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Quantifying blood flow and wall shear stresses in the outflow tract of chick embryonic hearts

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Abstract

Wall shear stresses (WSS) exerted by blood flow on cardiac tissues modulate growth and development of the heart. To study the role of hemodynamic conditions on cardiac morphogenesis, here, we present a methodology that combines imaging and finite element modeling to quantify the *in vivo* blood flow dynamics and WSS in the cardiac outflow tract (OFT) of early chicken embryos (day 3 out of 21-day incubation period). We found a distinct blood flow field and heterogeneous distribution of WSS in the chicken embryonic heart OFT, which have physiological implications for OFT morphogenesis.

Keywords

Chick embryonic heart; Cardiac outflow tract; Computational fluid dynamics; Wall shear stress

1. Introduction

Stresses exerted by blood flow on cardiac walls modulate growth and development of the heart in such a way that altered blood flow patterns could lead to cardiac defects, which affect 1% of newborns in the US each year [1]. Underlying mechanisms by which blood flow affects cardiac development remain elusive, partly due to a lack of methodologies to accurately quantify wall stresses in the beating embryonic heart. In particular, wall shear stresses (WSS) are known to regulate the response of endocardial cells (EC), which line the lumen of the heart [2–5]. *In vitro* studies have shown that ECs sense and respond to WSS by activating signaling pathways that modulate EC function [5, 6]. To understand the role of WSS in heart development, quantification of spatial and temporal distributions of WSS as the heart beats are needed. Here, we used the chick embryonic heart as our animal model of cardiac development, focusing on the chick heart outflow tract (OFT), and quantified the distribution of WSS on the wall of the OFT during the cardiac cycle using a combination of physiological data, imaging, and finite element (FE) modeling.

The chick embryo is frequently used to study cardiac development because of structural and functional similarities between chick and human embryonic hearts at early developmental

stages [7, 8] and easy access of chick embryos inside the egg-shell [6, 7]. At the early chicken developmental stage (i.e., Hamburger-Hamilton stage HH18 [9, 10]) that we studied here, the chick embryonic heart is an s-shape tube, with no valves or chambers, but with distinguishable heart segments: the sinus venosus, primitive atrium, primitive ventricle, and OFT [9, 11]. The OFT is the distal region of the heart, which connects the ventricle to the aortic sac, from where arterial branches bifurcate (see Fig. 1A). The OFT wall has 3-layers: i) myocardium, an external muscle layer that actively contracts; ii) endocardium, a monolayer of ECs lining the heart wall; and iii) cardiac jelly, an extracellular matrix that interposes between the myocardium and endocardium. In the OFT, the cardiac jelly forms cardiac cushions, which are protrusions of the cardiac wall toward the lumen (see Figs. 1B and 1C) and which distribute spirally along the OFT [12]. During myocardial contraction, the cardiac cushions deform to close the OFT lumen, acting as primitive valves to regulate blood flow through the OFT [13, 14]. At HH18, the OFT, which will eventually develop into valves and septa of the heart, is very sensitive to changes in blood flow dynamics.

To understand how the blood flow dynamics affect the morphogenesis of the OFT, it is important to quantify the distribution of WSS on the OFT wall. WSS is a hemodynamic force that blood flow imposes tangentially on the surface of the heart wall. It cannot be measured directly in the OFT *in vivo*, but can be derived from the flow velocity field:

$$\boldsymbol{\tau} = \mu \cdot d\mathbf{V}/d\mathbf{n} \quad (1.1)$$

where μ is the blood viscosity; $\mathbf{V}$ is the blood flow velocity vector; $\mathbf{n}$ is a unit vector normal to the lumen-wall surface; and $d\mathbf{V}/d\mathbf{n}$ is the directional derivative of the velocity in the direction normal to the lumen-wall surface.

Accurately quantifying the blood velocity field is critical for estimating WSS. Several flow-imaging techniques, such as Doppler ultrasound, and particle image velocimetry (PIV), have been used to measure intracardiac blood flow in the chick embryonic heart [15–20]. However, none of these techniques gives sufficient temporal and spatial resolution of 3D velocity fields in the tiny dynamic beating embryonic heart for accurately quantifying WSS.

Computational fluid dynamic (CFD) models have been developed to compute blood flow in human and chick embryonic hearts [4, 21–24]. Early CFD models of embryonic hearts assumed a steady-state flow and a rigid, simplified geometry [21, 24]. These simplified models suggested that blood flow dynamics is affected by the heart morphology, which varies significantly as the heart develops. Therefore, realistic models of the embryonic heart at specific developmental stages are needed to understand how blood flow dynamics affect heart development. To take the complex geometry of the embryonic heart into account, DeGroff and colleagues [22] pioneered the use of image-based FE models to simulate the blood flow in stages 10 and 11 human embryonic hearts, followed by Groenendijk and colleagues [4] who generated a FE model of an HH14 chick embryonic heart [4, 22]. Both image-based FE models showed 3D spatial distribution of WSS in early human and chick embryonic hearts; high WSS were identified at narrow lumen regions and the inner curvature of the lumen surface. However, the calculated WSS corresponded to a static geometry of the embryonic heart. To study the effects of the cardiac wall dynamics on the blood flow, Taber and colleagues generated a simplified 2D dynamic model of the chick embryonic heart [23]. The model indicated that the presence of cardiac cushions and peristaltic motion of the heart wall in early developing hearts renders pulsatile cardiac blood flow.

In our previous study, we generated dynamic models of the OFT, which included pulsatile pressures and dynamic wall motion [25, 26]. Our previous models have complementarily

showed that the 3D curvature of the OFT, the shape of the luminal cross-section, and the peristaltic wall motion of the OFT affect the distribution of WSS on the OFT wall. However, these models simplified the geometry of the OFT, and thus, they did not fully characterize blood flow dynamics and WSS in the OFT. In this study, we present a methodology to quantify the temporal and spatial distribution of WSS using image-based FE models of the developing heart OFT, combined with physiological data.

2. Experimental methods

To develop image-based FE models of the OFT, we imaged the OFT of an HH18 chick embryo *in vivo* using optical coherence tomography (OCT) and developed image analysis algorithms to extract the cardiac OFT wall geometry and motion. To provide proper boundary conditions to our OFT model, we measured blood pressures in the chick embryonic heart ventricle and aortic sac, which are the heart sections that are immediately upstream and downstream of the OFT, respectively (see Fig. 1A). Finally, to validate the blood flow velocity field predicted by our FE models, we measured blood flow at representative locations in the OFT using Doppler ultrasound.

