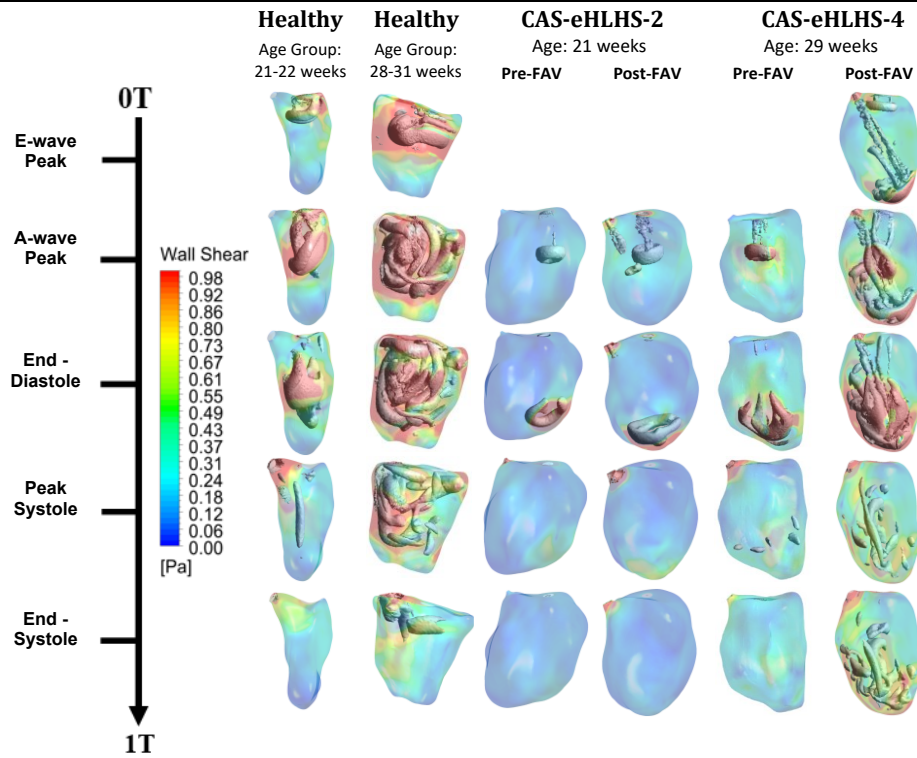


Figure 25: Visualization of flow dynamics at various points in the cardiac cycle using λ_2 vortex criterion iso-surfaces and wall shear stress colour contour plots. This figure compared two representative healthy left ventricles (LVs) with two representative critical aortic stenosis and evolving hypoplastic left heart syndrome (CAS-eHLHS) LVs cases before and after FAV, incorporating data from Chapter 3.

controls (Table 13). As flow rate is proportional to both velocity and orifice area, total inflow volume was likely reduced, even though it was not explicitly computed. This limited inflow, combined with the small vortex rings formed by the narrow jet, likely contributed to the relatively quiescent vorticity patterns observed in pre-FAV cases, particularly in the context of a dilated, under-contractile LV.

Following FAV, the complexity of vorticity dynamics in the LV increased drastically in a number of cases. In all cases, the intervention resulted in AV regurgitation, which created an additional vortex ring that travelled swiftly to the apex during early diastole. This vortex ring's impact at the apex produced chaotic secondary vorticity patterns in the region. MV inflow was biphasic in all cases except for CAS-eHLHS-2 and CAS-eHLHS-7 which had no inflow. Peak A-wave velocities were comparable to pre-FAV peak velocities. Consequently, two MV inflow vortex rings formed, with the slower E-wave vortex ring preceding the faster A-wave vortex ring. These vortex rings also moved rapidly toward the apex, albeit more slowly than the AV regurgitation vortex ring. Upon reaching the apical region, the MV vortex rings interacted with the secondary vorticity structures generated

by the earlier AV vortex ring, thereby increasing the overall complexity and turbulence of the vorticity field.

Compared to the pre-intervention flow field, most post-intervention cases displayed more pronounced vorticity dynamics that occupied a larger fluid space, driven by increased flow rates and the additional AV vortex ring. These vorticity structures persisted longer, lasting until the end of systole in most cases, in contrast to the pre-intervention cases. As these vorticity structures near the walls are the primary mechanism for inducing WSS, post-intervention cases appeared to have higher overall WSS levels. These changes were less noticeable in CAS-eHLHS-2, as demonstrated in Figure 25 and similarly in CAS-eHLHS-7, shown in Figure A3, where high-velocity AV regurgitation jet impingement did not really result in large-scale changes in LV vortex dynamics.

While this post-intervention flow field was more complex than in pre-FAV cases, comparison with healthy fetal hearts reveals a more nuanced picture. Healthy LVs typically exhibit broad, coherent vortex rings during diastole, with smooth propagation and efficient washout. The flow complexity observed post-FAV spans a spectrum. In several post-FAV cases—particularly those with significant improvements in LV function (e.g., CAS-eHLHS-3, -4, -5)—the restored flow rates and vortex formation approached, and in some instances rivalled, the level of chaoticity observed in healthy cases. In these examples, the chaoticity and temporal persistence of the secondary vortices were comparable to age-matched controls.

However, the apex-directed inflow and superimposed AV regurgitation vortex remain distinct features of post-FAV flow patterns. Although the resulting vorticity may appear visually similar to healthy cases, it does not necessarily reflect efficient flow organisation. This distinction becomes more apparent when examining haemodynamic energy loss, explored in the following section.

4.2.3 LV energy dynamics

TABLE 20. COMPUTATIONAL FLUID DYNAMICS-DERIVED HAEMODYNAMIC PARAMETERS BEFORE AND AFTER SUCCESSFUL FETAL AORTIC VALVULOPLASTY (FAV) OF FETUSES WITH CRITICAL AORTIC STENOSIS AND EVOLVING HYPOPLASTIC LEFT HEART SYNDROME (CAS-EHLHS), WITH HEALTHY COMPARISON

<i>Parameter</i>	<i>Healthy Cohort</i>	<i>Disease Cohort</i>	
	Overall Mean	Pre-FAV (n = 8)	Post-FAV (n = 5)
LV Systolic (Apex – AV) IVPG, Pa	242 ± 89	2147 ± 2009 †	1663 ± 1198 †
LV Systolic (Apex – MV) IVPG, Pa	-	1165 ± 721	1328 ± 711
LV Diastolic (Apex – MV) IVPG, Pa	41 ± 25	85 ± 63	655 ± 467 †‡
LV Diastolic (Apex – AV) IVPG, Pa	-	-	710 ± 415
Normalised Systolic Ejection Work Done, J/m ³	164 ± 69	2684 ± 856 †	2365 ± 1508 †
Normalised Systolic Energy Loss, J/m ³	22 ± 8	177 ± 52 †	196 ± 80 †
Normalised Diastolic Energy Loss, J/m ³	17 ± 10	101 ± 48 †	622 ± 341 †‡
Time- & Surface-Ave WSS, Pa	0.77 ± 0.26	0.47 ± 0.32 †	1.02 ± 0.58 ‡
Diastolic Peak Surface-Ave WSS, Pa	0.90 ± 0.38	0.86 ± 0.72	2.03 ± 1.23 ‡
Diastolic Time- & Surface-Ave WSS, Pa	0.67 ± 0.33	0.46 ± 0.39 †	1.31 ± 0.84 ‡
Systolic Peak Surface-Ave WSS, Pa	1.52 ± 0.36	0.73 ± 0.47 †	1.67 ± 1.17 ‡
Systolic Time- & Surface-Ave WSS, Pa	0.98 ± 0.27	0.49 ± 0.29 †	0.82 ± 0.44 ‡

Note: LV - left ventricle; MV – mitral valve; IVPG – intraventricular pressure gradient; WSS – wall shear stress. Disease cohort data from the current study were combined with pre-FAV and healthy cohort data from the previous chapter. All data are presented as mean ± standard deviation.

†P<0.05 comparing diseased pre-FAV to healthy and post-FAV to healthy; ‡P<0.05 comparing diseased pre-FAV and corresponding post-FAV cases.

Despite the low Reynolds numbers, ranging from 206 to 1360, which indicate a viscosity-dominated environment, strong vorticity dynamics were still present. This suggests significant fluid shear stresses and energy losses. Additionally, instability in the trailing shear layer of both AV and MV inflow vortex rings was observed, leading to the formation of secondary rolled-up vortices. This instability was likely due to the asymmetry of the orifice and the leading vortex ring (162, 163).

Table 20 provides details of CFD-derived haemodynamic parameters for fetuses with CAS-eHLHS before and after FAV, with a comparison to the healthy cohort. Figure 26 provides a visual summary of these findings, highlighting key differences in various haemodynamic parameters across the groups. Figures 26A and 26B present a comparison of the IVPG, systolic work, and energy losses during systole and diastole across healthy, pre-FAV, and post-FAV groups. The IVPG was determined as the peak instantaneous pressure difference between the apex and the MV or AV orifice, reflecting the energy losses linked to inflow and outflow. In Chapter 3, it was reported that pre-FAV LVs exhibited elevated systolic apex-AV IVPG due to CAS. This remained unchanged

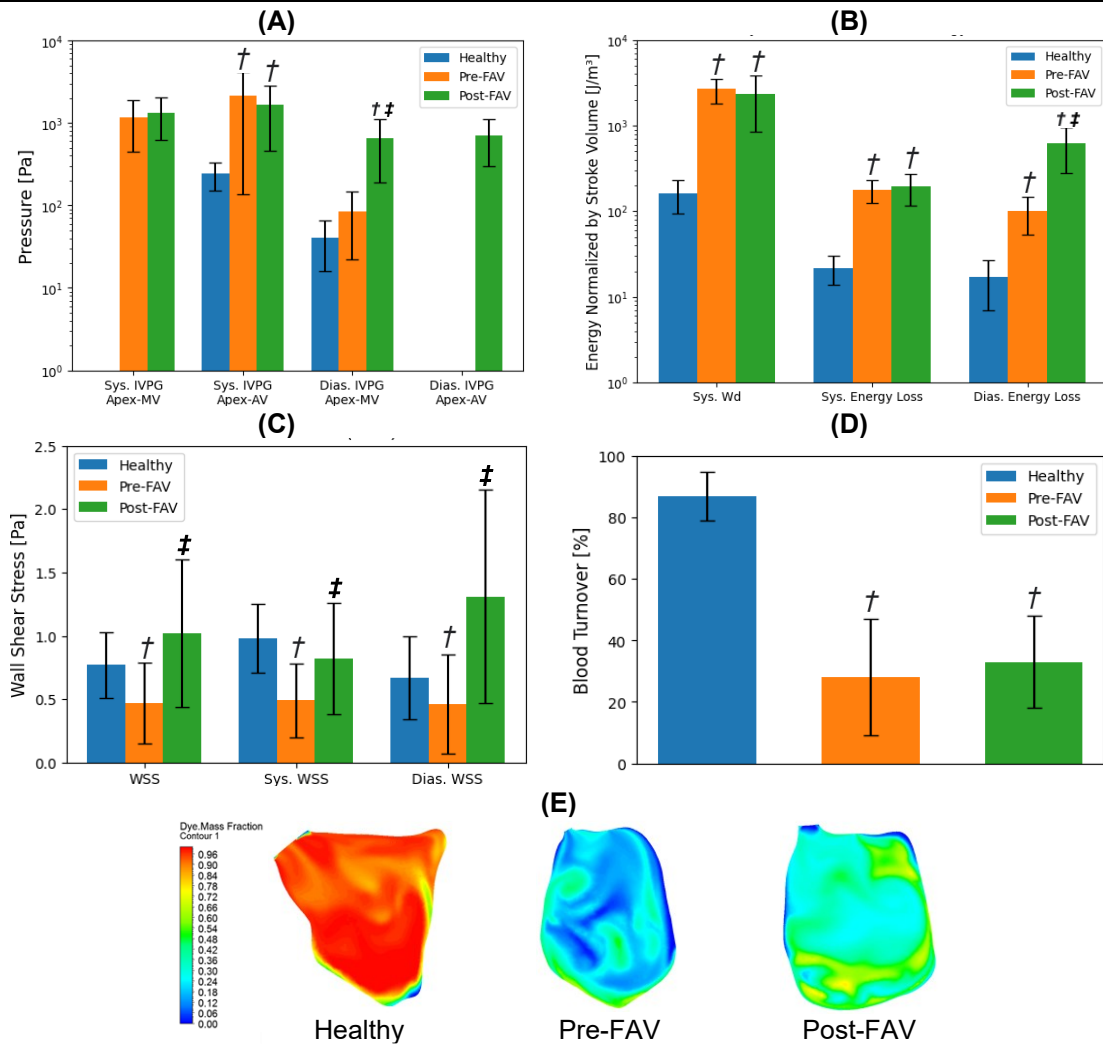


Figure 26: Haemodynamic parameters derived from computational fluid dynamics simulations are shown for healthy, pre- and post-fetal aortic valvuloplasty (FAV) cohorts. (A) Logarithmic plot of systolic intraventricular pressure gradients (IVPG) between the apex and mitral valve (MV) or aortic valve (AV), and diastolic IVPG between the apex and MV/AV. (B) Logarithmic plot of systolic work done alongside systolic and diastolic energy losses. (C) Time- and surface-averaged wall shear stress (WSS) magnitudes during the cardiac cycle, including systole and diastole. (D) Blood turnover percentage, calculated by inflow dye mass fraction inside the left ventricle after three cardiac cycles. (E) End-state in-plane contour maps displaying the passive dye mass fraction after three cardiac cycles for three representative cases from each cohort. † $P < 0.05$ comparing diseased pre-FAV to healthy and post-FAV to healthy; ‡ $P < 0.05$ comparing diseased pre-FAV and corresponding post-FAV cases.

post-FAV, as outflow velocities stayed high due to the increased flow rates following stenosis reduction. As previously reported in Chapter 3, there was no statistical significance in diastolic apex-MV IVPG between pre-FAV and healthy LVs. However, diastolic IVPG increased significantly post-FAV, primarily due to high-velocity AV regurgitation, raising apical pressures and because MV inflow velocities remained elevated compared to the pre-FAV state. These findings indicate that while the pre-FAV flow field already exhibited substantial fluid-mechanical inefficiency, the introduction of

AV regurgitation post-FAV further intensified this, leading to increased intracavitary pressure gradients and higher flow rates.

A similar pattern was observed in the normalized systolic and diastolic energy loss results. Pre-FAV LVs exhibited significant increases in fluid-mechanical energy losses compared to normal LVs. Instead of improving, energy losses worsened after FAV, with diastolic losses showing a marked increase. These additional losses were attributed to increased fluid shear and viscous dissipation due to enhanced vorticity dynamics, driven by AV regurgitation and higher LV flow rates. As a result, the normalised systolic fluid-mechanical work done by the LV remained unchanged post-FAV, staying significantly higher than in healthy LVs.

In summary, these findings suggest that FAV did not enhance flow energy efficiency; rather, it exacerbated energy losses. Post-FAV energy losses (diastolic and systolic) were $46.8 \pm 28.5\%$ of systolic work done, compared to $10.4 \pm 2.5\%$ in pre-FAV LVs.

4.2.4 LV Wall Shear Stress

Figure 27 shows a comparison of the spatial distributions of TAWSS and OSI for CAS-eHLHS cases with their respective age group controls. Other cases are shown in Figure A4. As seen in Chapter 3, high TAWSS and low OSI were observed in large areas of the mid-ventricle zone in the healthy cohort due to the wider diastolic vortex rings that propagate and interact with the expanding LV walls. In pre-FAV CAS-eHLHS LVs, high TAWSS and low OSI were observed only around the apical zone and valve orifices, and these patterns remained generally similar in post-FAV LVs. This was a consequence of the high-velocity vortex rings that impinged at the apical site, generating flow disturbances there, and elevating WSS across greater areas of the wall surface. This was particularly noticeable in CAS-eHLHS-5 (Figure A4), where flow disturbance caused by secondary vortices was quite substantial, compared to other cases, owing to much better flow function and EF (37.3%).