2.1 4D imaging and reconstruction of the OFT

The OFT of chick embryos at HH18 was imaged *in vivo* using OCT. Current state-of-the-art OCT and 4D imaging techniques allow studying heart dynamics in 4D [29–32]. OCT is a high resolution (2–20 μm) non-invasive (and non-contact) tomographic imaging technique that can image up to a 2-mm depth in biological tissues [27]. Therefore, OCT is well suited for imaging the chick embryonic heart during early development (<2 mm in size) [27, 28]. For this study, we imaged the chick OFT *in ovo* using a spectral OCT [17] with axial spatial resolution of 10 μm , lateral spatial resolution of 20 μm , and image acquisition rate of 140 frames per second.

Prior to imaging, fertilized White Leghorn eggs were incubated to HH18 (~3 days of incubation) [9]. A small window was opened on the egg shell and the overlying membrane was removed to expose the embryonic heart and its OFT. During imaging, the temperature of the chick embryo was maintained at $37.5 \pm 0.5^\circ\text{C}$ within a warming chamber, since temperature affects cardiac function. 2D image sequences of the OFT were obtained over a 2-second time interval, which spanned 4–5 cardiac cycles. These 2D image sequences were acquired at contiguous OFT cross-sections 10- μm apart, spanning the entire OFT. Since image acquisition gating was not used (i.e., the beginning of image sequence acquisition was not triggered at the same phase during each cardiac cycle) the image sequences were out of phase. To synchronize these imaging sequences and reconstruct 4D images of the OFT (the OFT 3D structure over time), we employed our developed image-based, post-acquisition synchronization procedure [32], which uses similarity of adjacent image sequences and a phase correction that accounts for peristaltic-like wall motion. Reconstructed 4D images were used to extract the geometry of the OFT lumen over time and generate FE models of the chick heart OFT.

We developed a set of image-analysis algorithms in order to extract the dynamic geometry of the OFT from the 4D OCT image data sets. We first chose a 3D image dataset corresponding to a phase in the cardiac cycle in which the OFT was most constricted, and using our algorithms we segmented (delineated) the OFT lumen-wall surface and myocardium from the 3D image, and calculated the OFT centerline. To characterize the wall dynamics of the OFT, we used the 4D image reconstructions to extract image sequences from 5 equally spaced cross-sections perpendicular to the OFT centerline: 1) OFT inlet, 2) middle upstream, 3) middle, 4) middle downstream, and 5) outlet (see Figs. 2A and 2B). Segmentations of the OFT lumen-wall interface over time in these 5 cross-sections were

used to build the geometry of our FE models of the OFT lumen (see Sections 3.1.1 and 3.2.1).

2.2 Measuring intracardiac pressures

We used a servo-null micro-pressure system (Model 5A-LN, Instrumentation for Physiology and Medicine, San Diego, CA) to measure blood pressures in the ventricle, and in the aortic sac of normal chick embryos at HH18 (see Fig. 1A). Pressure data were collected following standard procedures [33]: while maintaining the chick embryos at $37.5 \pm 0.5^\circ\text{C}$, real-time pressure traces were sampled at 100 Hz in the ventricle, and in the aortic sac of normal chick embryos ($n = 26$ and $n = 9$, respectively) over at least 10 cardiac cycles. Representative intracardiac pressures that had peak values closest to measured averages were used as boundary conditions in our FE models of the OFT.

2.3 Measuring blood flow in the OFT

We used a Vevo 2100 ultrasound imaging system with an MS-550S transducer (VisualSonics, Inc., Toronto, Canada) to measure blood flow in the OFT of normal chick embryos at HH18 ($n=10$). In pulsed wave Doppler mode, we measured blood flow centerline velocities over time near the inlet, middle, and outlet of the OFT of chick embryonic hearts (Fig. 1A, dots along the OFT centerline). During data acquisition, embryos were submerged in pre-warmed chick Ringer's solution, and temperature was maintained with a heat lamp and temperature controller. The Doppler angle, the angle between the sound wave and the blood flow direction, was adjusted within 30° by tilting the angle of the transducer with respect to the embryo. The acquired blood flow velocities were corrected for the Doppler angle, approximately determined from a static image of the embryonic heart.

At each selected location of the OFT (inlet, middle, outlet; I, M, O in Fig. 1A), centerline velocities were obtained by tracing the Doppler ultrasound images. After normalizing in time and aligning these velocity traces at their peak within a cardiac cycle, we calculated the average velocity trace and its standard deviation over three cardiac cycles and among 10 chick embryos. The average velocity traces that represented the blood flow at the OFT inlet, middle and outlet were compared with the centerline velocities predicted by our FE models of the OFT at corresponding locations.

3. Finite element models

We developed two FE models of the chick embryonic heart OFT at HH18: a 2D axisymmetric model and a 3D model. Both models included the dynamics of blood flow over the cardiac cycle, and featured OFT wall motions that were extracted from 4D OCT images of the OFT (Section 2.1). Because OCT images of the OFT, and pressure measurements in the ventricle and the aortic sac, could not be acquired simultaneously nor in the same embryos (due to technical difficulties), the 2D models were used as an initial approximation of the blood flow in the embryonic OFT, to aid in the determination of proper time phases among the measured OFT wall motion, OFT inlet pressure (ventricular pressure) and OFT outlet pressure (aortic sac pressure). A 3D model of the chick heart OFT was then developed to more accurately quantify blood flow dynamics in the OFT of living embryos. 2D and 3D models were implemented using the FE software Adina (ADINA R & D, Inc. Watertown, MA) [34].

3.1 2D dynamic models of the OFT

3.1.1 Model geometry—In our 2D FE models, the OFT was modeled as a straight tube with circular cross-sections (see Fig. 3). The geometrical parameters of the OFT model were obtained from OCT images. Specifically, to extract geometrical parameters, we used the 5

representative cross-sections perpendicular to the OFT centerline (see Figs. 2A and 2B). After segmenting the lumen-wall interface of each cross-section over time, we calculated the lumen area A' . Since it was difficult to distinguish blood and the thin layer of the endocardium in OCT images due to their similar reflective index, our segmentation included the endocardium, and thus A' overestimated the OFT luminal area. To correct for the thickness of the endocardium, we approximated the area of the endocardium as the minimal A' assuming that the OFT lumen is closed (zero luminal area) when the OFT is fully constricted; thus we subtracted the minimal area from A' (see Fig. 2C). Since the OFT at location 5 does not close completely, we estimated for this position the area of endocardium from locations 1 to 4. Further, to avoid a transient zero luminal area, which would cause numerical difficulties in FE analysis, we artificially added a very small area (0.0003 mm^2). From the corrected luminal area, A , we calculated a model radius R as follows,

$$R(t, \lambda_i) = [A(t, \lambda_i)/\pi]^{1/2} (i=1, 2, 3, 4, 5) \quad (3.1)$$

where $t = [0, T]$ is time, and T is the cardiac period; the subindex i represents each of the 5 representative cross-section, with λ_i indicating the axial location of each cross-section. To remove discontinuities in wall motion, we smoothed the radii over time and space with a moving average filter (see Fig. 4)

To correctly impose measured pressure boundary conditions, we extended the axial dimensions of our 2D FE models of the OFT to a total length of 1.0 mm (see Fig. 3). The pressure boundary conditions were measured outside the range of the imaged OFT with an axial distance of ~ 1.0 mm, while the axial length of the imaged OFT was ~ 0.6 mm (see Fig. 1A). To account for this discrepancy, we extended the model 0.2 mm before the OFT inlet and ~ 0.2 mm after the OFT outlet. We assumed that the extended 'inlet region', which was the distal region of the ventricle, had the same motion as the OFT inlet (λ_1). Because the aortic sac does not actively contract during the cardiac cycle, in the extended 'outlet region' we modeled a rigid aortic sac and a transition region from the aortic sac to the moving OFT (Fig. 3). We approximated the radius of the aortic sac as the average of the mean and largest radius of the OFT outlet over the cardiac cycle, as observed from our CCD images of the chick heart.

3.1.2 Model boundary and initial conditions—We chose representative pressure traces measured at the ventricle and aortic sac as boundary conditions of the 2D FE models of the OFT (see Fig. 5). The measured pressures were imposed on the extended inlet and outlet of the 2D FE models as normal tractions. The wall motion of the OFT (moving boundary) was imposed as a displacement (D) in the radial direction of the model, with respect to the initial configuration,

$$D(t, \lambda) = R(t, \lambda) - R(0, \lambda). \quad (3.2)$$

A non-slip condition was applied at the moving boundary representing the lumen-wall interface. An arbitrary Lagrangian-Eulerian formulation was used to account for the effects of wall motion on blood flow dynamics in the OFT models.

In our 2D FE models of the OFT, resulting blood flow velocities depended on the blood pressures imposed at the OFT inlet and outlet, and the prescribed OFT wall motion. Due to measurement procedures we were uncertain of the phase lag between imposed inlet and outlet pressure, as well as the phase lag between the ventricular (inlet) pressure and the wall

motion of the OFT. We used the 2D models to test the effects of applying different phase lags among pressure boundary conditions and wall displacements. To quantify phase lags for comparison, we defined Φ_p as the phase lag between the peak aortic sac pressure and the peak ventricular pressure, and defined Φ_m as the phase lag between the peak ventricular pressure and the peak inlet cross-sectional area of the OFT (see Fig. 5). We assumed $\Phi_p = 0$ to $0.2T$, and $\Phi_m = 0$ to $0.2T$, which we will discuss in Section 5.2. Figure 6 shows different combinations of the two phase relationships.

We assumed that the OFT was initially (reference configuration) at its most constricted state with zero blood flow velocity.

3.1.3 Material properties—The blood of chick embryos was modeled as a continuous, incompressible Newtonian fluid with a density $\rho = 1060 \text{ kg/m}^3$ and a viscosity $\mu = 3 \times 10^{-3} \text{ kg/(ms)}$ [25]. The non-Newtonian behavior of blood increases with the blood hematocrit, and at 0% hematocrit blood presents a Newtonian behavior. The assumption of Newtonian fluid is justified for embryonic chick blood, because it has low hematocrit (20 % for embryonic blood vs. 45% for mature whole blood) [35, 36]. The embryonic blood flow was modeled as a laminar ($Re \ll 2000$), transient, and incompressible flow.

3.1.4 Finite element discretization—The 2D blood flow model of the OFT was discretized with axisymmetric 2D 4-node quadratic flow-condition-based-interpolation (FCBI) elements [34, 37]. Mesh-independent solutions were obtained with a mesh consisting of 946 nodes and 850 elements. Time independent dynamic solutions were achieved with 200 time steps per cardiac cycle ($T = 400 \text{ msec}$, $\Delta t = 2 \text{ msec}$). To fully damp initial transient effects, we simulated two cardiac cycles in the 2D FE models and used the second cycle as our results.

Blood flow velocities calculated with our 2D OFT models were analyzed and compared to measured blood flow velocity data. We used these comparisons (see Section 4.4) to approximate the phase lags Φ_p and Φ_m to impose as boundary conditions in more accurate 3D models of the developing heart OFT.

3.2 3D dynamic model of the OFT

To more accurately quantify the blood flow dynamics in the cardiac OFT of chick embryos at HH18 and the distribution of WSS on the OFT walls, we developed a 3D FE model of the OFT. This model included the 3D curvature and non-circular cross-sections of the OFT lumen, as well as the heart wall motion and pulsatile pressures.

3.2.1 Model geometry—In our 3D models of the OFT, the OFT lumen (blood flow domain) was modeled as a 3D curved tube with elliptical cross-sections, with geometrical parameters extracted from 4D OCT images (Section 2.1). Specifically, to construct the 3D models of the OFT, we used the OFT centerline and image sequences from the 5 representative locations (see Fig. 2). Elliptical cross-sections were chosen to represent the geometry of the lumen-wall interface, as they approximately represent the shape of the lumen as observed with OCT (see Figs. 1C, 1D and 2B). To follow the motion of the OFT lumen-wall surface over the cardiac cycle, the length of the ellipse major semi-axis, a , and minor semi-axis, b , were allowed to change with time.

To determine the ellipse parameters from imaging data, we used the segmented OFT lumen-wall interface over time from the 5 representative cross-section image sequences (see also Section 3.1.1). Using the segmented curves, we calculated and corrected the area of the lumen, $A(t, \lambda_i)$, over time as explained in Section 3.1.1. The orientation of the ellipse major and minor semi-axes in 3D were calculated (at a phase when the OFT was most constricted),

and initial values of the major and minor semi-axes, $a'(t, \lambda_i)$ and $b'(t, \lambda_i)$, over time, were determined by fitting the segmented lumen curve to an ellipse. However, $a'(t, \lambda_i)$ and $b'(t, \lambda_i)$ did not preserve the lumen area. To enforce the lumen area, we first calculated $e(t, \lambda_i)$, the ratio between a' and b' that describes the shape of the ellipse at each as it changes from a fully open OFT configuration ($e \cong 1$, close to a circle), to a contracted OFT configuration ($e \cong 2.5$) over the cardiac cycle (see Fig. 7A). We then calculated the ellipse model parameters as follows,

$$a(t, \lambda_i) = \sqrt{A(t, \lambda_i)e(t, \lambda_i)/\pi} \quad (i=1, 2, 3, 4, 5), \quad (3.3)$$

$$b(t, \lambda_i) = a(t, \lambda_i)/e(t, \lambda_i) \quad (i=1, 2, 3, 4, 5), \quad (3.4)$$

where $a(t, \lambda_i)$ and $b(t, \lambda_i)$ are the major semi-axis and minor semi-axis at λ_i used in our model. $a(t, \lambda)$ and $b(t, \lambda)$ were then smoothed in time and space to remove discontinuities (see Figs. 7B and 7C).