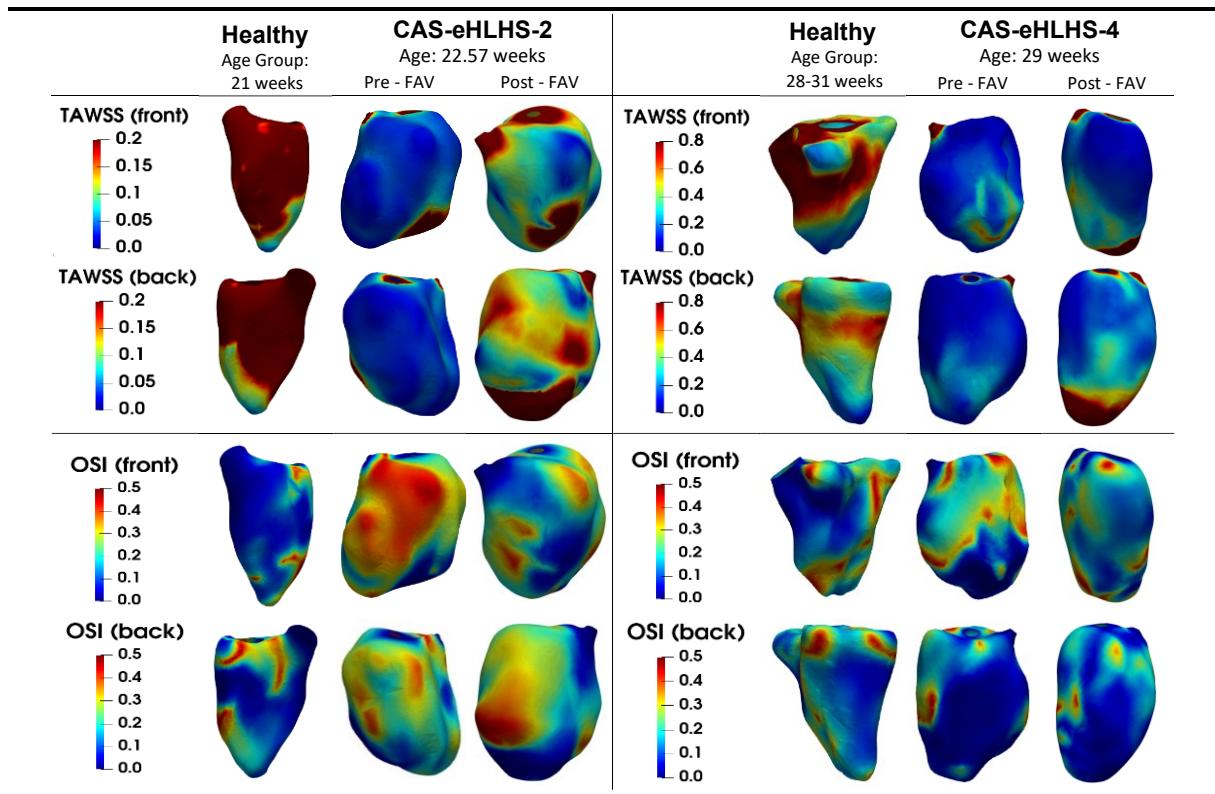


Figure 27: Comparative maps of time-averaged wall shear stress (TAWSS) and oscillatory shear index (OSI) from front and back views in healthy left ventricles (LVs) and critical aortic stenosis and evolving hypoplastic left heart syndrome (CAS-eHLHS) LVs, both pre- and post-fetal aortic valvuloplasty (FAV). The figure includes two different age groups: (1) 21 weeks and (2) 28-31 weeks. The maps highlight distinct differences in the distribution and magnitude of shear stress between healthy LVs and disease cohorts, with significant alterations observed after FAV intervention.

There is likely no substantial increase in inflow through the MV, given that there were no significant changes in MV orifice sizes and only a slight increase in MV inflow duration, the observed elevation in WSS and flow disturbances were most likely caused by strong AV regurgitation. Along with broader coverage of elevated WSS, post-FAV cases exhibited higher WSS magnitudes across all measures, as illustrated in Figure 26C and Table 20. This increase was more pronounced during diastole compared to systole. The rise in systolic WSS can likely be attributed to the persistence of diastolic vortices into systole, as well as the increased stroke volume and flow rates observed after the intervention.

4.2.5 Blood Turnover and Washout Efficiency

Figure 26D illustrates the percentage of fresh blood introduced into the LV in healthy, pre-FAV, and post-FAV cases after three cardiac cycles. Figure 26E indicates that the inefficient blood turnover observed in pre-FAV cases did not significantly improve following FAV. This limited improvement can be attributed to only a marginal

enhancement in MV inflow, with a partial portion of the increase in stroke volume being accounted for by AV regurgitation, which does not contribute to introducing fresh blood into the LV. These findings have potential implications for the oxygen content within the LV chamber and the development of EFE, as was previously discussed in Chapter 3. CAS-eHLHS-7 was not included in blood turnover analysis as post-FAV LV had no MV inflow and so would have theoretically no turnover of blood.

To assess whether this CFD-based turnover analysis offers meaningful improvements over a basic “complete mixing” model, a comparison was made between the CFD-simulated dye-based turnover and theoretical turnover assuming ideal instantaneous mixing. Results are summarised in Table 21. In this simplified model, turnover is determined purely by the ratio of SV and EDV, with the theoretical dye fraction after n cycles, f_n , is given by:

$$f_n = 1 - \left(1 - \frac{SV}{EDV}\right)^n \quad (23)$$

However, in post-FAV cases with AV regurgitation, SV includes backward flow that does not contribute to fresh inflow. Hence, the effective turnover was recalculated using:

$$f_n = 1 - \left(1 - \frac{SV(1 - f_{AVregurg.})}{EDV}\right)^n \quad (24)$$

where $f_{AVregurg.}$ is the fraction of the SV attributable to AV regurgitation, as quantified from the CFD simulations.

Across all cases, CFD-derived turnover fractions were, on average $1.44 \pm 8.5\%$ higher than theoretical predictions. Discrepancies arise from two main factors embedded in the CFD methodology. First, the simulations employ dynamic valve switching based on the direction of flow to simulate physiological valve closure if backflow is detected such as flow into the LV through the AV orifice or flow out of the LV through the MV orifice. This nuanced approach more accurately captures transitional valve dynamics and can influence the precise timing and volume of dye entering the LV, ultimately affecting the calculated turnover.

Second, non-uniform dye distribution within the LV and the selective ejection of blood further contribute to the observed differences. In healthy LVs, vortex ring formation

TABLE 21. COMPARISON OF BLOOD TURNOVER BETWEEN CFD-BASED AND THEORETICAL MIXING MODELS FOR THREE CARDIAC CYCLES.

<i>Patient Case</i>	<i>CFD-simulated dye fraction</i>	<i>Theoretical dye fraction</i>	<i>Percentage Change,%</i>
<u>Healthy Cases</u>			
H1	0.791	0.796	-0.57
H2	0.942	0.876	+7.51
H3	0.909	0.979	-7.20
H4	0.929	0.945	-1.70
H5	0.789	0.935	-15.6
<u>Pre-FAV Cases</u>			
CAS-eHLHS-1	0.193	0.193	-0.20
CAS-eHLHS-2	0.142	0.134	+5.98
CAS-eHLHS-3	0.199	0.193	+3.16
CAS-eHLHS-4	0.197	0.173	+13.9
CAS-eHLHS-5	0.644	0.638	+1.02
CAS-eHLHS-6	0.304	0.332	-8.28
<u>Post-FAV Cases</u>			
CAS-eHLHS-2	0.185	0.161	+15.1
CAS-eHLHS-3	0.295	0.281	+4.95
CAS-eHLHS-4	0.289	0.261	+10.8
CAS-eHLHS-5	0.596	0.672	-11.3
CAS-eHLHS-6	0.294	0.279	+5.43

during diastole tends to concentrate dye near the base of the LV, close to the outflow tract, which facilitates its preferential ejection during systole. In contrast, CAS-eHLHS cases often exhibit narrow, high-velocity MV inflow jets that direct dye toward the apex, where it is less likely to be expelled. As a result, a larger fraction of dye remains trapped in the LV over successive cardiac cycles, reducing turnover efficiency and bringing the CFD-derived results closer to the theoretical baseline, particularly in severely dysfunctional cases.

4.3 Discussion

These image-based flow simulations provided detailed insights into the acute changes in LV flow patterns, WSS, and energy dynamics induced by FAV. The results indicated that SV was restored to normal levels and EF showed improvements. Although a significant portion of the increased SV was likely due to AV regurgitation, these changes may have contributed to the restoration of myocardial deformational stimuli, enhanced flow stimuli to the heart valves and aorta, and facilitated the transition from abnormal

retrograde systolic flow in the transverse aortic arch to a normal antegrade flow in most cases. The findings also revealed an increase in WSS stimuli to the LV walls, which could potentially support improved morphological development.

Unexpectedly, it was found that FAV exacerbated the already inefficient flow energy dynamics within the LV, leading to additional and significant energy losses, with losses reaching up to 49.9% of the haemodynamic work done by the heart on LV fluid. This substantial energy loss within the flow field may help explain why the calculated systolic work done on the blood remained elevated after FAV, highlighting persistent inefficiencies in haemodynamic energy transfer. The primary cause of these excessive energy losses was the high-velocity AV regurgitation. It resulted in a highly chaotic diastolic flow pattern that persisted throughout the cardiac cycle, leading to substantial viscous energy losses.

One crucial consideration in FAV interventions is ensuring adequate resolution of stenosis, as cases of AV re-stenosis requiring a second intervention have been reported, which poses significant risks to the fetus (127, 164). Consequently, balloon sizes are often chosen to be large, which commonly results in severe AV regurgitation (10, 128). This diastolic dysfunction and the associated high energy requirements for systolic ejection of the LV could be detrimental. In addressing this issue, fetal transcatheter AV replacement might offer a solution. Recent advances, such as those demonstrated by Zakko et al., have shown the successful transcatheter delivery of a tissue-engineered heart valve in a fetal ovine model, with survival to birth (158).

On the other hand, there are opposing views that AV regurgitation may not necessarily be detrimental to LV development. AV regurgitation usually resolves itself several weeks post-FAV, potentially due to AV remodelling, which could resolve poor diastolic function and excessive energy losses. Secondly, AV regurgitation may enhance the heart's SV, increase mechanical stimuli and even allow for greater deformation of the LV myocardium, serving as a critical biomechanical stimulus for continued LV growth and normalisation of LV morphological development (130). Past studies have suggested that increased LV strains post-FAV correlate with a BV outcome rather than a UV outcome, supporting this idea (94). However, further research is necessary to substantiate these findings.

FAV did not alleviate the high AV outflow velocities seen in the pre-FAV cases. Despite the AV flow orifice area being doubled on average after the procedure, it remains approximately six times smaller than in a normal heart. This was unsurprising since mid-gestation CAS-eHLHS fetuses have low AV annulus z-scores (-4.2 to -1.94) (73), and FAV balloon sizes are selected to closely match the annulus size (117, 118). Although the AV poses less of a restriction to outflow after FAV, it can still contribute to elevated systolic energy losses within the flow and more energy is required to eject blood from the LV.

In terms of WSS, FAV significantly elevated WSS, restoring it to levels similar to or even higher than those observed in a healthy LV. It can be hypothesised that this increase in WSS could stimulate LV growth, potentially leading to a BV birth outcome, as WSS is thought to be a stimulus for gestational cardiac growth. The increased WSS on heart valves and the aorta may improve their growth rates (10, 14), which could further support the achievement of BV outcomes. Interestingly, CAS-eHLHS-2 and CAS-eHLHS-7 post LVs with UV postnatal outcome exhibited comparably lower WSS levels than other cases. However, the limited number of UV cases available makes it difficult to substantiate a definitive link between poor WSS and the likelihood of a UV outcome.

The simulations indicated that FAV did not significantly enhance LV blood turnover within the first two days post-procedure, suggesting that systemic blood delivery is still somewhat compromised. This raises concerns that FAV may not effectively reduce the risk of EFE in CAS-eHLHS due to the activation of hypoxia-induced EndMT (156, 165), particularly if systemic or coronary oxygen delivery remains inadequate. Inadequate perfusion of the fetal brain was reported even after technically successful FAV, where cerebral blood flow profiles remained abnormal. Additionally, severe AV regurgitation following FAV may lead to LV distension, potentially triggering stretch-induced EndMT, which could contribute to the development or worsening of EFE (157). It remains unclear which factor, if either, is the primary driver of EFE in these cases.

4.4 Limitations

The study in this chapter has similar limitations as that mentioned in Chapter 3. Mainly the idealisation of the MV as a simple orifice without valve leaflets, due to the inability of fetal echo imaging to capture detailed valve structure and dynamics, leaving

the cause of mild MV stenosis unaddressed. Image noise and signal losses could also lead to errors in segmentation and motion tracking. Besides that, the current study focuses solely on the acute changes in flow biomechanics following FAV, without considering the positive AV remodelling that often occurs several weeks after the procedure. The study does not address the potential for significant heart remodelling during the remainder of gestation, given the fetal heart's plasticity. Additionally, the results suggested that the high energy losses observed during diastole were related to AV regurgitation, which, according to single-centre experience, occurred in 92% of FAV cases. However, the absence of AR is also possible after successful FAV, but such cases are not analysed here and are less seen. In such cases, energy losses would remain like pre-FAV levels, as transaortic velocities tend to remain the same across FAV, but future work is necessary to ascertain this. With a greater number of UV and BV cases, future image-based CFD simulations should explore how fluid dynamic parameters can be predictors of postnatal outcomes.

4.5 Conclusion

In summary, the impact of FAV on LV flow, WSS and energy dynamics was investigated using patient-specific flow simulations. The findings indicate that FAV improved stroke volume, EF and WSS stimuli to the LV walls mainly by the introduction of AV regurgitation. This resulted in chaotic diastolic flow patterns, significantly reduced flow energy efficiency, and excessive energy losses immediately post-FAV. Additionally, the analysis suggests that while the AV orifice was enlarged following FAV, there is still a substantial restriction of flow from hypoplastic AV and there was no significant improvement in blood turnover within CAS-eHLHS LVs.

Chapter 5

Aim 3 : Flow Reconstruction via Physics-Informed Neural Network from Echocardiography guided by 3D Colour Doppler

In the previous chapters, it was shown that fluid dynamics of the LV can provide critical biological cues for understanding cardiac health and conditions such as CAS-eHLHS. It has great potential for supporting diagnosis and prognosis. However, directly acquiring fluid dynamics information from clinical imaging was challenging, as they are often noisy and have limited resolution, preventing accurate detailed fluid dynamics analysis. Image-based flow simulations offer high detail but are typically difficult to align with clinical velocity measurements, and as a result, may not accurately depict true fluid dynamics. Inverse-computing LV velocity fields from images via VFM have been reported, but they can become inaccurate when faced with missing or noisy measurement data, a common issue with modalities such as ultrasound. Here, a PINN framework is used that can accurately reconstruct detailed 3D flow fields of the cardiac LV within a localised time window, using supervision from colour Doppler measurements, despite their low resolution and signal-to-noise ratio. This framework couples PINN solvers at consecutive time frames with discrete temporal numerical differentiation and is thus named the "Coupled Sequential Frame PINN" or CSF-PINN.

Image-based flow simulations of fetal and adult hearts to generate synthetic colour Doppler velocity data for testing the framework. Results show that CSF-PINN can accurately predict high levels of fluid dynamics detail, including flow patterns, IVPG, vorticity structures, and WSS. This accuracy was maintained even when colour Doppler

velocity data were spatially and temporally sparse and noisy, and when complex motions of the MV were modelled. These are scenarios in which previous methodologies, including image-based flow simulations and VFM, have struggled. Additionally, a scheme for advancing fluid dynamics predictions to subsequent time windows was proposed, by using trained predictions from the previous time window to initialise networks for the subsequent window, further minimising errors.

5.1 Introduction

The study of cardiac fluid dynamics offers biomechanical insights for assessing cardiovascular health (132). Detailed cardiac fluid dynamics, such as those presented in previous chapters, were derived using image-based CFD flow simulations, where echocardiographic or MRI images are used to build dynamic mesh models for flow simulations (166, 167). Such simulations can give a general idea of energy dynamics and IVPGs. To obtain more realistic flow features near the base, cardiac flow simulations need to include heart valves as they exert great influence on the flow, and this often requires complex FSI simulations (132, 168). However, flow simulations can have discrepancies with clinical observations of flow velocities obtained from Doppler ultrasound or Phase-Contrast Magnetic Resonance Imaging (PC-MRI), and it is difficult to tweak CFD simulations to improve the match.