The 3D geometrical model of the OFT was constructed starting from the OFT centerline. We first divided the centerline into 61 equally spaced points (the 5 representative cross-sections were uniformly located at center points $i=1, 16, 31, 46, 61$). Then, at each of these points we generated cross-sections that were perpendicular to the centerline. To describe the elliptical shape of a cross-section, at a point (X_c, Y_c, Z_c) in the centerline, we built a local Cartesian coordinate system based on the direction of the major semi-axis (denoted as a unit vector $\mathbf{L}$), the direction of the minor semi-axis (denoted as a unit vector $\mathbf{S}$), and the tangential direction of the centerline (denoted as a unit vector $\mathbf{N}$) at this point. In the local coordinate system (x, y) , the elliptical cross-section was described using a general formulation of a 2D elliptical curve:

$$\begin{aligned} x(t) &= a(t)\cos(\theta) \\ y(t) &= b(t)\sin(\theta) \end{aligned} \quad (3.5)$$

where $\theta = [0, 2\pi]$ indicates a closed curve. To implement the 3D curvature of the OFT in a global Cartesian coordinate system, we calculated the coordinates of the elliptical cross-sections in a global coordinate system (X, Y, Z) ,

$$[X, Y, Z]^T = \mathbf{R} \cdot [x+X_c, y+Y_c, Z_c]^T \quad (3.6)$$

where $\mathbf{R} = [\mathbf{L}, \mathbf{S}, \mathbf{N}]^T$ is the transformation matrix between the local and global coordinate systems at any given centerline point (X_c, Y_c, Z_c) . The 3D OFT lumen surface at any given time was then generated from the 61 elliptical contours which were lofted and interpolated between adjacent elliptical contours in 3D space using Rhinoceros 4.0 (Robert McNeel & Associates, Seattle, WA).

3.2.2 Boundary and initial conditions—We used pressures and wall motion as boundary conditions in our 3D model of the OFT. The wall motion of the OFT was modeled by imposing displacements on the lumen-wall surface. We assumed that the displacement in the axial direction was negligible. For each elliptical cross-section, the displacement was calculated in the local coordinate system using:

$$\begin{aligned}
 D_x(t, \lambda) &= x(t, \lambda) - x(0, \lambda) \\
 D_y(t, \lambda) &= y(t, \lambda) - y(0, \lambda) \\
 D_z(t, \lambda) &= 0
 \end{aligned}
 \tag{3.7}$$

Displacements were prescribed on the lumen surface in the local coordinate system (i.e., skew system in Adina [34]). To account for the motion of the wall and moving mesh within the blood fluid domain, an arbitrary Lagrangian-Eulerian formulation was employed.

The phase relationships between the pressures and wall motion (Φ_p and Φ_m) used in the 3D FE model were determined from the 2D models and physical arguments (see Section 4.4). To account for the discrepancy in axial distance of pressure measurement and imaged OFT axial length (see Section 3.1.1 and Fig. 3), we extracted pressures at both OFT inlet and outlet from the 2D model with the same Φ_p and Φ_m ; and imposed the pressures uniformly at the inlet and outlet of the 3D FE model as normal tractions. Similar to our 2D models, we assumed that initially the OFT was at its most constricted state with no blood flow.

3.2.3 Material properties—In our 3D FE model, we used the same material properties for the embryonic blood as in the 2D models and assumed incompressible, Newtonian, laminar flow (see Section 3.1.3).

3.2.4 Finite element discretization—The flow domain of the 3D FE model was discretized with 3D 4-node tetrahedral FCBI elements. Mesh-independent solutions were obtained using 66400 nodes and 353000 elements. To calculate WSS, we defined a very thin boundary layer (a built-in function in Adina [34]) at the OFT wall in the fluid model. The thickness of the boundary layer was specified as 0.0004 mm, which was less than 1/5 of the element size in the fluid model. We simulated two cardiac cycles and used 200 time steps per cardiac cycle ($T = 400$ msec; $\Delta t = 2$ msec). For further analysis, we extracted results at 3 cross-sections: near the OFT inlet about 0.16 mm away from the inlet, at the middle, and near the outlet about 0.16 mm away from the outlet (see Fig. 11B).

4. Results

To quantify blood flow dynamics and WSS in the chick embryonic heart OFT, we generated 2D and 3D FE models of the OFT. Boundary conditions were obtained from pressure measurement and 4D OCT imaging of the OFT *in vivo*. Our 2D models characterized the effects of different phase relationships among the pressures and wall motion. We then used our 3D FE model to more accurately quantify the blood flow field and distribution of WSS in the OFT.

4.1 Wall motion of the OFT

Figure 2C shows the temporal variations of the luminal areas of the 5 representative cross-sections of the OFT over the cardiac cycle; the 5 cross-sections were extracted about evenly spaced along the OFT from the 4D OCT images (see Figs. 2A and 2B). Using the OFT luminal area as an indicator of the OFT wall motion, we found that the motion of the OFT wall changes from the inlet to the outlet. Towards the inlet (A1), the OFT wall motion showed a gradual expansion and steep contraction; in contrast, toward the outlet (A5), the OFT wall motion showed a relatively steep expansion and gradual contraction. The middle region of the OFT had the greatest wall motion and the OFT outlet had the smallest wall motion. The traces of the luminal areas at the 5 locations suggested that the entire OFT wall opened and closed peristaltically, with a phase lag with respect to the OFT inlet.

From each of the 5 cross-sections that we extracted along the OFT, we found the OFT lumen underwent eccentric deformation, deforming from an almost circular shape when the OFT was fully expanded, to a slit-like shape when the OFT was fully constricted (e.g., Figs. 1C and 1D). The lumen shape can therefore be approximately represented by an ellipse. Due to the presence of the cardiac cushions, the OFT lumen was fully closed (slit-like shape in Fig. 2B) at locations 1 to 4 during OFT contraction, but not at location 5 (the OFT outlet). The orientation of the major axis was also observed to change counter-clockwise from the OFT inlet to the outlet (see Fig. 2B), indicating helical distribution of the cardiac cushions along the OFT.

4.2 Blood pressure measurements

Figure 5 shows representative ventricular and aortic sac pressures used in our 2D models. We found that the ventricular pressure and aortic sac pressure were very similar in shape and magnitude. Both pressure waveforms were pulsatile and showed a peak.

4.3 Centerline velocity profiles

Figure 9 shows the blood flow average velocity traces and their variation ranges measured with Doppler ultrasound near the OFT inlet, middle and outlet, from 10 chick embryos (Section 2.3). We termed blood flow as *positive* when blood flowed from the inlet toward the outlet (also referred to as *forward flow*) and *negative* when blood flowed back towards the inlet (*backflow*). At the OFT inlet (Fig. 9A), the velocity profile exhibited a slow increase (i.e., a *shoulder*) followed by a fast increase in velocity to a peak, then a gradual decrease in velocity. At the OFT middle (Fig. 9B), the velocity profile first exhibited a sharp increase in velocity and then a gradual decrease after the velocity peak. At the OFT outlet (Fig. 9C), a backflow was observed *before* the velocity peak and a shoulder followed the peak velocity. Deviations from these general trends were observed in our Doppler ultrasound flow measurements due to the biological variations of chick embryos and operator's uncertainties (i.e., reproducible selection of measurement locations and determination of Doppler angles).

Figure 10 gives the average peak forward velocity and peak backward velocity measured near the OFT inlet, middle and outlet for 10 chick embryos. Although the peak velocities were not significantly different, the average magnitude of the peak forward flow tended to be higher near the OFT outlet ($70 \pm 10 \text{ mm/s}$) than near the inlet ($60 \pm 10 \text{ mm/s}$), this is consistent with the tapering of the OFT lumen towards the outlet. The magnitude of the peak backflow exhibited the same trend.

4.4 2D FE models of the OFT

Our 2D FE models were generated to study blood flow velocities under different combinations of phase relationships within the range of 0 to $0.2T$ between the representative ventricular pressure and aortic sac pressure (Φ_p), and between the ventricular pressure and wall motion of the OFT inlet (Φ_m), see also Fig. 5.

Figure 8 shows examples of the velocities predicted from our 2D models for different phase relationships within the ranges: $\Phi_p = 0.1$ to $0.2T$ and $\Phi_m = 0.1$ to $0.2T$ (see Fig. 6). For all combinations of $\Phi_p = 0$ (data not shown), the magnitudes of the predicted velocities were lower than those of the velocities that we measured using Doppler ultrasound. For all combinations of $\Phi_m = 0$ (data not shown), the 2D FE models predicted a dramatic backflow that was not observed in the velocity profile measurements (Fig. 9). For the particular cases depicted here, when Φ_m is constant, as Φ_p increases ΔP increases, and thus centerline velocities increase. When Φ_p is constant, the peak ΔP moves towards larger t/T as Φ_m

increases, and the differences in the peak flow among the inlet, middle and outlet reduce because the differences in luminal areas at the peak ΔP reduce (see Fig. 6).

4.5 3D dynamic model of the OFT

We chose the cases (1) $\Phi_p = 0.1T$ and $\Phi_m = 0.15T$, and (2) $\Phi_p = 0.1T$ and $\Phi_m = 0.2T$, and applied these phase lags in boundary conditions to more accurate but time consuming 3D models of the OFT. We found that the case of boundary conditions $\Phi_p = 0.1T$ and $\Phi_m = 0.2T$, yielded velocity profiles that better resemble those of ultrasound flow measurement.

Figure 9 shows a comparison of blood flow velocity profiles between our 3D FE model prediction ($\Phi_p = 0.1T$ and $\Phi_m = 0.2T$) and Doppler ultrasound flow measurement. The centerline velocities predicted from our 3D FE model captured some characteristics of the velocity traces measured from Doppler ultrasound. Near the OFT inlet and middle region, the predicted peak forward and backward centerline velocities were within the ranges of our Doppler ultrasound measurements, but near the OFT outlet the predicted peak forward flow was higher than the measurements (see Figs. 9 and 10).

As shown in Figure 11, the 3D FE model predicted a skewed distribution of velocity profile at the OFT inlet towards OFT inner curvature; the effects of the 3D curvature on velocity profiles diminished towards the OFT middle region. In-plane flow near the OFT wall was also observed, reflecting the effects of the wall motion and helical orientation of the elliptical cross-sections. No noticeable vortex was observed.

Figure 12 shows the heterogeneous distribution of WSS magnitude on the OFT wall at peak forward flow. When the OFT was expanding, a high level of WSS magnitude transiently located at the outlet; the maximal WSS (~ 11 Pa) occurred at the outlet of the OFT where the peak blood velocity was highest (see Fig. 9). Low level of WSS was found at the middle region of the OFT, which had a large lumen area. At each OFT cross-section, WSS were higher at the cushion side (the minor axis of the elliptical lumen) than the region without the cushion (the major axis). Near the OFT inlet, WSS was higher towards the inner curvature than the outer curvature, a result of the skewed velocity towards the inner curvature.

5. Discussion

In this paper, we present image-based 2D and 3D dynamic FE models of the heart OFT of normal chick embryos at HH18; these models were generated based on our 4D OCT images of the OFT and *in vivo* pressure measurements. Our 2D FE models of the OFT were used to characterize the effects of phase relationships in boundary conditions (pressures and wall motion). Our 3D dynamic FE model took into account the 3D curvature of the OFT, cardiac cushions, dynamic wall motion, and pulsatile pressures imposed at the OFT inlet and outlet. Within the scope of the model assumptions, the 3D FE dynamic model quantified the distinct blood flow field and tempo-spatial distribution of WSS in the normal chick OFT at HH18. Thus we expect that our 3D models of the cardiac OFT will contribute to understand the role of hemodynamics in heart development.

5.1 Assumptions and limitations of the 3D FE Model

In our 3D FE model of the OFT, we simplified the OFT geometry. To model the effects of cardiac cushions, we assumed that the cross-section of the OFT lumen was elliptical. The shape of the OFT luminal cross-section does not strictly resemble an ellipse because the cardiac cushions are not evenly distributed around the OFT cross-section; and the effects of the cardiac cushions become more noticeable when the OFT contracts (see Figs. 1C and 2B). To minimize the effects of the assumed elliptical shape of OFT lumen on blood flow simulations, while we modeled the segmented lumen using ellipses, the areas of the ellipses

were kept the same as those of the segmented lumens. In addition, the shape of the ellipses (e, see Fig. 7A) was allowed to vary during the cardiac cycle and along the OFT to better model the dynamic deformation of the OFT lumen. Therefore, we expected that the elliptical cross-section might locally and transiently affect the distribution of the blood flow within the cross-section during OFT contraction, but not the general pattern of blood flow.