On the other hand, clinical measurements generally cannot provide sufficient details of cardiac fluid dynamics. Doppler ultrasound technology is capable of measuring blood flow velocities but is limited to providing one-dimensional velocities along the direction of the ultrasound beam (169). PC-MRI or 4D flow MRI have poor resolution and velocity measurements near wall boundaries can suffer from errors from averaging effects (170). It will be difficult to use these sparsely sampled field data for detailed analysis, such as calculating energy losses, or ventricular WSS.

To improve clinical measurements, past investigators have proposed VFM to generate velocity maps from colour Doppler images in 2D (171) and 3D (172) using some physics constraints. 3D VFM frameworks solve an optimization spline fitting problem to back-compute flow fields and pressures, using free-slip boundary conditions and mass conservation as constraints, and in some studies, momentum conservation constraints

as well (173). Unlike image-based CFD simulations, they have the advantage of not requiring precise flow boundary conditions, and they provide flow results with high-fidelity to image measurements. However, VFM approaches conform fully to imaged velocity and are susceptible to noise errors in measurements, which can be substantial for ultrasound Doppler. They also do not work well where there are regions of signal losses and missing measurements. Therefore, for the ultrasound modality, a high Doppler gain is needed, leading to substantial aliasing and reduced accuracy velocity measurements (174).

Recently, PINNs have emerged as a promising way for semi-supervised deep learning reconstruction of biomedical flow fields (18). Neural network optimisation is achieved by integrating the full governing laws of flow physics as constraints, allowing them to effectively leverage sparse data alongside physics constraints to reconstruct the solution field (19, 20), and derive realistic hemodynamic predictions even with real, noisy clinical data (175). This makes it ideal for application on clinical measurements of cardiac flow, which is sparse and noisy and yet enables the extraction of detailed fluid dynamics, including near-wall blood flow and WSS (21). This is possible even without explicit specification of inlet and outlet boundary conditions, which avoids the problem of error propagation from boundary condition to ventricular flow fields.

These advantages have prompted investigators to incorporate PINN in 2D VFM, where PINN was applied to 2D Doppler echocardiography (176). Investigators verified PINN's ability to address spatial and temporal data scarcity and noise in colour Doppler data and showed that PINN is consequently more accurate than the traditional VFM. Although the 2D approach is an idealization of the problem by assuming no out-of-plane flow, these initial findings are promising and motivated the current study, which aims to extend PINN-VFM to 3D.

In this chapter, PINN was employed using 4D echocardiography images and synthetic colour Doppler flow imaging (CDFI) velocity data to demonstrate its capabilities to reconstruct high-quality in-vivo cardiac flow dynamics for a short time window of 2-4 time frames. To enable this, the framework coupled PINN solvers at consecutive time frames with discrete temporal numerical differentiation. Synthetic 4D echocardiography

Doppler data to train and test the current approach so that the ground truth flow fields are available for assessing errors.

5.2 Methods

5.2.1 Fetal LV CFD model and Adult LV FSI model

Diastolic intracardiac flows are emphasized because flow reconstruction errors are more significant during this period (172). Two datasets were utilized to generate synthetic Doppler data: an echo-based dynamic mesh CFD simulation of a healthy human fetal LV, where the MV was not modelled, previously established in earlier chapters, and a smoothed particle hydrodynamic (SPH) simulation of a healthy human adult LV model where the MV was included.

The methodology for performing image-based CFD simulations of healthy fetal LVs was previously described in Section 3.1 of Chapter 3. A minor modification was made, where simulations employ a Newtonian blood viscosity of $0.0035 \text{ kg}/(\text{m}\cdot\text{s})$ as an approximation, rather than the more physiological Carreau-Yasuda model for simplicity. The human adult LV model, utilising an FSI framework to simulate MV dynamics on blood flow, was provided by Dr. Wenbin Mao, a collaborator from the University of South Florida, United States, and has been previously published (15).

Briefly, the adult LV and cardiac wall motions are reconstructed based on multi-slice CT scans, capturing different time phases. The MV geometry used in this model is not subject-specific but is adapted from a prior study (177). The material parameters for valve tissues are obtained from biaxial and uniaxial testing of healthy human MV tissues (177). The passive mechanics of the MV leaflets are modelled using a modified anisotropic hyperelastic Holzapfel-Gasser-Ogden model (178). The MV chordae tendineae is modelled using an isotropic hyperelastic Ogden model.

Intraventricular fluid dynamics is modelled via SPH while the fluid-solid interaction between blood and the MV is modelled by a nonlinear FE method (15). SPH is implemented in Abaqus/Explicit 2016 (SIMULIA, Providence, RI), with further details explained in the Appendix. The reference density is $\rho = 1056 \text{ kg}/\text{m}^3$ and shear viscosity is $\mu = 0.0035 \text{ kg}/(\text{m}\cdot\text{s})$.

The FSI model predicts physiologic MV leaflet kinematics, in terms of opening and closing times and leaflet angles, and the model shows good prediction of AV peak systolic velocities and ejection times. The predicted MV inflow waveform pattern matches echo measurements but underestimates peak velocity compared to Doppler measurements (0.74m/s vs 1.17m/s) (15).

5.2.2 CSF-PINN architecture

A PINN framework was utilised to simulate a few consecutive time instances during diastole, referred to as the “Coupled Sequential Frames PINN” (CSF-PINN) framework, as shown in Figure 28. This framework utilizes a time-marching approach to simulate the temporal evolution of intracardiac flows, where the flow field is resolved at 2-4 discrete and consecutive time instances within a window that matches the time instances of Doppler imaging. The framework can be subsequently repeated for the next time window to resolve flow fields in later time instances, using the existing PINN solution as the initial condition for this subsequent time window.

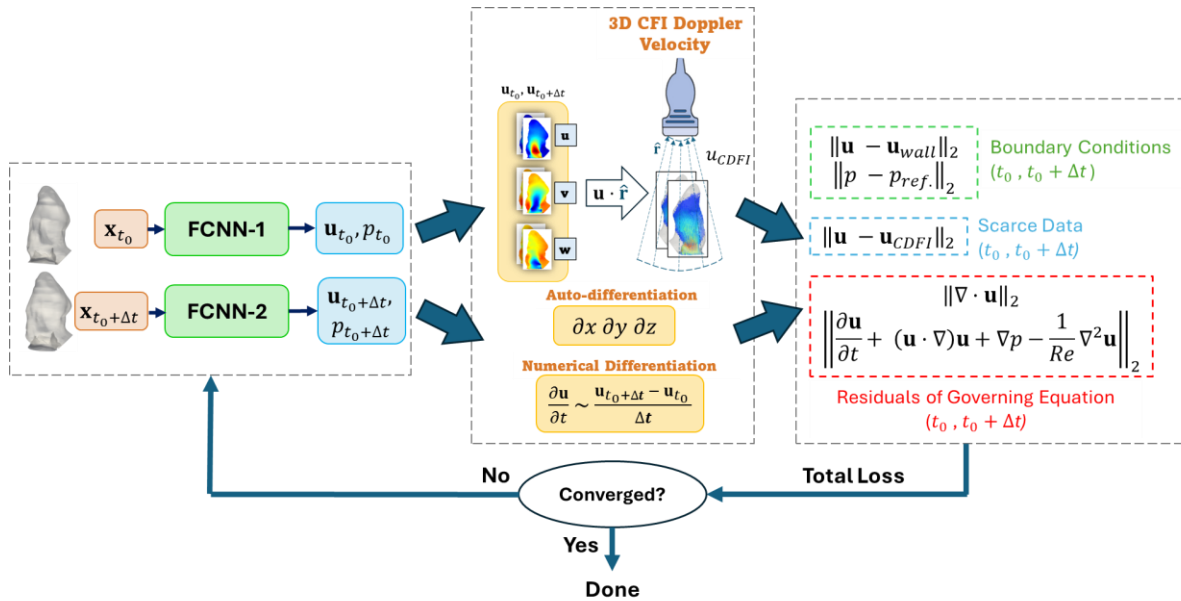


Figure 28: Schematic of the Semi-Supervised CSF-PINN Framework for simulating transient velocity (u) and pressure (p) fields within the LV at two consecutive time instances. Two fully connected neural networks are used to predict flow and pressure fields at each of the two time points (FCNN-1 for time t_0 and FCNN-2 for time $t_0 + \Delta t$). The spatial input coordinates of the LV volume (x) for respective time steps are fed into each FCNN. The output velocities are projected onto Doppler beam directions for loss computations. Temporal velocity derivatives ($\frac{\partial u}{\partial t}$) are calculated using a numerical scheme between the two FCNNs (i.e. finite difference scheme), while auto-differentiation is used to derive spatial derivatives within each FCNN to compute the residuals of the Navier-Stokes equations. Colour Doppler velocity data (u_{CDFI}) are incorporated into the loss function to ensure the PINN predictions are consistent with observed flow patterns, together with constraints of the Navier-Stokes equations and boundary conditions.

Figure 28 illustrates the two-time-point configuration of the CSF-PINN. This involves two fully connected neural networks (FCNN-1 and FCNN-2) that output flow $\vec{u}$ and p fields at two discrete time points with a time step of Δt . The framework can be adapted to incorporate additional time frames by adding additional FCNN blocks. CSF-PINN computes temporal derivatives (such as for unsteady terms in Navier-Stokes) via discrete numerical schemes between the two FCNNs, but spatial derivatives of velocity and pressures are computed using auto-differentiation within each FCNN. Temporal gradients are calculated via Lagrange polynomials: given n velocity data points $(t_0, \vec{u}_0), (t_1, \vec{u}_1), \dots, (t_{n-1}, \vec{u}_{n-1})$, the temporal gradient of velocity, $\frac{\partial \vec{u}}{\partial t}$, is calculated as:

$$\left. \frac{\partial \vec{u}}{\partial t} \right|_{t=t_i} = \sum_{i=0}^{n-1} \vec{u}_i \left. \frac{\partial l(t)}{\partial t} \right|_{t=t_i} \quad (23)$$

where, $l_i(t)$ is the i -th Lagrange basis polynomial, defined as:

$$l(t) = \prod_{\substack{j=0 \\ j \neq i}}^{n-1} \frac{t - t_j}{t_i - t_j} \quad (24)$$

Having additional time frames can enhance estimations of temporal gradients by having more discretized points in the numerical differentiation.

For each time point, the predictions of $\vec{u}$ and p is formulated as a constrained optimization problem, where the network is trained with loss functions for conforming to governing equations and boundary conditions, and for matching CDFI velocity data. The loss function $\mathcal{L}(\theta)$ is structured as a weighted sum of Mean Squared Error (*MSE*) terms across different components, each corresponding to a specific aspect of the model's performance at multiple time points, $t \in \{0, 1, \dots, n\}$, expressed as:

$$\mathcal{L}(\theta) = \sum_{t=0}^n (\omega_{physics,t} \mathcal{L}_{physics,t} + \omega_{BC,t} \mathcal{L}_{BC,t} + \omega_{CFDI_data,t} \mathcal{L}_{CFDI_data,t}) \quad (25)$$

where $\mathcal{L}_{physics,t}$ is the *MSE* of the physics-based residuals, ensuring the Navier-Stokes equations are satisfied for all collocation points that are randomly sampled at each time instance; $\mathcal{L}_{BC,t}$ measures the *MSE* of the discrepancies in the boundary conditions; and $\mathcal{L}_{CFDI_data,t}$ calculates the *MSE* of the differences between the predicted velocity vector

in the direction of the transducer source and the actual observed CDFI velocity data, u_{CDFI} . These are given as:

$$\mathcal{L}(\theta) = \sum_{t=0}^n (\omega_{physics,t} \mathcal{L}_{physics,t} + \omega_{BC,t} \mathcal{L}_{BC,t} + \omega_{CDFI_data,t} \mathcal{L}_{CDFI_data,t}) \quad (26)$$

where $\mathcal{L}_{physics,t}$ is the *MSE* of the physics-based residuals, ensuring the Navier-Stokes equations are satisfied for all collocation points that are randomly sampled at each time instance; $\mathcal{L}_{BC,t}$ measures the *MSE* of the discrepancies in the boundary conditions; and $\mathcal{L}_{CDFI_data,t}$ calculates the *MSE* of the differences between the predicted velocity vector in the direction of the transducer source and the actual observed CDFI velocity data, u_{CDFI} . These are given as:

$$\mathcal{L}_{physics,t} = \frac{1}{V_s^{-2} N_{domain}} \sum_{i=1}^N |\nabla \cdot \hat{\mathbf{u}}|^2|_{\Omega,t} + \frac{1}{(V_m V_s^{-2})^2 N_{domain}} \sum_{i=1}^N \left| \frac{\partial \hat{\mathbf{u}}}{\partial t} + (\hat{\mathbf{u}} \cdot \nabla) \hat{\mathbf{u}} + \frac{1}{\rho} \nabla \hat{p} - \nu \nabla^2 \hat{\mathbf{u}} \right|^2|_{\Omega,t} \quad (27)$$

$$\mathcal{L}_{BC,t} = \frac{1}{(V_m V_s^{-1})^2 N_{wall}} \sum_{i=1}^N (\hat{\mathbf{u}} - \bar{\mathbf{u}}_{motion\ tracking, wall})^2|_{\Gamma_{wall,t}} + \frac{1}{(V_{kg} V_m^{-1} V_s^{-2})^2 N_{inlet}} \sum_{i=1}^N (\hat{p} - p_{reference})^2|_{\Gamma_{inlet,t}} \quad (28)$$

$$\mathcal{L}_{CDFI_data,t} = \frac{1}{(V_m V_s^{-1})^2 N_{CDFI}} \sum_{i=1}^N (\hat{\mathbf{u}} \cdot \hat{\mathbf{r}} - u_{CDFI})^2|_{\Omega,t} \quad (29)$$

where V_{kg} , V_m and V_s are the unit normalization constants for mass, length, and time, respectively, $\hat{\mathbf{u}}$ is the neural network prediction for the velocity vector, $\hat{p}$ is the network prediction for the pressure, $\bar{\mathbf{u}}_{motion\ tracking, wall}$ represents the wall velocities from the motion tracking algorithm applied to B-mode images, $p_{reference}$ is an arbitrary reference pressure at the inlet boundary, $\hat{\mathbf{r}}$ represents the direction vector pointing to the transducer source, N is the number of randomly selected collocation points in the domain Ω , at specific boundaries Γ or point locations with u_{CDFI} data within the domain. These loss terms are weighted by their respective weights: $\omega_{physics,t}$, $\omega_{BC,t}$ and $\omega_{CDFI_data,t}$. The PINN framework used equal weights for all the different loss terms as it works well, but it is likely that further fine-tuning of these hyperparameters may enhance learning performance.

Although collocation points are randomly sampled at each time step from the geometry at that specific time instance, temporal derivatives remain valid because the networks are queried at shared spatial coordinates across time. Table 22 illustrates how each neural network is used to compute temporal derivatives and enforce the Navier-Stokes equations at each time point for a 3-time-point configuration. For example, suppose a random collocation point (x, y, z) is sampled from the geometry at time t_t . The corresponding Navier-Stokes residual at time t_t is computed at this location using $\frac{\partial u_0}{\partial t}$, evaluated from network outputs u_0, u_1 and u_2 at (x, y, z) . Even if this point lies just outside the geometry at $t_{t+\Delta t}$ and $t_{t+2\Delta t}$, the network can still be queried at that coordinate, allowing temporal derivatives to be computed consistently.

TABLE 22. COMPARISON OF BLOOD TURNOVER BETWEEN CFD-BASED AND THEORETICAL MIXING MODELS FOR THREE CARDIAC CYCLES.

<i>Time point</i>	<i>Network</i>	<i>Outputs</i>	<i>Temporal Derivative</i>	<i>Physics Constraint</i>
t_t	Flow_net_1st	u_0, v_0, w_0, p_0	$\frac{\partial u_0}{\partial t} = \frac{-3u_0 + 4u_1 - u_2}{2\Delta t}$	$\left \frac{\partial u_0}{\partial t} + (u_0 \cdot \nabla) + \frac{1}{\rho} \nabla p_0 - \nu \nabla^2 u_0 \right ^2$
$t_{t+\Delta t}$	Flow_net_2 nd	u_1, v_1, w_1, p_1	$\frac{\partial u_1}{\partial t} = \frac{u_2 - u_0}{\Delta t}$	$\left \frac{\partial u_1}{\partial t} + (u_1 \cdot \nabla)u_1 + \frac{1}{\rho} \nabla p_1 - \nu \nabla^2 u_1 \right ^2$
$t_{t+2\Delta t}$	Flow_net_3 rd	u_2, v_2, w_2, p_2	$\frac{\partial u_2}{\partial t} = \frac{u_0 - 4u_1 + 3u_2}{2\Delta t}$	$\left \frac{\partial u_2}{\partial t} + (u_2 \cdot \nabla)u_2 + \frac{1}{\rho} \nabla p_2 - \nu \nabla^2 u_2 \right ^2$

Due to the dynamic nature of the LV, there was a potential for points near the edges of the geometry at one time step to fall outside the domain at neighbouring time steps. One possible strategy to avoid this issue would be to restrict collocation sampling to the spatial intersection of all geometries across the time window (i.e., points guaranteed to lie within the LV at all time steps). However, this conservative approach would significantly reduce the number of usable points, particularly near the boundaries, where important flow features often occur. In this study, temporal derivatives were calculated and loss constraints were still imposed at fixed spatial coordinates, even if those locations extended marginally beyond the geometry at adjacent time steps. This choice preserved broader spatial coverage and was considered acceptable given the small temporal spacing Δt , which limited geometric variation between time frames. However, its impact on velocity prediction accuracy was not systematically tested.

5.2.3 Synthetic Colour Doppler Dataset Preparation

Synthetic CDFI velocity data was generated from the ground truth simulations to allow for comparison with PINN predictions. This approach is necessary because applying CSF-PINN to clinical Doppler scans does not provide access to the ground truth flow field data. The synthetic CDFI velocity data was created while considering the temporal and spatial resolution of clinical echocardiography, with low-velocity data points excluded. Additionally, appropriate velocity magnitude noise was incorporated into the CDFI dataset.

In the fetal cases, 4D image acquisition was previously performed using a General Electric RM6C 3D/4D curved array transducer with a frequency range of 2.0MHz-6.0MHz at capture rates of 70-90 frames per second, equating to a time step of 0.014 to 0.011 seconds (139). For adult echocardiography, typical frame rates noted in the literature range from 40 to 90 frames per second, resulting in a time step of 0.025 to 0.011 seconds (179). For the PINN model, a time step of 0.012s for fetal cases and 0.02s for adult cases was adopted based on these parameters.

Velocity data from CFD or FSI models are sparsely sampled at points arranged in a fan-shaped ultrasound zone, with points that were $0.2 \pm 0.1\text{mm}$ or $0.7 \pm 0.3\text{mm}$ apart on average. Doppler values at each location were obtained by projecting the velocity onto the direction vector pointing towards the transducer source point, which is specified to be 18cm below the centroid of the fetal hearts, mimicking the mitral commissural view, and 4cm below the apex of the heart for the adult heart, mimicking the long-axis transapical view. These distances are informed by literature reports of the distance between the transducer and the adult (180) or fetal heart (181).

Velocity data are filtered to exclude low-velocity data points, which will typically be observed at the typical gain setting, such that only 50-70% of the LV volume would have Doppler information. Typically, the temporal resolution in colour Doppler is lower than that of B-mode imaging. To address this, test scenarios were included in which the synthetic CDFI dataset exhibited temporal scarcity, with the time interval between available u_{CDFI} data being double that of the B-mode.

Errors in Doppler ultrasound velocity measurements can vary, depending on several factors, including the signal-to-noise ratio, the frequency of ultrasound used, Doppler gain settings, beamforming methods, and flow velocity. Assuming a signal-to-noise ratio (SNR) of 15-20 dB, past studies suggest that velocity errors are approximately around 2-4% (182). As a conservative estimate, the CDFI data was augmented with 5% Gaussian white noise defined as the root-mean-square noise-to-signal ratio.

5.2.4 PINN Network Training

In the FCNN (a series of fully connected layers), the output $\mathbf{y}(\mathbf{x})$, is computed from the network inputs, $\mathbf{x}$, which consists of weights $\mathbf{W}_i$ and biases $\mathbf{b}_i$, for each of the n layers. The output is formulated as:

$$\begin{aligned} \mathbf{y}(\mathbf{x}) &= \mathbf{W}_n \Phi_{n-1}(\mathbf{x}) + \mathbf{b}_n \\ \Phi_i(\mathbf{x}) &= \text{SiLU}(\mathbf{W}_i(\Phi_{i-1}(\mathbf{x})) + \mathbf{b}_i), \quad \text{for } 2 < i < n - 1 \end{aligned} \quad (30)$$

where Φ_i represents outputs of the i th layer in the network. The network size is 200 neurons per layer across 10 hidden layers. The training is conducted using the Adam optimizer (183), with an exponential learning rate decay strategy implemented in TensorFlow. The initial learning rate is set at 10^{-3} and decays to 10^{-6} after 1500 epochs, using a single NVIDIA Quadro RTX 5000 graphics processing unit (GPU) for training.

The Sigmoid Linear Unit (*SiLU*) function is used as the activation function. Networks and losses were constructed using NVIDIA's Modulus framework v22.09 (184) to speed up training via the optimization of the differentiation process. At each time point, each FCNN is trained with 100,000 wall collocation points for wall velocity boundary conditions (with a mini-batch size of 10,000), 50,000 inlet collocation points for reference pressure boundary conditions (with a mini-batch size of 10,000), and 4,000,000 fluid domain points for governing equation residuals (with a mini-batch size of 4,000). The number of collocation points used for CDFI velocity data matching depends on the spatial resolution of the synthetic CDFI dataset. For the fetal LV, at the finer spatial resolution of $0.2 \pm 0.1\text{mm}$, 60,000 observation samples are used, and at the coarser spatial resolution of $0.7 \pm 0.3\text{mm}$, 1,500 samples are used. For the adult LV, only 1 resolution setting is investigated, utilizing 25,000 samples at the resolution of $0.7 \pm 0.3\text{mm}$, as the adult LV is substantially larger than the fetal LV. A mini-batch size of 10,000

is used for CDFI velocity matching unless the number of available points is below this threshold. The PINN codes are available at <https://github.com/HSW15/CSF-PINN>.

5.2.5 Error Analysis and Haemodynamic Quantification

The performance of the PINN model is evaluated by comparing its predictions directly with data from CFD and FSI simulations at corresponding time points and locations. The accuracy of the PINN outputs is quantified using the *MSE* and the normalised root mean square error (RMSE) expressed as a percentage, as follows:

$$MSE = \frac{1}{N} \sum_{i=1}^N |y_{PINN_i} - y_{CFD_i}|^2 \quad (31)$$

$$Normalised\ RMSE = \frac{1}{\max|y_{CFD}|} \sqrt{\frac{1}{N} \sum_{i=1}^N |y_{PINN_i} - y_{CFD_i}|^2} \quad (32)$$

where y_{PINN_i} and y_{CFD_i} are the outputs from the PINN and CFD/FSI models, respectively, at each i -th test point for N number of test points, $\max|y_{CFD}|$ is the maximum value of the variable y observed from the entire CFD flow field at that time frame. The coefficient of determination, R^2 , between y_{PINN} and y_{CFD} is also evaluated.

Visualization of the vortex volumes is done using the Q -criterion with a minimum pre-defined threshold, where areas with a Q -value larger than $8000\ 1/s^2$ were considered part of the vortex volume. The vorticity of a cross-section through the LV is also calculated by taking the curl of $\vec{u}$. $\overline{WSS}$ was also evaluated, as defined previously in Section 3.1 of Chapter 3.

5.3 Results

5.3.1 Predicting Mid-Diastole Fetal Heart Flow and Vorticity Field

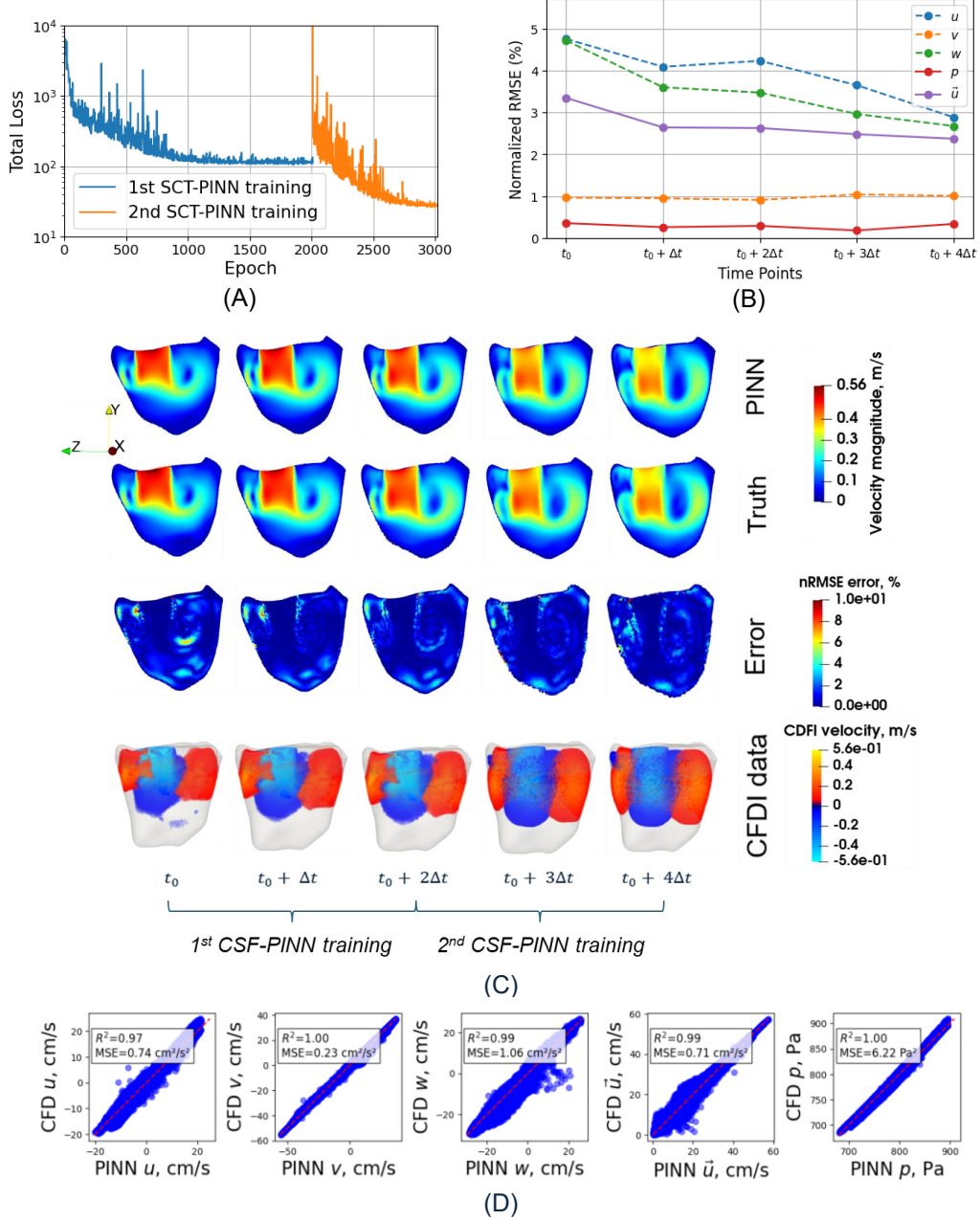


Figure 29: Evaluation of the Coupled Sequential Frames Physics-Informed Neural Network (CSF-PINN) for Intracardiac Flow Dynamics. (A) Training loss profiles over epochs for the first and second training phases of the CSF-PINN, indicating convergence behaviour. (B) Normalized Root Mean Square Errors (RMSE) for different flow variables (u , v , w , velocity component of the velocity vector, the 3D velocity vector, $\vec{u}$, and pressure, p) averaged across 5 consecutive time points. (C) The temporal sequence of flow velocity magnitude predictions by CSF-PINN compared to ground truth data from computational fluid dynamics (CFD) simulations at multiple diastolic phases (t_0 to $t_0 + 4\Delta t$), demonstrating the network's capacity to capture dynamic evolution of diastolic vortices. Below the flow fields, normalized RMSE is plotted spatially to visualize locations of errors. (D) Scatter plots between the CSF-PINN predictions and CFD data for three velocity components (u , v , w), the velocity vector, $\vec{u}$, and pressure, p . Strong linear correlations with coefficients of determination (R^2) close to 1.00 and low mean squared error (MSE) indicate high model accuracy.

Figure 29 demonstrates the good performance of the CSF-PINN applied to synthetic CDFI data from a fetal LV CFD simulation. The CSF-PINN is trained to predict flow fields during mid-diastole, where a vortex ring emanates from the MV annulus towards the apex. CSF-PINN is applied twice, to two consecutive time windows. Each application includes three-time frames, with a Δt of 0.012s, where CDFI is available for all three frames. The trained FCNN of the final time frame of the first window serves as the initial condition for all the FCNNs in the second window, and $t + 2\Delta t$ is not retrained during the second window.

Figure 29A illustrates the successful training convergence of the networks and demonstrates that applying CSF-PINN to the second time window converges better than that in the first, due to the use of results from the first application being used as the initial condition for the second. Figure 29B shows the normalised RMSE values across all three time points for the first application for u , v , w , $\vec{u}$ and p (noting that for $\vec{u}$, the norm of velocity vectors is used in the calculation as shown in equation 32). Since the primary direction of flow and vortex ring propagation are along the y -direction, magnitudes of v velocity are much higher than those of u and w , and the low errors for v can demonstrate the good performance of CSF-PINN. As u and w are much smaller than v , normalised RMSE are naturally higher, due to normalization by a smaller number. The results show that v velocity errors are in the order of 1%, while those for u and w are in the order of 2-5%, suggesting good accuracy.

Figure 29C shows that the velocity magnitude field predicted by CSF-PINN closely resembled that of the CFD ground truth at all time frames. Errors tended to be higher at low-velocity regions without Doppler guidance, such as near the apex and within the vortex. Figure 29D shows the correlation between flow variable predictions by CSF-PINN versus those from CFD truths, again demonstrating that the reconstructed flow has good accuracy, where the coefficient of determination, R^2 , was between 0.97-1.00 and the MSE was low. Similar results were obtained from investigations in a second fetal heart, as shown in Figure A5.

5.3.2 Optimal Configuration for Applying CSF-PINN

Various CSF-PINN configurations were explored to identify the optimal setup. Initially, the impact of the number of time frames used in concurrent training was examined by comparing networks involving 2, 3, and 4 time frames for fetal heart fluid dynamics. Results in Figures 30A and B show that increasing the number of time points from 2 to 3 reduces the normalized RMSE for the fetal heart case but increasing further to 4 time points does not improve accuracy, suggesting that 3 time frames are sufficient. The improvement from 2 to 3 time frames is likely due to a better time-derivative $\frac{\partial \bar{u}}{\partial t}$ estimation, where a higher-order discrete numerical time differentiation can be achieved.