We also simplified the wall motion of the OFT. To model the OFT wall motion, we obtained wall dynamics from the 4D OCT image data of the OFT. Full implementation of the wall motion demands a 4D segmentation algorithm for the 4D image data of the OFT, which is still under development. Therefore, we estimated the wall motion from 5 representative OFT locations and captured the heterogeneous wall motion along the OFT at those points. We expected that our 3D FE model qualitatively predicted the effects of the heterogeneous wall motion on the blood flow pattern and distribution of WSS in the OFT.

To prevent numerical difficulties in FE analysis, in our 3D FE models we simulated the closure of the OFT lumen using a very small area. This procedure might result in blood flow (though low in magnitude) driven by the pressure difference between the OFT inlet and outlet when the OFT was closed. Since the OFT wall contracted sequentially along the OFT from 0 to 0.5T, calculated blood flow and thus WSS during this time period is not accurate.

In studying the effects of the 3D curvature of the OFT on blood flow distribution, we assumed that the OFT was a curved tube and that the OFT centerline was static. However, the curvature of the centerline changes during the cardiac cycle. To evaluate the changes in the curvature of the OFT centerline during the cardiac cycle, we extracted and compared two OFT centerlines: one centerline when the OFT was fully contracted and one, when fully expanded. We found that the maximal curvature of the OFT centerline changes slightly (<15%). Therefore, we expected that the changes in 3D curvature only modestly affected the skewed distribution of the velocity profile, and thus heterogeneous distribution of WSS during the cardiac cycle.

In our FE models, we used blood pressures as both inlet and outlet boundary conditions, rather than commonly used blood flow as inlet boundary condition. This was based on the following two reasons: (1) detailed and accurate blood flow velocity data are not available due to challenges of measuring the blood flow in the tiny beating embryonic heart (dimension $\cong 1$ mm and heart rate at 2 to 2.5Hz) and a lack of flow measurement techniques with sufficient spatial and/or temporal resolution; (2) the assumption of Poiseuille- or Womersley-type blood flow, which is a common and good inflow condition for simulating vessels, may not be valid for the inflow to the OFT ejecting from the primitive ventricle. At this time, we consider that our pressure data are more accurate. Our underlying assumption of using blood pressure as boundary conditions is that the blood pressures reflect the effects of both upstream ventricle and downstream aortic sac on the OFT inlet and outlet, respectively. However, besides increasing the ventricular pressure, the contraction of the ventricle provides momentum driving the blood flow to the OFT. The limitation might affect the magnitude of the velocities at the shoulder region during ventricular contraction transiently. Considering that the predicted velocities captured the flow features observed from measurement, we expected our 3D models were qualitatively valid.

5.2 Phase relationships between blood pressure and wall motion of the OFT

For the chick OFT, which is essentially a curved tube, blood flow is regulated by two factors: (1) the pressure difference between the OFT inlet and outlet; and (2) the motion of the OFT wall. However, we were uncertain of the phase relationship between the ventricular pressure and aortic sac pressure (Φ_p), and the phase relationship between the ventricular pressure and wall motion (Φ_m) due to technical difficulties.

To determine a suitable range of values for Φ_p and Φ_m , we used physical arguments. Since during the cardiac motion blood pressure waves travel from the ventricle to the aortic sac, the peak pressure at the aortic sac (outlet) has to occur later than the peak ventricular pressure (inlet). The magnitude of the pressure wave speed is on the same order of the propagating speed of contractile wave of the OFT wall. Inspections of Figure 2C shows that the phase lag between the maximal lumen areas in the OFT inlet and outlet is about $0.2T$; this finding is consistent with the reported impulse conductance speed of the OFT wall (~ 7 mm/s [38]) that travels along the OFT (~ 1 mm), inducing sequential contraction of the OFT wall from the OFT inlet to the OFT outlet. From these arguments, a reasonable range of Φ_p is 0 to $0.2T$.

We assumed that the peak radius of the OFT inlet occurred earlier in cardiac phase than the peak ventricular pressure within the range of $\Phi_m = 0$ to $0.2T$. The assumption was reasonable based on the following arguments. First, the peak ventricular pressure has been reported to occur during ventricular contraction, in other words, the largest ventricular area occurs before the peak ventricular pressure [33]. Since the conducting speed of depolarizing impulse, which induces consecutive contraction of the embryonic heart tube, is fast in the ventricle ($V \sim 40$ mm/s for HH18 chick embryos [38]), the electric impulse reaches the OFT inlet and induces the contraction of the OFT inlet almost synchronized to that of the ventricle. Therefore we assumed that the peak area of the OFT inlet occurs before the peak ventricular pressure, and with approximately the same phase lag as that of the ventricular walls. Second, we approximated the pressure-area (P-A) loops using the ventricular pressure and area of the OFT inlet with different phase relationships Φ_m varying between $[0, 2\pi]$, and compared them to the published P-A loop for HH18 chick embryos [33]. We were aware that the areas used in [33] were different from ours: the areas in [33] were measured by tracing the epicardial border of the ventricle from light microscopic images, while ours were measured by tracing the interior boundary of the myocardium of the OFT inlet cross-section in the OCT images. We expected, however, that their temporal relationship with the ventricular pressure were similar over the cardiac cycle. We found that only P-A loops within $\Phi_m = 0.1$ to $0.2T$ were comparable to the reported P-A loops. Therefore we considered that $\Phi_m = 0$ to $0.2T$ was a reasonable range for the phase relationship between the ventricular pressure and wall motion at the OFT inlet.

Given the assumed ranges for Φ_p and Φ_m , we generated 2D FE models of the OFT to study the effects of different combinations of phase relationships among the imposed pressures and wall motion on blood flow. We compared qualitatively the centerline velocities predicted from 2D models with those measured using Doppler ultrasound. We expected the general patterns of the velocity profiles to be similar between the 2D and 3D models since the luminal areas were the same in both models. We also expected that the magnitudes of centerline velocities predicted from 2D models would be higher than those from our more accurate 3D models. This is because under the same pressure conditions blood flow in our 3D models with an elliptical lumen, spiral distribution of cardiac cushions, and 3D curvature of the OFT tube would have larger momentum loss than that in the 2D models that were essentially consisted of a straight tube with circular cross-section. We found that the phase relationships of $\Phi_p = 0.1T$ and $\Phi_m = 0.2T$ best reproduced our flow measurement with Doppler ultrasound (see Fig 9), based on 2 selective criteria: (1) the shape of the centerline velocities and (2) relative ratio among peak velocities at three locations. We considered that the phase relationships ($\Phi_p = 0.1T$ and $\Phi_m = 0.2T$) approximated well the coupled relationships between the pressures and wall motion of the OFT for normal chick embryos at HH18.