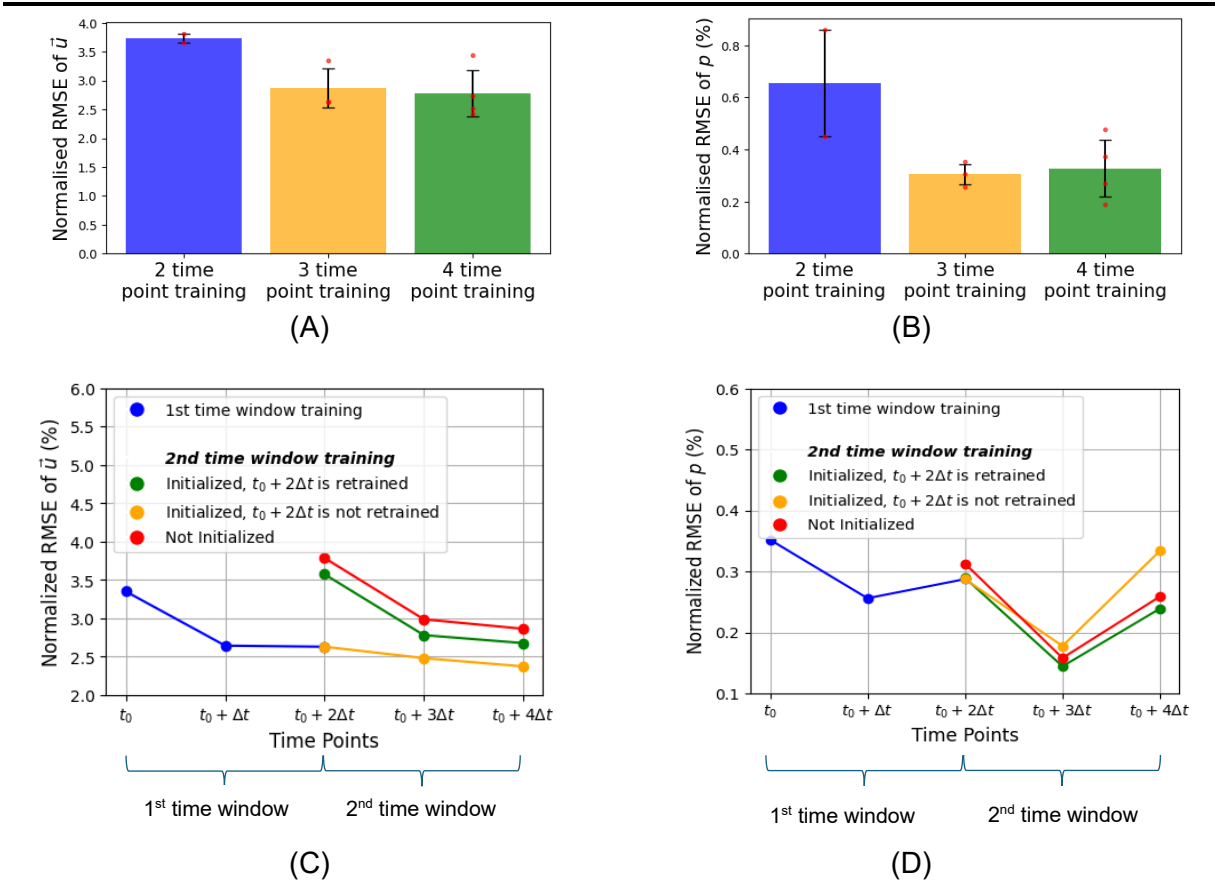


Figure 30: Performance of the CSF-PINN framework across different configurations and training approaches. This figure compares the normalized RMSE for (A) the velocity vector magnitude and (B) pressure, for CSF-PINN involving one time window of 2, 3, or 4 time points. Panels (C) and (D) explore strategies for training the second time window. After the initial training phase, networks for subsequent time points ($t_0 + 3\Delta t$, $t_0 + 4\Delta t$) are initialized with the trainable parameters from the network trained at $t_0 + 2\Delta t$. During the 2nd time window training, the network for $t_0 + 2\Delta t$ can either be retrained (green) or results for this time point from the previous time window's training can be reused without further amendments (yellow). These approaches are compared to a scenario with no initial training and no network initialization (red).

Next, various approaches for applying the 3-time-frame CSF-PINN to a second time window were compared after its application to the immediately preceding time window. The last time frame of the first window ($t_0 + 2\Delta t$) corresponds to the first time frame of the second window. This scheme allows the framework to be marched over time to obtain flow results for a wider duration. The option of initialising all time frames of the second time window using the training results from the last frame of the first time window was explored. This is accomplished by directly utilising the trained FCNN weights of $t_0 + 2\Delta t$ after the first time window training in all FCNNs during the second time window, as an initialisation before further refinement training. The option of retraining the FCNN at $t_0 + 2\Delta t$ together with other time frames in the second window, versus fixing the FCNN at $t_0 + 2\Delta t$ as trained in the first window without retraining, were also tested.

The resulting velocity errors are shown in Figure 30C. When no initialisation is performed (red line), velocity errors are the highest. When initialisation was performed and the FCNN at $t_0 + 2\Delta t$ was further trained as part of CSF-PINN training for the second time window (green line), and velocity errors were reduced marginally. However, the best results were obtained when initialisation was performed, but the trained FCNN at $t_0 + 2\Delta t$ from the first time window is not retrained (orange line). With this approach, velocity errors can be reduced to be lower than those in the first window. Figure 30D shows the corresponding pressure prediction errors. Here, normalised RMSE errors were consistently low (below 0.35%) regardless of the training strategy used, and the options considered are not consequential. Overall, the initialisation and no-retraining approach is found to be optimal, as it reduces velocity errors.

Table 23 summarises the computational times and GPU memory usage associated with different CSF-PINN training configurations. As expected, increasing the number of time frames in the first training window (from 2 to 4) results in a near-linear increase in both training duration and GPU memory demand, reflecting the growth in network complexity and parameter count. For the second time window, initialising the network with previously trained weights significantly reduces training time. Interestingly, omitting retraining of the shared FCNN at $t_0 + 2\Delta t$ not only yielded the lowest velocity errors (as shown in Figure 30C) but also led to faster training times.

TABLE 23: COMPUTATIONAL TIMES (USING 1 NVIDIA RTX4090 GPU) AND GPU MEMORY USAGE FOR DIFFERENT CSF-PINN TRAINING EXPERIMENTS.

Experiment	Computational Times, (days)	GPU memory (GB)
<u>1st time window training</u>		
2 time point training (726,406 hyperparameters)	2.04	16.1
3 time point training (1,089,609 hyperparameters)	3.11	22.2
4 time point training (1,452,812 hyperparameters)	4.37	28.4
<u>2nd time window training</u>		
Initialized, $t_0 + 2\Delta t$ is retrained	1.80	16.4
Initialized, $t_0 + 2\Delta t$ is not retrained	2.04	16.6
Not initialized	3.11	22.2

5.3.3 Effects of Sparse and Noisy Doppler Observation Data

Following this, the robustness of the 3-time-frame CSF-PINN was evaluated under conditions of sparse and noisy ultrasound Doppler measurements, where data quality and availability were systematically varied to simulate realistic clinical CDFI scenarios. Fetal heart fluid dynamics are investigated for this purpose.

Results are shown in Figures 31A and B. In the baseline scenario, high-resolution CDFI data ($0.2 \pm 0.1\text{mm}$ average data point spacing) were available for all three time points (blue line), and this produced the lowest errors. To test the model's ability to handle missing data or scenarios where colour Doppler frame rates were lower than B-mode, CDFI datasets at the second time frame ($t_0 + \Delta t$) were omitted, effectively halving the frame rate (orange line). This leads to a modest increase in average normalized RMSE, $0.4 \pm 0.1\%$ for $\bar{u}$ and $0.6 \pm 0.4\%$ for p from the baseline.

Next, the impact of reduced spatial resolution was explored, by adopting CDFI data with $0.7 \pm 0.3\text{ mm}$ average data point spacing, but where Doppler data was available for all three time frames (red line). This resulted in increases of $0.89 \pm 0.07\%$ for $\bar{u}$ and $0.11 \pm 0.04\%$ for p from the baseline. In the third scenario, both reduced Doppler frame rate and lower spatial resolution (green line) were combined and tested. This leads to a $0.98 \pm 0.10\%$ increase in $\bar{u}$ error and a $0.29 \pm 0.11\%$ increase in p error from baseline. Lastly, 5% Gaussian white noise was added to the reduced Doppler frame rate and low spatial resolution scenario (black line). This elevated $\bar{u}$ errors by $1.29 \pm 0.13\%$ and p errors by $0.34 \pm 0.11\%$ compared to baseline, which are not substantial. Figure 31C

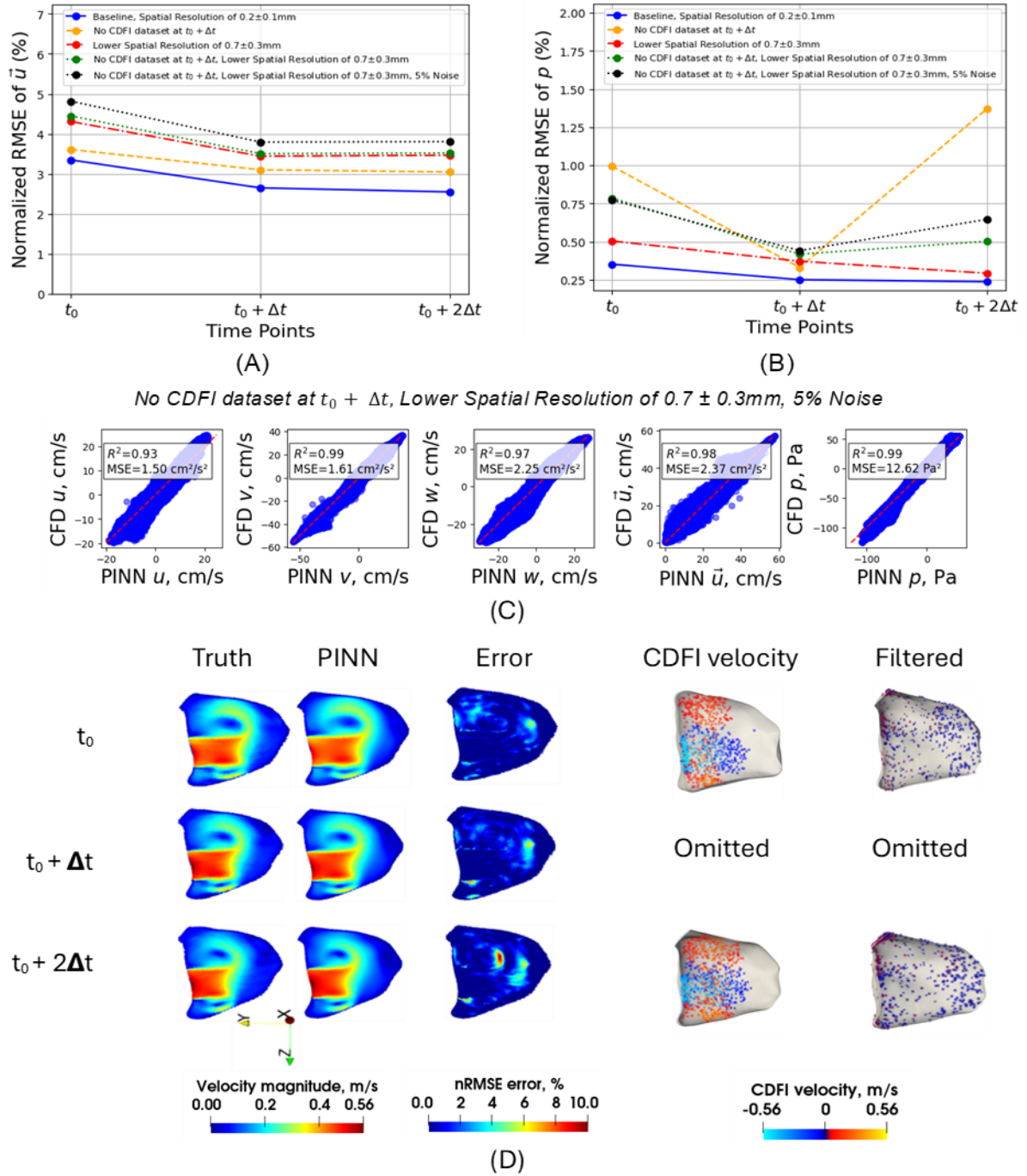


Figure 31: Impact of Data Availability and Resolution on CSF-PINN Performance. Panels (A) and (B) display the normalized RMSE for velocity vectors and pressure across different data scenarios using a 3-time-point training CSF-PINN configuration. These scenarios include training with high-resolution CDFI data with an average data spacing of 0.2 ± 0.1 mm (blue, yellow) or with low-resolution CDFI data with an average data spacing of 0.7 ± 0.3 mm (red, green, black); and training with CDFI being available at all time points (blue, red), or with CDFI not being available in the second time point (yellow, green, black); and training with CDFI data with the addition of 5% random noise (black), or without the addition of this noise (blue, yellow, red, green). Results show that CSF-PINN remains robust with accurate predictions despite suboptimal data conditions. Panel (C) illustrates the scatter plot between CFD simulation results and PINN predictions at $t_0 + \Delta t$ under the conditions of lower spatial resolution, missing CDFI data and 5% noise, again demonstrating good accuracy despite suboptimal training data, with coefficients of determination (R^2) close to 1.00 and low mean squared error (MSE). Panel (D) shows the flow field visualizations for CSF-PINN predictions, ground truth, and the

corresponding error at three time points. The predicted velocity magnitude fields show substantial similarity to the ground truth.

further shows that under this worst scenario, the correlation between PINN predicted flow parameters and ground truth values remained high, with R^2 of 0.91-0.99, and a good, low MSE . Figure 31D further shows that the flow field predicted by CSF-PINN remains reasonably like the ground truth.

The same investigations were repeated for a second fetal LV, as shown in Figure A5. The effects of sparse data conditions and noise were stronger for the second fetal heart, resulting in some flow parameters having normalised RMSE above 10%. This is because the second fetal LV was much smaller than the first, having an LV volume that is approximately half of the first LV. Consequently, only 350 CDFI data points per time step are available for training, compared to 1500 data points for the first heart. However, as Figure A5 shows, the flow fields retain substantial visual similarity to the ground truths.

The worst scenario of 5% noise with reduced Doppler frame rate and low spatial resolution was used for the adult heart in Figure 32. Nonetheless, convergence is still achieved quickly (Figure 32A), which is faster than in the fetal LVs, and normalised RMSE errors were around 5%, similar to those in the first fetal LV (Figure 32B). This was despite the time step used in the adult LV, set according to the typical frame rate of adult echocardiography. However, the higher error at the second time frame, compared to the first and third, is likely due to the unavailability of CDFI data for that time point. Figure 32C showed that the CSF-PINN predicted flow fields had substantial similarity to the FSI ground truth. Errors are localized around the valve leaflets but were more pronounced in the inflow region at $t_0 + \Delta t$. A maximum of 12% normalised RMSE error was observed in this region. Higher errors at $t_0 + \Delta t$ is likely due to the larger time gap of 0.04 s between CDFI data in adult heart imaging, compared to 0.024 s in fetal cases, and may be related to the highly dynamic nature of the MV.

Figure 32D shows that despite sparse data, large time intervals and noise, the predicted flow parameters maintained strong linear correlations with ground truths, with R^2 of 0.93-0.98. The MSE is higher than in the fetal case (Figure 32D) due to the greater

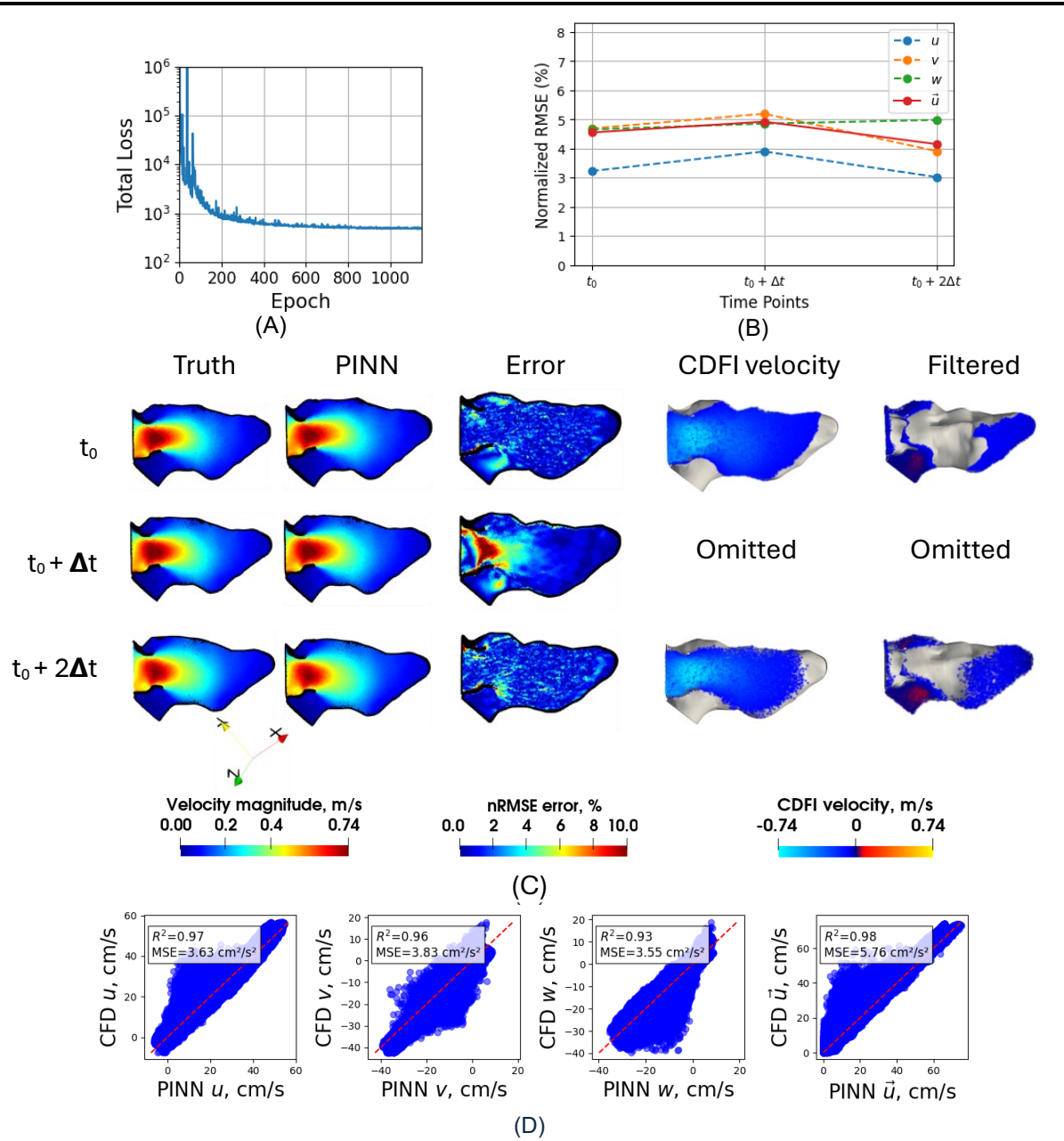


Figure 32. Effectiveness of the CSF-PINN in modelling adult heart dynamics with mitral valve. Here, CDFI data is assumed unavailable for $t_0 + \Delta t$, and 5% random noise is added to CDFI. Panel (A) displays the training loss profile, indicating convergence after approximately 500 epochs. Panel (B) illustrates the normalized RMSE for various flow components (u , v , w) and the velocity vector ($\vec{u}$) across three time points, demonstrating reasonably low errors and stability over time. Panel (C) showcases the temporal progression of velocity magnitude predictions by the CSF-PINN compared to the CFD ground truth across three diastolic phases (t_0 to $t_0 + 2\Delta t$), with error plots below indicating spatial variability of errors. Finally, panel (D) features correlation plots for velocity components (u , v , w) and velocity vector $\vec{u}$ between CSF-PINN predictions and CFD data. These plots show strong linear relationships with high coefficients of determination (R^2) and low mean squared error (MSE) values, demonstrating reasonable accuracy of the CSF-PINN predictions.

velocity range, with peak velocities reaching 0.74m/s compared to 0.56m/s in the fetal case.