5.3 Comparison of blood flow velocity between model and experimental data

Blood flow dynamics regulates the morphogenesis of the OFT [39]. To quantify blood flow dynamics in HH18 chick heart OFT, we generated 3D FE models of the OFT based on *in vivo* pressure measurements and the dynamic geometry derived from the 4D OCT imaging data. Our FE models reproduced qualitatively some trends and values (within the same order of magnitude) of the centerline velocity profiles over the cardiac cycle measured from our Doppler ultrasound (see Fig. 9) and Oosterbaan's Doppler ultrasound [19], despite some discrepancies that we will discuss in the following paragraph. Our 3D FE model also qualitatively reproduced the skewed blood flow towards the inner curvature of the OFT, which was previously observed using PIV [16], and using Doppler OCT [18]. In addition, our 3D dynamics FE model of the OFT revealed a heterogeneous flow field in the OFT, which is important for estimating WSS distribution on the OFT wall.

Discrepancies between Doppler ultrasound velocity measurements and modeled velocities (see Fig. 9) could be due to experimental errors in flow measurements, simplifying geometrical assumptions of our FE models, and uncertainties in the phase relationships between pressures and wall motion. Experimental errors in flow measurements are mainly due to three factors. First, Doppler ultrasound acquires the component of blood flow velocity in the direction of the incident sound wave within a small sample volume ($30\mu\text{m} \times 30\mu\text{m} \times 30\mu\text{m}$) that is fixed in space (rather than moving with the heart); thus velocity data measured with Doppler ultrasound images reflect the flow within the small volume that is manually selected from a static image. Second, to obtain the absolute velocity, measured velocity data needs to be corrected for the Doppler angle, which is difficult to determine *in vivo* since the OFT is a curved tube. The Doppler angle is also manually estimated from a static image. The Doppler angle was especially difficult to determine accurately towards the OFT outlet where the OFT changes its orientation sharply (ref to Fig. 1A); this difficulty may partly explain discrepancies with the model results (see Fig. 9C). Third, it is difficult to choose reproducible locations along the OFT *in vivo* (center lumen point near the OFT inlet, middle and the outlet). Variations in flow measurements, as well as the biological variations of the chick embryos, may artificially broaden the average blood flow traces (see Fig. 9).

The simplifying geometrical assumptions and uncertainties in the boundary conditions of our 3D FE models also contributed to the discrepancies observed. Our models did not properly consider the contractile effects of the ventricular wall as well as the influence of the curvature of the upstream ventricle on the inflow. The simplified OFT wall motion may also affect blood flow. These limitations of the models may partly explain the discrepancy between the model prediction and flow measurement, especially, the shoulder region after the peak flow. Considering the errors in blood flow measurements, model limitations, and biological variations of chick embryonic hearts, we did not expect modeled and measured velocity traces to match exactly but to qualitatively capture the characteristics of the flow.

5.4 Blood flow in the OFT

The blood flow in the OFT is characterized by three dimensionless numbers: Reynolds (Re), Womersley (Wo), and Dean (De) numbers. Re is a measure of the ratio between inertia forces and viscous forces. Wo is a measure of the ratio between pulsatile flow frequency and viscous forces. De is a measure of curvature effects (centrifugal forces) with respect to flow viscous forces. Calculated from our 3D model of the OFT, the maximum De (max De $\approx$ 3.0) of the blood flow in the OFT occurred near the OFT inlet where the OFT centerline had the largest curvature. There the blood flow profile slightly skewed toward the inner curvature, which is consistent with *in vivo* flow measurements [16, 40]. The estimated Re (max Re $\approx$ 5.0) and Wo (max Wo $\approx$ 0.4) of the blood flow in the OFT were low, which is consistent with the estimates from previous flow measurements [20] and computational models of

chick embryonic hearts [25, 26]. Low Re and Wo imply that blood flow in the OFT is laminar, with viscous forces dominating the blood flow.

The effects of initial or boundary conditions in our FE models only locally and temporally affected the accuracy of the calculated flow field. Boundary effects were limited to 0.1 mm, so the hemodynamic parameters that we extracted about 0.16 mm away from the OFT inlet or outlet in our 3D FE model were not affected by boundary effects. Initial transient effects were estimated to last for less than 0.03 cardiac cycles (0.03T).

In our models, the blood flow pattern in the OFT is mainly regulated by the pressure difference imposed on the OFT from the ventricle and aortic sac, and heterogeneous wall motion. We found that along the OFT the effects of the pressure difference and wall motion varied. At the inlet, the centerline velocity profile followed closely the shape of the pressure difference (see Figs. 6, 8, and 9A), indicating that the pressure difference dominated the velocity profiles near the OFT inlet. Towards the outlet of the OFT, the effects of the heterogeneous wall motion on the blood flow increased. Other factors such as the 3D curvature, elliptical cross-sections, and orientation of the elliptical sections also locally affected the flow field in the OFT, including the skewed flow towards the inner curvature and in-plane flow, which may be important for the WSS distribution on the OFT wall. These local and global effects affect the distribution of WSS on the OFT wall.

5.5 WSS on the wall of the OFT

To understand the role of WSS in the morphogenesis of the OFT, it is necessary to quantify the spatial distribution of the WSS on the OFT wall over the cardiac cycle. Comparing to rigid wall, steady state models [4, 21, 22, 24], the WSS distribution predicted by our current 3D model was more realistic because the geometry and motion of the 3D model were obtained from the 4D OCT images of the OFT, and the pressures imposed at the inlet and outlet were measured *in vivo* at the ventricle and aortic sac.

From the 3D FE model of the OFT, we found the maximal WSS (~ 11 Pa) occurred near the outlet when the OFT was expanded (0.16 mm away from the outlet). Note that the high level of the WSS at the inlet and outlet (< 0.1 mm from the inlet/outlet surface) may be due to the boundary effects and thus is not analyzed. The magnitude of calculated WSS was consistent with WSS derived from the blood flow field measured using PIV [16, 20] and Doppler OCT [41]. Our predicted distribution of the WSS was also consistent with the literature [4, 22, 25, 26]: the high level of WSS was located at the narrower lumen regions, inner curvature of the OFT wall, and at the cardiac cushions. In addition, our 3D FE model of the OFT predicted a transient variation of WSS along the OFT. The transient heterogeneous distribution of WSS implies that ECs are subject to differential biomechanical stimuli, which may modulate differential growth and morphogenesis in the OFT.