Overall, it can be concluded that despite a substantial reduction in Doppler data availability and resolution, along with the introduction of noise, CSF-PINN's performance remains robust, maintaining high accuracy and fidelity even under suboptimal data conditions.

5.3.4 Vortex Dynamics, Wall Shear Stress, and Pressure Gradient Predictions

Figure 33 provides additional comparisons between CSF-PINN and CFD results for the first fetal heart, demonstrating a good match, and thus highlighting CSF-PINN's ability to capture detailed intraventricular fluid dynamics. Figure 33A presents the Q -criterion iso-vorticity contour surfaces while Figure 33B shows vorticity magnitudes along a cross-section of the heart. They both demonstrate that essential vortex structures and shear layers of various scales are captured by the PINN predictions. However, subtle differences from ground truth can be observed in more quiescent regions such as at the apical region of the heart.

Figure 33C shows the comparison of WSS patterns. There is generally a satisfactory match in terms of critical zones of high and low WSS magnitude, however, there are more pronounced differences with ground truth than is observed for vorticity, particularly for $t_0 + 4\Delta t$, where certain regions of high shear stress are under-predicted by CSF-PINN. This is likely because velocities near the walls are small, and thus lack Doppler velocity supervision, leading to higher errors. Further, as WSS are computed as gradients of velocities, there is propagation of velocity errors during gradient computation to amplified errors. To mitigate this, future work can explore methods like importance sampling to prioritize near-wall regions (185) or Coupled Automatic-Numerical PINN, which uses a stencil-based method that combines numerical and automatic differentiation to enhance velocity gradient approximations (186).

Figure 33D shows that wall pressure distributions are faithfully replicated by CSF-PINN. At time point t_0 , the predicted IVPG, a clinically relevant parameter (187)) is 1.14 mmHg, which is closely aligned with the CFD ground truth of 1.12 mmHg. Similarly, at $t_0 + 4\Delta t$, the model accurately predicts an IVPG of 0.71 mmHg, which matches the CFD ground truth of 0.72 mmHg well.

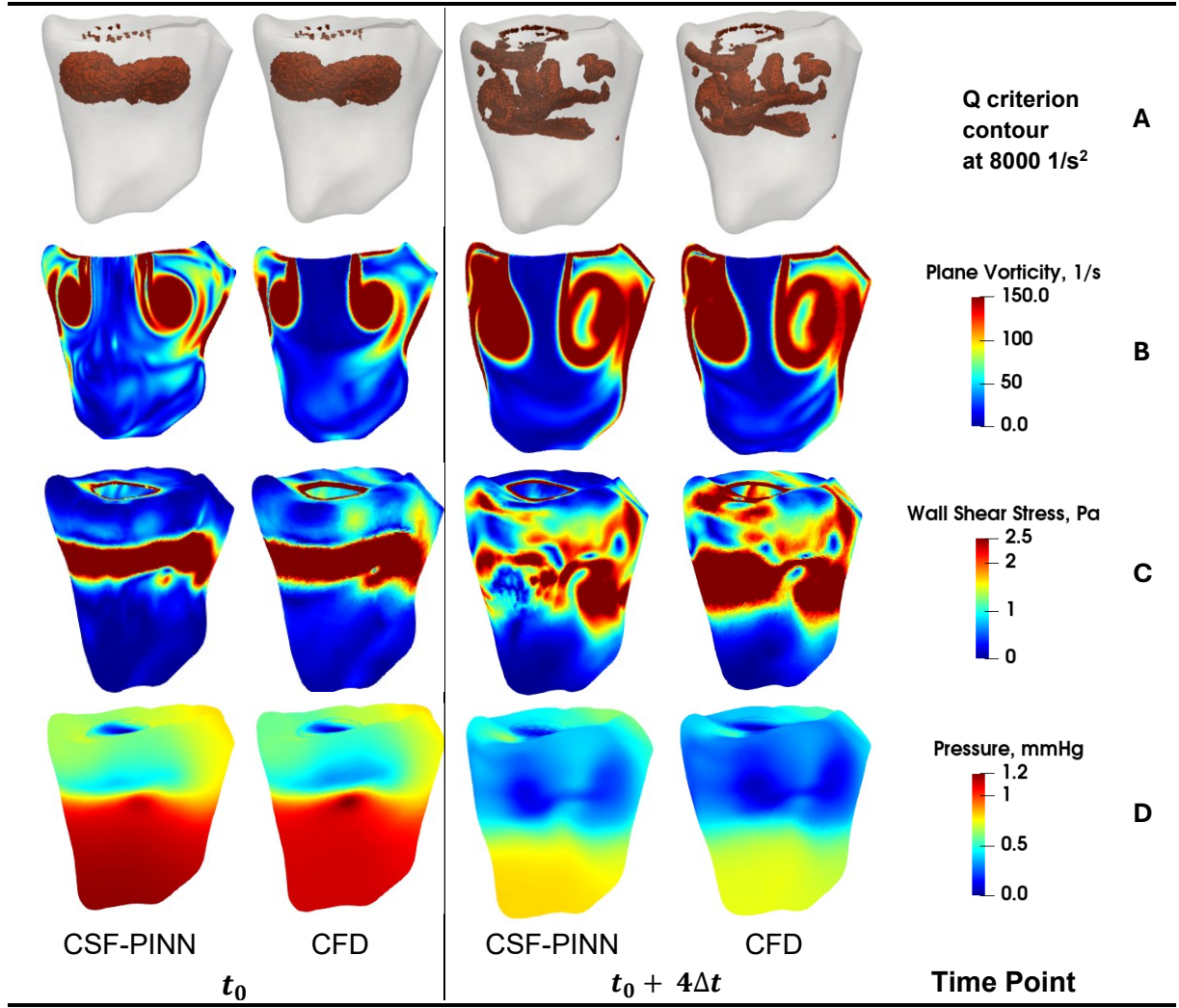


Figure 33: Hemodynamic metrics derived from CSF-PINN and CFD methods. The figure compares the CSF-PINN predictions and CFD simulations at the initial time point, t_0 and at $t_0 + 4\Delta t$. Panel (A) illustrates the Q criterion contours used to identify vortices within the heart, set at 8000 1/s^2 , revealing the diastolic vortex ring and vortical structures. Panel (B) and (C) rows display plane vorticity and wall shear stress distributions, respectively. Panel (D) shows the pressure distribution within the ventricular wall, measured in mmHg . Results demonstrate the PINN's ability to reasonably accurately reproduce vorticity, wall shear stress and wall pressure variations observed in CFD simulations.

5.3 Discussion

The CSF-PINN framework was proposed for reconstructing 3D velocity and pressure fields within the cardiac LV over localized time windows, achieving a strong alignment with synthetic 4D clinical colour Doppler measurements. This approach discretises the time domain, instead of using it as an input dimension to the PINN and introduces a time-marching method to solve consecutive time windows, ensuring continuity and consistency across them. The findings show that the CSF-PINN framework achieves high accuracy when compared to CFD and FSI references, effectively predicting large-scale flow patterns, vorticity structures, and intraventricular pressure fields, while

reasonably estimating WSS. The accuracy of CSF-PINN is influenced by the noise level and volume of Doppler observation data; however, it remains robust even with the sparse and noisy Doppler measurements typically encountered in clinical settings.

Alternative methods for reconstruction include image-based biomechanics simulations like CFD, SPH, and FSI, as well as flow mapping techniques that interpolate Doppler measurements within physics constraints. The CSF-PINN approach offers distinct advantages over these alternatives.

VFM involves regularising colour Doppler measurements with conservation laws and boundary conditions via a linearized least square optimisation scheme (171). Its computation is very fast, and results can be obtained quickly, while the PINN approach requires substantial time and advanced GPU computational equipment. However, PINN enables better and smarter regularisation. It strives for flow field results with a best match to Doppler measurements instead of directly adopting Doppler measurements like VFM, and as such it is less susceptible to errors and noise in Doppler measurements. Errors from the CSF-PINN compare well with a previous 3D VFM report (172), especially for the u and w velocities (non-radial velocities), where the r^2 between CFD and VFM is reported to be $0.57^2 - 0.78^2$, while that between CFD and CSF-PINN is found to be above 0.90. Further, VFM performs poorly when there are large spatial gaps of Doppler measurements (176), which can be difficult to avoid clinically, while PINN can function well even with sparse Doppler measurements.

Flow simulations such as CFD are highly accurate methods for computing fluid dynamics, and achieving similar accuracy via PINN approaches can be challenging. However, compared to CFD, PINN can achieve a best-fit match with clinical Doppler measurements, ensuring high fidelity of the reconstructed flow fields to those occurring in the patient. This is not easily achieved with traditional CFD, because CFD is controlled by boundary conditions, and iteratively adjusting these conditions at the boundaries to enable a match with Doppler measurements in the middle of the flow field is challenging. Additionally, the accuracy of CFD flow simulations often depends on accurate inflow and outflow boundary conditions, which can be difficult to obtain from clinical scans (132). With a PINN approach, these boundary conditions are not required, as the algorithm will seek the best-fit boundary conditions complying with physics and Doppler matching loss

functions (19, 21). Furthermore, accurate simulations of flow require that the MV is modelled in the simulation, as the MV extends substantially into the LV fluid chamber, and exerts substantial influence on the flow field (15). Modelling the MV may require complex efforts, necessitating FSI concurrent modelling of valve tissue mechanics and blood fluid mechanics (168). PINN on the other hand, retains the ability to reconstruct ventricular flow fields well even without careful consideration of valve motion and mechanics.

The current CSF-PINN approach is designed for investigations of specific time instances, decoupling the time dimension from the spatial dimension in the inputs, employing discrete numerical differentiation for time differentials, and modelling flow fields at different time instances using separate networks. The typical PINN architecture models time as an input dimension but doing this requires a network with a much larger size and higher complexity, which will further increase the already very high computational burden of PINN training. With the current time discretisation scheme, it enabled easier convergence and good results with a reasonably low computational burden. Past investigators have also proposed a similar reduction of the time dimension in PINNs. For example, SeqPINN reconstructs steady-state solutions for each time frame while refining the solution to match ultrafast Doppler image data (188). Furthermore, the PPINN is proposed to solve time-dependent differential equations in a time-marching fashion similar to the CSF-PINN. It uses a fast coarse-grained solver for network initialization, before refining the predictions by each time subdomain network (189).

Despite this dimensional reduction, CSF-PINN remains limited by the substantial time required for training, as is the case for all PINN algorithms generally. However, there are several promising approaches to reduce computational time through GPU parallelisation. For example, the PINN algorithm can be parallelised by concurrently dividing different mini batches to different GPUs during the training. Further, parallelisation through domain discretisation can be performed. For example, hard domain decomposition to both the space and time domains for parallelisation is demonstrated by the cPINN and XPINN algorithms (190, 191). Additionally, a soft domain partitioning approach using a gating network that allowed for flexible and trainable domain decompositions is demonstrated by Hu et al. (192).

5.4 Limitations

Our work has the following limitations. First, we assumed that the anatomic reconstruction and wall boundary motions were accurately retrieved for use in CSF-PINN. In reality, there are likely errors in both, which may increase PINN errors. Although CSF-PINN is capable of remaining robust under noisy velocity and location measurements, these errors can have stronger effects on WSS estimations. An appropriate model of these errors is required to test their effects on PINN accuracy in future.

Secondly, the adult heart FSI model used is based on SPH simulations. SPH is a meshless method based on computations at unstructured node locations, without rerunning the simulation, it is difficult to accurately extract spatial gradients of parameters. We have thus not evaluated the accuracy of parameters requiring spatial gradients. Further, SPH does not solve the continuity equation as accurately as CFD, and this likely contributed to the higher observed errors.

Thirdly, while our framework has been validated using simulated data, it has not been tested on real in vivo data, which will be essential to fully evaluate its robustness. Clinical acquisitions often introduce additional complexities such as noise, motion artefacts, and velocity aliasing, which may affect the model's performance. Further development is thus likely needed before CSF-PINN is ready for clinical translation. Addressing these challenges, including aliasing, will be an important next step, and deep learning approaches can potentially help in this regard (193).

5.4 Conclusion

In conclusion, the study demonstrates that the CSF-PINN framework, which discretises the time domain rather than using time as a PINN input dimension, is a promising approach for accurately reconstructing 3D velocity and pressure fields within the LV in localised time windows, closely matching Doppler echocardiography data. This method offers advantages over traditional CFD and VFM techniques, particularly when Doppler observational data are sparse or noisy. However, the computational burden

remains high, leading to lengthy training times, which warrants further investigation for improvement.

Chapter 6

Conclusions

One of the primary objectives of this thesis was to characterize the acute haemodynamic changes in the LV following the FAV procedure in the CAS-eHLHS cohort. The rationale behind FAV is to relieve the stenosis of the AV and increase blood flow through the left side of the heart. In doing so, it promotes the growth of left-sided heart structures and changes the trajectory of LV growth for the remainder of gestation. This approach is grounded in the “no flow, no growth” aetiology of HLHS, which suggests that appropriate flow stimuli are essential for normal cardiogenesis.

Chapter 3 demonstrated that the native flow haemodynamic in CAS-eHLHS LVs differ significantly from those in healthy LVs. These differences are characterised by a high-velocity inflow vortex ring that travels through the LV with minimal interaction with the LV walls, remaining relatively unperturbed until it reaches the apex. To ensure CFD-simulated peak velocities matched the elevated patient-specific Doppler peak flow velocities, the MV inlet orifice had to be considerably smaller which indicates some inflow restriction is inherently present, which resulted in low WSS areas in many regions inside the LV. The lack of WSS mechanical stimuli on the ventricular walls is compounded by its significant increase in energy that must be expended to squeeze flow out of the LV and poor blood turnover. These findings offer up potential culprits in future investigations into the mechanotransduction pathways that lead to poor LV growth and eventual hypoplasia.

Chapter 4 provided further insights into the changes following FAV, particularly highlighting the role of AV regurgitation in increasing stroke volume and likely EF. It also significantly elevated WSS, at least doubling it, while further exacerbating diastolic energy losses. Although there were common features across all post-FAV cases, those with a UV outcome exhibited more quiescent flow, reminiscent of pre-FAV LVs. In contrast, flow changes in post-FAV cases with a BV outcome were more pronounced,

with substantial secondary flow vortices being generated. It can be postulated that there exist inherent differences in mid-gestational haemodynamics between CAS-eHLHS fetuses that are born with UV versus BV circulation. However, any deductions or conclusions about this topic are limited due to the availability of UV cases with a full set of scans. Identifying predictors of UV and BV postnatal outcome amongst the CAS-eHLHS cohort has been key in improving FAV efficacy.

Chapter 5 pivoted into the use of PINNs to extract in-vivo haemodynamics, instead of image-based CFD simulations. The iterative tuning of CFD models was a time-intensive process that took at least two or three weeks to complete for each case. While a good match was obtained, there are slight mismatches in the flow velocity waveform and magnitude. In contrast, the PINN framework presented in this chapter enabled the reconstruction of 3D velocity and pressure fields directly from colour Doppler data. Although training a PINN across 30–40 time points to fully represent the cardiac cycle could take 4–6 weeks in its current implementation, the approach shows strong potential. With further optimisation, PINNs could provide accurate and robust haemodynamic reconstructions over localized time windows, offering better consistency with Doppler observations and addressing limitations of CFD and VFM methods, especially in cases of sparse or noisy input data. Unlike conventional PINN architectures, where time and spatial coordinates are used as inputs to a single network, this approach represents each time point with its network, which likely facilitates easier network optimization.

Future Work

On the topic of CAS-eHLHS, several areas of future work emerge from the findings of this thesis. Firstly, investigating the differences in fluid mechanics between UV and BV natural history cohorts for CAS-eHLHS cases that meet the criteria for FAV can address a significant gap in the current knowledge. This gap involves distinguishing between fetuses that would naturally develop a BV postnatal outcome without intervention and those who truly require FAV to avoid progression to HLHS. Applying, the current image-based CFD methodology to analyse the haemodynamic features of CAS-eHLHS fetuses

and correlating these with natural history outcomes could provide critical insights that impact patient selection of FAV.

Secondly, increasing the number of pre-FAV CAS-eHLHS cases in the current CFD study could help further delineate differences in fluid mechanics between UV and BV cohorts. Identifying these differences would deepen our understanding of the factors that contribute to successful BV outcomes and could refine FAV candidate selection criteria.

Thirdly, longitudinal studies on post-FAV outcomes are essential. Monitoring these patients into later gestational stages would offer valuable insights into how the initial changes induced by FAV can rehabilitate cardiac development for the remainder of gestation. These studies could reveal whether early improvements in haemodynamics translate into long-term success, such as the likelihood of achieving BV circulation, and help provide predictive markers for patient prognosis.

Even if fluid mechanics can address some of these issues in the management of CAS-eHLHS, its clinical application will always be held back by the time-intensive process of image-based CFD simulations, which require significant computational resources and time to produce prospective data. This delay makes it difficult to incorporate fluid dynamic findings into real-time clinical decision-making. PINN-based flow reconstruction presents a compelling future direction, especially in light of advances in GPU parallel computing and hardware acceleration. Although the current implementation of CSF-PINN has a relatively slow training time (approximately 1 day per time point) and some subtle discrepancies in WSS pattern predictions, leveraging GPU parallelisation and gradient-enhanced methods provides promising avenues for improving the PINN framework.

These technological developments could greatly expedite the analysis of haemodynamics, which is particularly valuable in the context of CAS-eHLHS. PINN-based methods have the potential to scale up the number of cases analysed, providing clinicians with a more robust dataset for FAV criteria refinement. Moreover, by enabling rapid and precise haemodynamic assessments, PINN-based approaches could move toward near-real-time predictions, thus enhancing clinical decision-making and ultimately improving patient outcomes. This framework can support the extraction of

high-resolution haemodynamic metrics like wall shear stress (WSS), vorticity, and intraventricular pressure gradients (IVPG). These parameters are typically inaccessible using conventional imaging or current clinical software.

Looking forward, a long-term vision of this work would be to integrate PINN-based haemodynamic reconstruction into a clinical pipeline. A fetal cardiologist could acquire 4D colour Doppler scans during routine assessment, which could then be processed by an automated CSF-PINN module to produce a haemodynamic report within hours. This report would highlight abnormal flow features and quantify energy losses, pressure drops, and flow inefficiencies, potentially supporting decisions about FAV timing, candidacy, or post-procedural monitoring. For this to be clinically viable, further validation on real Doppler data, automation of preprocessing steps, and prospective clinical studies are needed.

One key factor affecting CSF-PINN accuracy is the resolution of the Doppler input. The effect of spatial versus temporal resolution depends on the imaging context. In adult hearts, where temporal resolution is often low, accuracy is more sensitive to timing. In fetal hearts, where spatial resolution is limited, spatial detail has a bigger impact on accuracy. Since Doppler imaging involves a trade-off between spatial and temporal resolution, there is likely an optimal configuration that minimises reconstruction errors. This trade-off may also vary depending on the pathology. For example, in cases with weak or slow flow like cardiomyopathy, spatial resolution may be more important to capture fine features. In highly dynamic cases like aortic regurgitation or volume overload, temporal resolution may be more important to resolve fast-changing flow patterns.

Identifying these optimal imaging settings will be key to improving the reliability of CSF-PINN in clinical workflows. Future work should focus on mapping how imaging resolution affects reconstruction quality across a range of cardiac conditions.

Chapter 7

Original Contributions

Here the list of peer-reviewed publications, publications in progress and conference presentations is presented.

7.1 Peer-Reviewed Publications

- 1) H.S. Wong, H. Wiputra, A. Tulzer, G. Tulzer, C.H. Yap, Fluid Mechanics of Fetal Left Ventricle During Aortic Stenosis with Evolving Hypoplastic Left Heart Syndrome, *Annals of Biomedical Engineering*, (2022).
- 2) H.S. Wong, B. Li, A. Tulzer, G. Tulzer, C.H. Yap, Fluid Mechanical Effects of Fetal Aortic Valvuloplasty for Cases of Critical Aortic Stenosis with Evolving Hypoplastic Left Heart Syndrome, *Ann. Biomed. Eng.*, 51 (2023) 1485-1498.
- 3) H.S. Wong, W.X. Chan, B.H. Li, C.H. Yap, Strategies for multi-case physics-informed neural networks for tube flows: a study using 2D flow scenarios, *Scientific Reports*, 14 (2024) 11577.
- 4) H.S. Wong, W.X. Chan, C.H. Yap, 3D velocity and pressure field reconstruction in the cardiac left ventricle via physics informed neural network from echocardiography guided by 3D color Doppler, *Computer Methods and Programs in Biomedicine*, (2025) 108671

7.2 Conferences Oral Presentations

1. **27th Congress of European Society of Biomechanics (ESB)**. Fluid mechanics of Fetal Aortic Valvuloplasty for Critical Aortic Stenosis with Evolving Hypoplastic Left Heart Syndrome. 26-29th June 2022, Porto, Portugal
2. **9th World Congress of Biomechanics (WCB)**. Fluid mechanics of Fetal Aortic Valvuloplasty for Critical Aortic Stenosis with Evolving Hypoplastic Left Heart Syndrome. 10-14 July 2022, Taipei, Taiwan.
3. **The Annual UK gathering of Biomedical Engineers, Medical Engineers and Bioengineers (BioMedEng23)**. Strategies for multi-case physics-informed neural networks for tube flows: a study using 2D flow scenarios, September 2023, Swansea.

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Appendix

Z-score computation method

Z-scores for fetal cardiac structures were computed using the formula and regression data provided by Schneider et al., which allow normalisation of cardiac measurements to fetal size using femur length (FL), biparietal diameter (BPD) or gestational age (GA). The Z-score quantifies how many standard deviations a measured cardiac dimension deviates from the predicted normal value for that gestational size. It is calculated using the following formula:

$$Z\ score = \frac{\ln(actual) - \ln(predicted)}{root\ MSE} \quad (Ai)$$

$$\ln(predicted) = m . \ln(FL , BPD\ or\ GA) + c \quad (Aii)$$

where actual is the measured cardiac dimension, predicted is the expected cardiac dimension, *root MSE* is root mean square error for the regression, as reported by Schneider et al (69). The values of *m* and *c* are established through log-linear regression based on measurements from a large normative dataset of healthy fetuses, allowing for gestation- and size-adjusted assessment of cardiac structures.

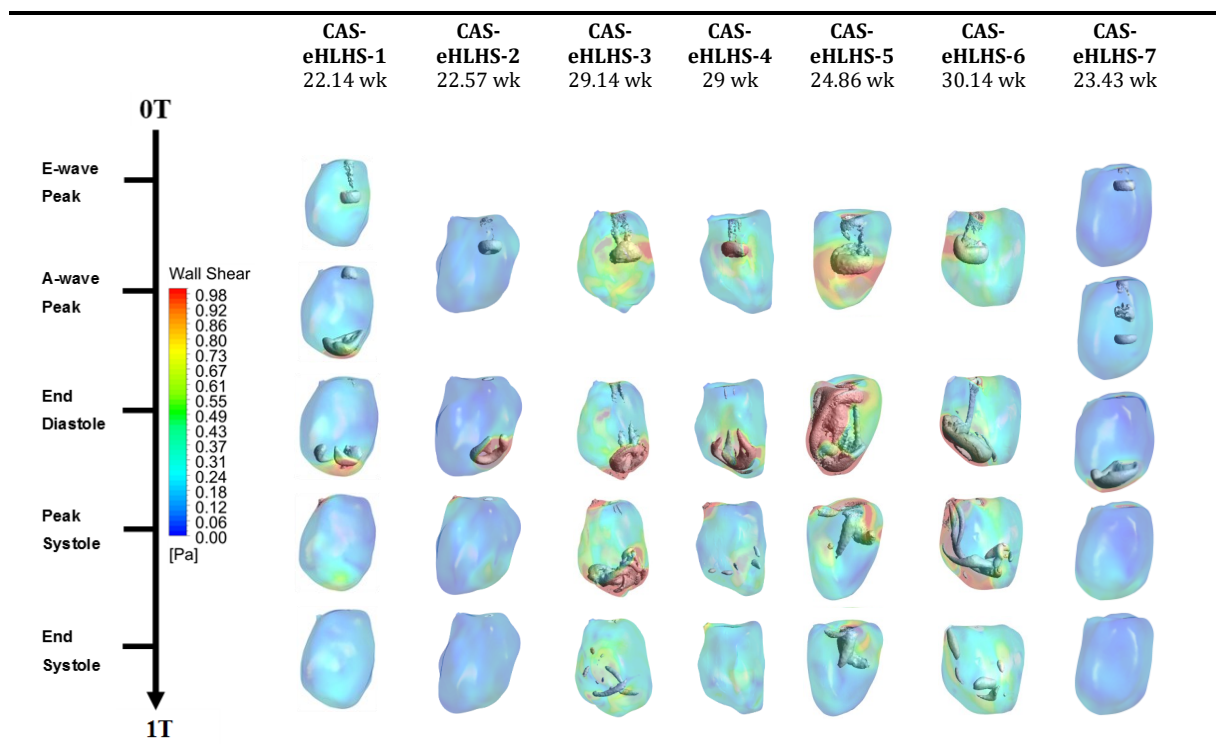


Figure A1: Wall shear stress colour contour and λ_2 iso-velocity surfaces in CAS-eHLHS left ventricles (before fetal aortic valvuloplasty).

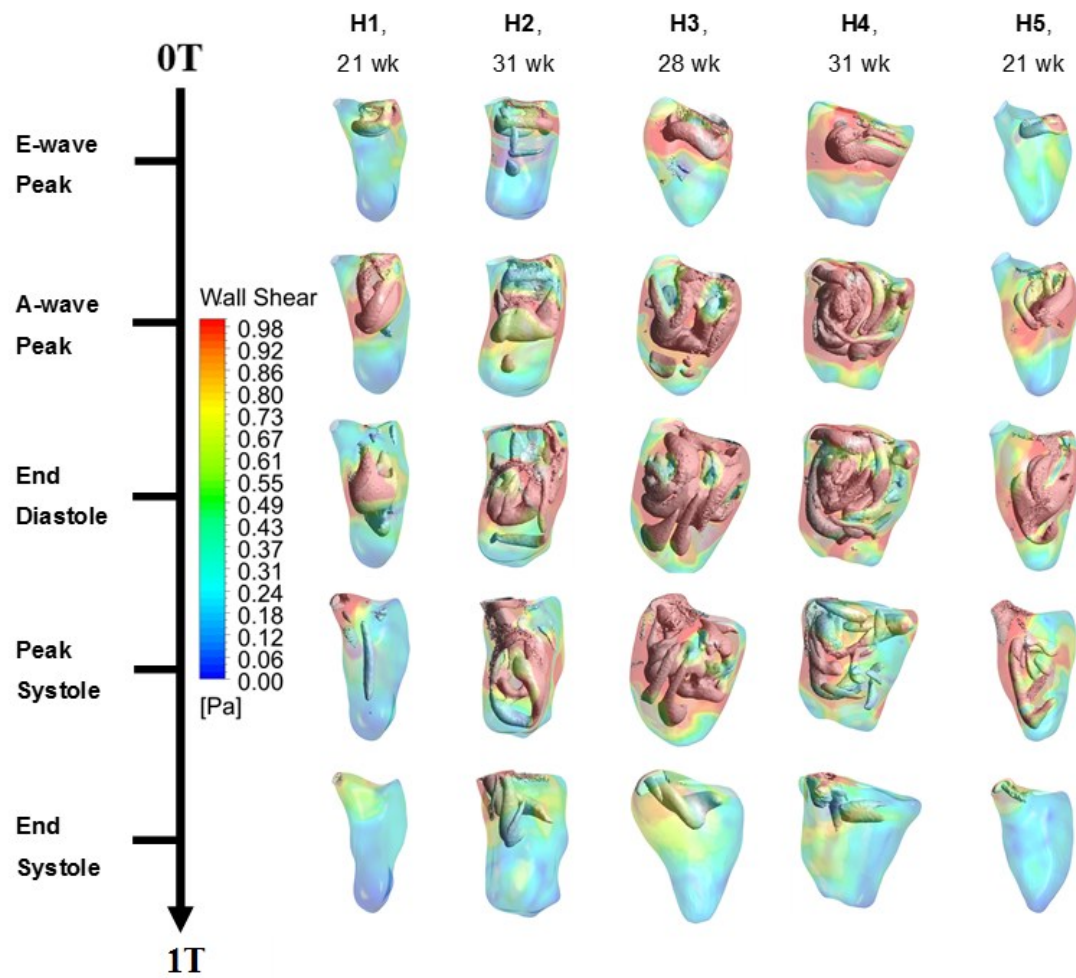


Figure A2: Wall shear stress colour contour and λ_2 iso-velocity surfaces in healthy left ventricles.