6. Conclusions

This paper provides a methodology to quantify subject-specific distributions of WSS in the living embryonic heart. We developed image-based dynamic FE models of the cardiac OFT of normal chick embryos at stage HH18. The FE models took into account physiologically pulsatile pressures imposed at the inlet and outlet of the OFT, and the dynamic wall motion obtained from the 4D OCT imaging. The 2D FE models of the OFT, integrated with Doppler ultrasound and OCT imaging techniques, aided in the determination of the phase relationships between the pressures and wall motion, which provided the boundary conditions for our 3D FE model. The 3D FE model of the OFT revealed a distinct blood flow field and transient heterogeneous distribution of WSS in the OFT over the cardiac cycle. We found that on the OFT wall the high level shear stress was located at the outlet

towards the inner curvature and at the cardiac cushions, and low level shear stress was located at the middle region and towards the outer curvature. In the future, correlation of WSS and shear-response gene expressions (e.g., [3, 4]) will lead to a better understanding of the role of WSS in cardiac development.

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Figure 1.

Stage HH18 chick embryonic heart and its outflow tract (OFT). (A) Sketch of a chick embryonic heart with the OFT region marked by dotted black lines; the upper right inset shows a sketch of the OFT cross-section. Red arrows indicate the approximate locations where blood pressures were measured: in the ventricle and aortic sac. The three purple dots indicate the approximate locations where blood flow was measured (near the OFT inlet, middle and outlet) using Doppler ultrasound. (B), (C) and (D) Optical coherence tomography (OCT) images of the HH18 chick heart, showing: (B) a transverse section of the OFT, (C) a cross-section of the OFT at the most constricted state, and (D) the same cross-section at the most expanded state. The dotted purple line in (B) indicates the location of the cross-section in (C) and (D). M, myocardium; C, cardiac jelly; L, lumen; A, atrium; V, ventricle; OFT, outflow tract; AS, aortic sac.

Figure 2.

OFT wall motion. (A) Transverse sectional OCT image of the OFT; the 5 lines show the locations where cross-sections were extracted from 4D OCT images. (B) The 5 extracted cross-sections at their most constricted state; the lines in image 1 show the major and minor semi-axes of the lumen, assuming an elliptical luminal area. (C) Calculated luminal areas (A1, A2, A3, A4, A5) for each of the 5 locations over the cardiac cycle. t , time; T , period of the cardiac cycle.

Figure 3.

2D axisymmetric model of the chick embryonic heart OFT. The three white dots at the OFT centerline indicate the locations where centerline velocities were extracted to compare with those measured with Doppler ultrasound. P_{as} , aortic sac pressure; P_v , ventricular pressure; AS, aortic sac; Tr, transition region; OFT, outflow tract; V, ventricle.

Figure 4.

OFT lumen radius (R) over the cardiac cycle and along the OFT used in our 2D models. R was calculated and interpolated from the luminal areas obtained from the 5 cross-sections shown in Figure 2. λ , is the axial distance along the OFT centerline from the OFT inlet to the outlet.

Figure 5.

Pressure and wall motion phase lags. The figure shows representative pressures measured at the ventricle and aortic sac of stage HH18 chick embryos, as well as the luminal area at the OFT inlet (A1 from Figure 2C). It also shows hypothetical phase relationships among the pressures and OFT wall motion. Φ_p , is the phase lag of the aortic sac pressure (P_{as}) with respect to the ventricular pressure (P_v); Φ_m , is the phase lag of the ventricular pressure (P_v) with respect to the wall motion of the OFT inlet (A1).

Figure 6.

Temporal relationships among the representative luminal areas and the pressure drop along the OFT for different phase lag combinations (Φ_p and Φ_m). A1, A3, and A5, are the luminal areas at the 3 cross-sections of the OFT (see Figure 2B); ΔP , is the pressure difference between the ventricle and aortic sac ($\Delta P = P_v - P_{as}$).

Figure 7. Geometric parameters used in 3D FE model of the OFT. (A) shape index ($e = a/b$) of the elliptical cross-section at the 5 selected cross-sections (see Figure 2) over a cardiac cycle. (B) Major semi-axis (a), and (C) minor semi -axis (b), of the OFT elliptical lumen cross-sections over a cardiac cycle and along the OFT. λ , is the axial distance along the OFT centerline from the OFT inlet to the outlet.

Figure 8.

Centerline velocity profiles calculated using 2D FE models under different phase relationships between pressures and OFT wall motion. VI, VM, and VO, are centerline velocity profiles extracted at the centerline near the OFT inlet (I), middle (M) and outlet (O), respectively (see also Figure 3).

Figure 9.

Comparison of velocities measured using Doppler ultrasound and calculated using our 3D FE model of the OFT. The comparisons are shown at 3 locations in the OFT lumen (see figures 1A and 11B): (A) near the OFT inlet (location I), (B) middle (location M), and (C) near the outlet of the OFT (location O). Measured centerline velocities were represented as the average velocity, V_{US} (solid black line) $\pm$ standard deviations (dotted lines above and below the solid line). The grey line in (B) was obtained from reference [19], and measured the centerline blood velocity at about the middle region of a representative HH18 chick OFT using Doppler ultrasound.

Figure 10.

Averaged peak forward velocities (top) and peak backflow velocities (bottom) measured using Doppler ultrasound. Velocities were measured at about the 3 locations shown in Figure 1A: near the OFT inlet (I), middle (M) and outlet (O) of HH18 chick embryos (n=10). Data are presented as mean and standard deviations.

Figure 11.

Blood flow patterns calculated using our 3D model of the cardiac OFT of HH18 chick embryos. (A) Centerline velocities over a normalized cardiac cycle, and (B) velocity profile along the major axis of the elliptical lumen cross-sections. Centerline velocities and velocities profiles are shown at the 3 locations shown in figure 1A: near the OFT inlet (I), middle (M), and the outlet (O). The vertical lines in (A) indicate the cardiac phase of the velocities shown in (B). The three dots in (B) indicate the locations from where the centerline velocities in (A) were extracted.

Figure 12. Distribution of wall shear stresses on the OFT wall at peak forward flow. The flow rate depicted was calculated from the flow at the middle cross-section of the OFT (Location M in figure 1A).