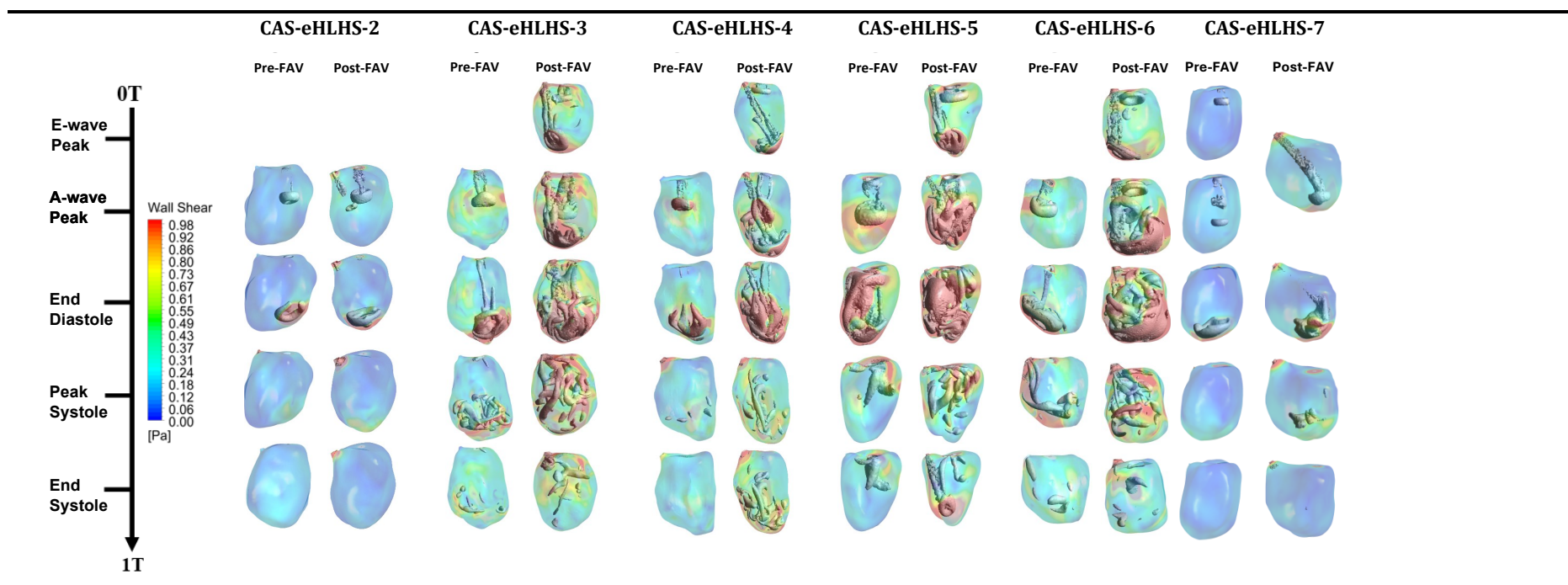


Figure A3: Wall shear stress colour contour and λ_2 iso-velocity surfaces for pre- & post-FAV cases

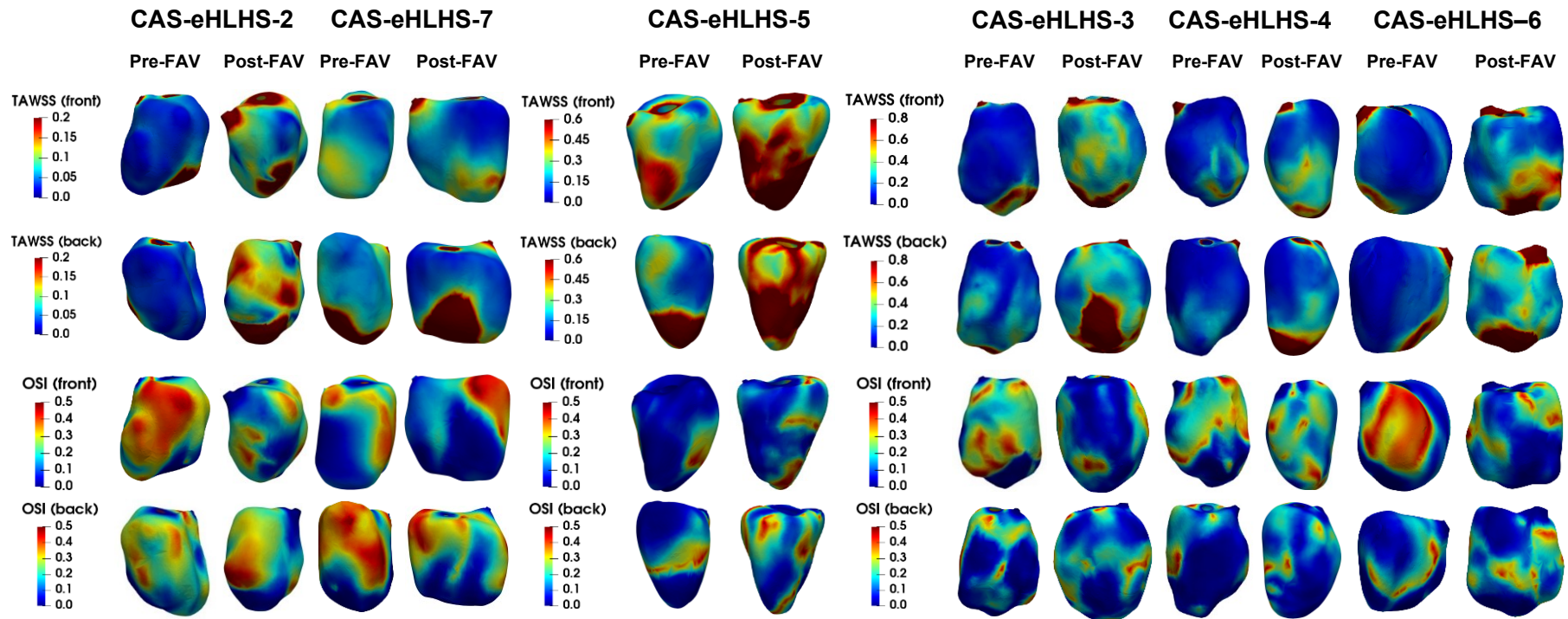


Figure A4: Time-averaged wall shear stress (TAWSS) and oscillatory shear index (OSI) distributions on the left ventricle (LV) surface for multiple CAS-eHLHS cases, both pre- and post-FAV intervention. The figure presents front and back views of each LV, demonstrating the spatial variation in shear stress and the extent of oscillatory shear experienced across different regions. The TAWSS and OSI maps reveal the localized differences in mechanical stress on the LV wall before and after the FAV procedure, highlighting the impact of the intervention on the hemodynamic environment within the LV.

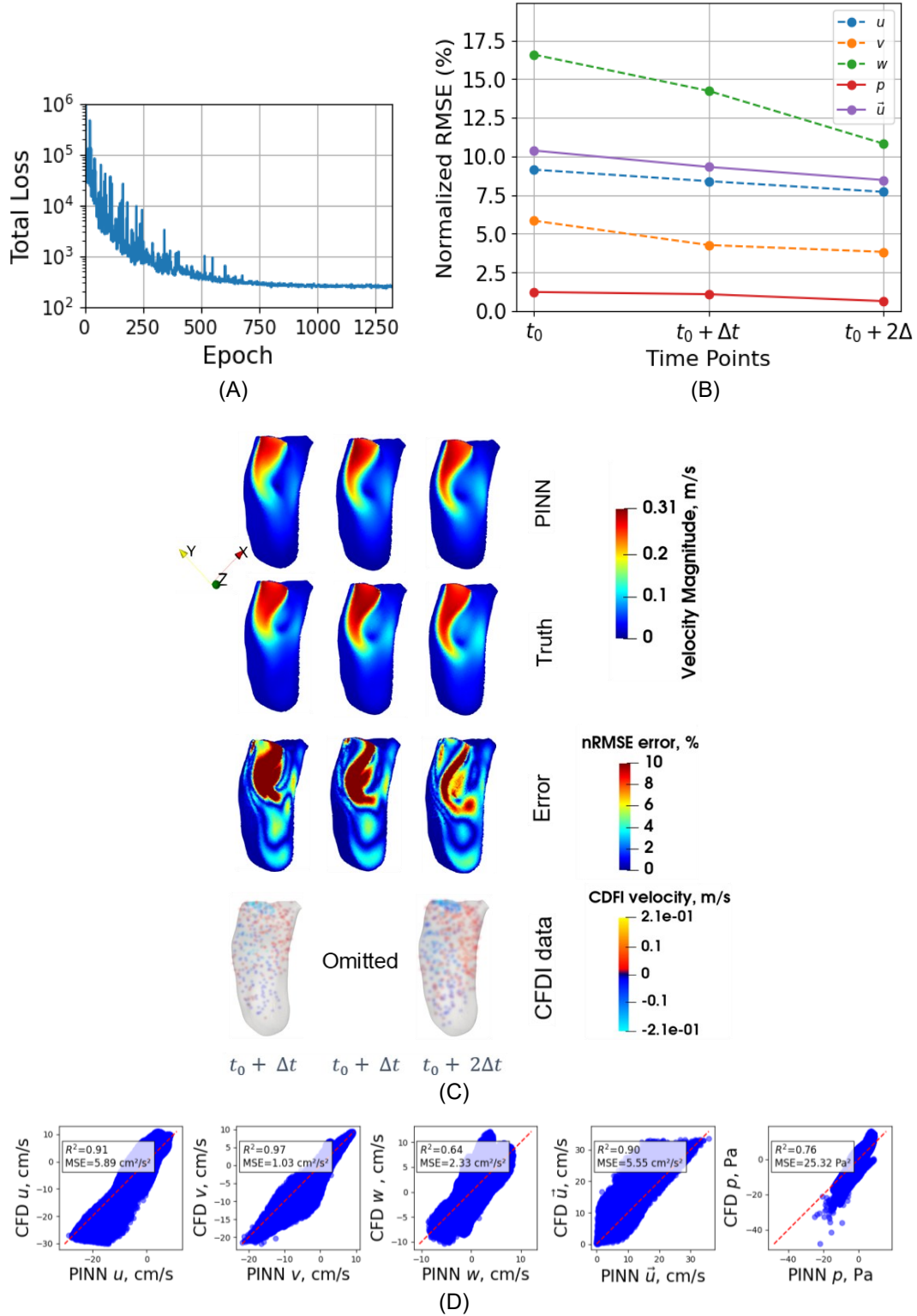


Figure A5: Evaluation of the CSF-PINN under suboptimal data quality conditions for a different fetal case. (A) The training loss profile across epochs shows model convergence at roughly 750 epochs. **(B)** The normalized RMSE for velocity components u , v , w , vector $\vec{u}$, and pressure p at each time point, highlighting greater prediction deterioration from 5% Gaussian white noise, and poorer spatial and temporal resolution. **(C)** Essential flow dynamics were captured even under challenging conditions, with a few subtle differences, that improved over time. **(D)** Scatter plots between CSF-PINN predictions and CFD data for velocity and pressure, combined across different time points. The results show moderate to strong linear correlations with coefficients of determination (R^2) ranging from 0.64 to 0.97. Despite some discrepancies and higher mean squared error (MSE) values, the model still demonstrates reasonable accuracy across the studied parameters.

TABLE A1: CASE-SPECIFIC COMPARISON OF COMPUTATIONAL FLUID DYNAMICS SIMULATED CARDIAC PARAMETERS AND CLINICAL MEASUREMENTS OF VALVE VELOCITIES BEFORE AND AFTER FETAL AORTIC VALVULOPLASTY (FAV).

Parameter	CAS-eHLHS-2		CAS-eHLHS-3		CAS-eHLHS-4		CAS-eHLHS-5		CAS-eHLHS-6		CAS-eHLHS-7		P-Value
	Pre - FAV	Post - FAV	Pre - FAV	Pre - FAV	Pre - FAV	Post - FAV	Pre - FAV	Post - FAV	Pre - FAV	Post - FAV	Pre - FAV	Post - FAV	
Peak AV, m/s	1.79	0.79	2.38	2.12	1.16	2.06	3.15	2.23	1.27	1.58	1.33	0.81	0.224
Diastolic E, m/s	-	-	-	0.90	-	0.40	-	0.60	-	0.60	0.50	-	-
Diastolic A, m/s	0.38	0.70	1.05	1.34	0.94	0.60	0.87	0.86	0.64	0.60	0.60	-	0.368
MV regurgitation, m/s	3.04	2.80	3.15	3.84	3.12	3.62	4.22	3.35	3.77	3.64	2.39	1.64	0.314
AV regurgitation, m/s	-	1.92	-	2.92	-	2.55	-	2.26	-	2.61	-	1.98	-
RV SV, ml	1.09	0.87	1.56	3.39	2.30	2.43	0.82	1.52	2.34	1.91	0.73	0.47	0.220
LV SV, ml	0.14	0.16	1.20	1.86	0.68	1.22	0.64	0.94	0.84	1.48	0.11	0.11	0.016*
RV EDV, ml	2.91	3.05	3.16	6.98	5.29	5.23	1.84	3.30	6.13	7.25	2.20	1.80	0.085
LV EDV, ml	2.99	2.53	17.40	12.61	11.1	8.23	2.23	2.53	6.68	7.32	2.40	2.10	0.105
LV EF, %	4.63	6.37	6.90	14.76	6.15	14.84	28.7	37.3	12.6	20.3	4.8	5.0	<0.001*
AR volume: LV SV, %	-	10.2	-	29.3	-	35.4	-	16.5	-	48.9	-	100	-
MV annulus z-score	-0.96	-1.13	3.35	3.04	0.67	0.70	0.79	0.16	1.67	1.98	0.74	0.77	0.198
MV flow orifice area, mm ²	3.50	2.46	16.46	9.82	9.82	13.46	7.40	11.31	20.41	23.01	1.60	-	0.408
AV flow orifice area, mm ²	0.11	1.82	0.55	3.97	1.71	2.19	0.18	0.59	6.58	6.99	0.02	0.81	0.028*
LV systolic (Apex – AV) IVPG, Pa	1607	331	2930	3227	697	2154	5884	2601	831	1313	932	354	0.254
LV systolic (Apex – MV) IVPG, Pa	949	197	1340	2310	838	1400	2480	1740	345	995	1036	1327	0.305
LV diastolic (Apex – AV) IVPG, Pa	-	228	-	447	-	601	-	960	-	1401	-	625	-
LV diastolic (Apex – MV) IVPG, Pa	22	220	98	278	105	539	194	899	47	1340	42	-	0.026*
Normalised Systolic Ejection Work Done, J/m ³	2846	461	2827	3632	2534	3655	4178	3732	1800	1885	1921	822	0.286
Normalised Systolic Energy Loss, J/m ³	163	74	216	276	161	253	263	239	124	212	137	124	0.275
Normalised Diastolic Energy Loss, J/m ³	34	156	171	917	97	733	135	284	71	1009	100	635	0.005*
Haemodynamic Inefficiency, %	6.9	49.9	13.7	32.8	10.2	27	8.32	14	10.8	64.8	12.3	92.3	0.012*
Time- & Surface-Ave. WSS (Pa)	0.15	0.27	0.56	1.35	0.39	1.01	1.02	1.38	0.52	1.72	0.19	0.38	0.011*
Diastolic Peak Surface-Ave. WSS (Pa)	0.18	0.48	0.91	2.40	0.56	1.53	2.17	3.05	1.00	3.68	0.33	1.01	0.009*
Diastolic Time- & Surface-Ave. WSS (Pa)	0.14	0.28	0.46	1.47	0.38	1.22	1.21	1.84	0.38	2.54	0.20	0.50	0.017*
Systolic Peak Surface-Ave. WSS (Pa)	0.23	0.33	1.03	1.88	0.50	1.35	1.37	2.41	1.00	3.44	0.24	0.58	0.019*
Systolic Time- & Surface-Ave. WSS (Pa)	0.17	0.27	0.62	1.25	0.40	0.83	0.84	1.02	0.76	1.24	0.17	0.30	0.007*

Note: *P-value<0.05, paired comparison between diseased pre-FAV and post-FAV.

AV - aortic valve; MV – mitral valve; RV - right ventricle; LV - left ventricle; EDV - end-diastolic volume; IVPG – Intraventricular pressure gradients; WSS – Wall Shear Stress; Time- & Surface-Ave. – Time- and Surface-averaged.

The SPH formulation, implemented in Abaqus/Explicit 2016 (SIMULIA, Providence, RI), discretizes the fluid domain into particles that carry mass, velocity, and pressure attributes. The physical properties at any point are interpolated from those of nearby particles using a cubic smoothing kernel function, $W(\mathbf{x}_a - \mathbf{x}_b, h)$ with an influence length h . The interpolation is defined as:

$$A(\mathbf{x}_a) = \sum_b m_b \frac{A_b}{\rho_b} W(\mathbf{x}_a - \mathbf{x}_b, h) \quad (\text{Aiii})$$

where $A(\mathbf{x}_a)$ represents a physical property of particle 'a' at position $\mathbf{x}_a$, m_b is the mass, ρ_b is the density, and A_b is the physical property of the particle 'b' at position $\mathbf{x}_b$.

Under the SPH formulation, the conservation of momentum is expressed as,

$$\frac{\partial \vec{u}_a}{\partial t} = - \sum_b m_b \left(\frac{P_a + P_b}{\rho_a \rho_b} \right) \nabla_a W_{ab} + \sum_b m_b \frac{(\mu_a + \mu_b) \vec{u}_{ab}}{\rho_a \rho_b \mathbf{x}_{ab}^2} \mathbf{x}_{ab} \cdot \nabla_a W_{ab} \quad (\text{Aiv})$$

where $\vec{u}_a$ is the velocity vector at particle 'a', $\vec{u}_{ab} = \vec{u}_a - \vec{u}_b$ is the relative velocity vector between particles 'a' and 'b', $\nabla_a W_{ab}$ is the gradient of the kernel function concerning the coordinates of the particle 'a', P is the pressure, μ is the viscosity, ρ is the density and $\mathbf{x}_{ab}$ is the vector from particle 'a' to particle 'b'. The conservation of mass is formulated as,

$$\frac{\partial \rho_a}{\partial t} = \sum_b m_b \vec{u}_{ab} \cdot \nabla_a W_{ab} \quad (\text{Av})$$

This setup treats the blood as weakly compressible, allowing for some variation in density driven by pressure changes.