Spatial velocity profile in mouse embryonic aorta and Doppler-derived volumetric flow: a preliminary model

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Phoon, Colin K. L., Orlando Aristizábal, and Daniel H. Turnbull. Spatial velocity profile in mouse embryonic aorta and Doppler-derived volumetric flow: a preliminary model. Am J Physiol Heart Circ Physiol 283: H908–H916, 2002.—Characterizing embryonic circulatory physiology requires accurate cardiac output and flow data. Despite recent applications of high-frequency ultrasound Doppler to the study of embryonic circulation, current Doppler analysis of volumetric flow is relatively crude. To improve Doppler derivation of volumetric flow, we sought a preliminary model of the spatial velocity profile in the mouse embryonic dorsal aorta using ultrasound biomicroscopy (UBM)-Doppler data. Embryonic hematocrit is 0.05–0.10 so rheologic properties must be insignificant. Low Reynolds numbers (<500) and Womersley parameters (<0.76) suggest laminar flow. UBM demonstrated a circular dorsal aortic cross section with no significant tapering. Low Dean numbers (<100) suggest the presence of minimal skewing of the spatial velocity profile. The inlet length allows for fully developed flow. There is no apparent aortic wall pulsatility. Extrapolation of prior studies to these vessel diameters (300–350 μm) and flow velocities (~50–200 mm/s) suggests parabolic spatial velocity profiles. Therefore, mouse embryonic dorsal aortic blood flow may correspond to Poiseuille flow in a straight rigid tube with parabolic spatial velocity profiles. As a first approximation, these results are an important step toward precise in utero ultrasound characterization of blood flow within the developing mammalian circulation.

cardiac development; embryonic blood flow; physiology; ultrasound

THE PHYSIOLOGICAL STUDY OF developing circulation remains technically challenging (for a review, see Ref. 37). The mouse is the primary model of mammalian development, but the assessment of developmental cardiovascular physiology, critical to the study of functional genomics, has lagged behind the recent advances in molecular cardiology. We recently (1, 36, 40) demonstrated that quantitative in utero study of mouse embryonic cardiovascular physiology is feasible using 40- to 50-MHz ultrasound biomicroscopy (UBM) and 43-MHz spectral pulsed-wave (PW) Doppler. UBM age-guided PW Doppler, in particular, is a powerful tool for the quantitative and noninvasive investigation of early mouse circulatory development, allowing for more physiologically relevant data than previously obtained (36).

Precise characterization of developing embryonic circulatory physiology requires information on cardiac output and volumetric blood flow, allowing for insights into blood flow distribution and oxygen delivery. Volumetric blood flow (Q) in a given blood vessel may be calculated as

\[ Q = \text{vessel cross-sectional area} \times \text{time-averaged mean velocity} \]

Overall, current analysis of Doppler signals to provide volumetric blood flow information is very poorly developed (13). In Doppler assessment of cardiovascular physiology, several assumptions are required to allow transformation of Doppler frequency shifts to blood flow velocities and, finally, to blood flow volume (5, 12, 16, 21). These may be conveniently categorized because those assumptions are related to the physical properties of the fluid (blood) and tube (blood vessel) and characteristics of Doppler beam transmission, reception, and processing. Of the blood and blood vessels, we generally assume the following: 1) blood is an incompressible, homogeneous, and Newtonian fluid, 2) the concentration of the erythrocytes across the vessel lumen is uniform, or the rheological properties of blood are negligible, 3) the flow is laminar, 4) the blood vessel does not taper; that is, it is of uniform cross-sectional area, 5) the blood vessel is a straight tube, 6) the cross-sectional shape of the blood vessel is circular, 7) there is no entrance effect; that is, there is enough tube length to allow for fully developed flow, and 8) the blood vessel is a rigid (nonpulsatile) tube. Such assumptions may not, in fact, hold true for the small arteries of the embryonic mouse.

Several assumptions are made of the Doppler signal itself, including exclusion of extraneous Doppler signals, uniformity and accuracy of Doppler beam transmission and reception, and accurate weighting of the

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Doppler power spectrum. In the final analysis, the reflected Doppler signal must represent only the velocities of the erythrocytes/fluid of interest in a specific blood vessel and not other signals (such as wall motion, nearby sources of blood flow, or flow at a suboptimal incident angle). Such assumptions are particularly relevant when a Doppler processor yields “mean” velocities because the weighting of the Doppler power spectrum will be influenced by nonrepresentative sources of flow. These assumptions, required in both experimental Doppler-derived phasic mean velocities (10, 29) and clinical spectral Doppler, are fraught with error (16).

The assumption that a Doppler power spectrum accurately represents mean spatial fluid velocity in a vessel can be circumvented by the use of the peak spectral velocity. If the spatial velocity profile is geometrically simple (flat or parabolic profile, for instance), then one can readily calculate the mean velocity from the peak spectral velocity, based on the assumed geometry of the profile. We sought to develop a reasonable first-order approximation model of the spatial velocity profile in the early mouse embryonic aorta, with the help of newly available UBM-Doppler imaging data. We hypothesized that peak spectral Doppler velocities may be related to mean velocities by a simple model of spatial velocity profile. Such a model would validate and/or circumvent many of the assumptions currently in use and allow more accurate calculation of volumetric blood flow than is currently available.

MATERIALS AND METHODS

It is important to note that there are currently no noninvasive methods that allow direct, accurate measurement of velocity profiles in small blood vessels. Therefore, we indirectly modeled the spatial velocity profile in the embryonic circulation using a combination of fluid biomechanical theory, published data, and our own UBM-Doppler data from middorsal aorta in normal midgestation Swiss-Webster mouse embryos. Thus results includes, in addition to experimental data from our laboratory, discussions of biomechanical theory and published results as appropriate.

The UBM system was used to determine the position of the embryo, to identify the heart and dorsal aorta, and to allow measurements of the dorsal aorta as described below. To obtain the sharpest possible images and the best possible orientation for measurement of aortic curvature, aortic diameter, and inlet length (see below), embryos were imaged in “semi-invasive” fashion (27, 35) (Fig. 1). After the pregnant mouse was sedated, the maternal abdomen was incised. The entire uterus was then gently pulled out of the abdomen, allowing both sides of the uterine horn to be examined and the embryos to be counted and identified. The embryos were then gently placed back into the abdomen. A thin 35-mm-diameter rubber membrane with a 5- to 15-mm central slit was stretched over a central 25-mm hole in a petri dish, and the petri dish was mounted over the pregnant mouse. Part of the uterus containing one or two embryos was carefully pulled through the slit into Dulbecco’s phosphate-buffered saline (37 °C; Sigma; St. Louis, MO) that filled the petri dish. Thus embryonal-placental continuity was maintained, and the embryo remained in situ, encased in its uterine sac and amniotic fluid. We have shown previously that this semi-invasive approach yields physiologically relevant data (35).

Figures 2 and 3 show various measurements made using the UBM system. Aortic dimension was measured at the middorsal aorta, where we recorded Doppler signals (36), as

Details of the preparation and imaging of mouse embryos, including UBM image-guided Doppler interrogation and analysis, have been previously described (1, 36, 40). In brief, timed pregnant Swiss-Webster mice (Taconic; Germantown, NY) were anesthetized with pentobarbital sodium (6 mg/100 g body wt ip), with added MgSO4·7 H2O (14 mg/100 g body wt) to reduce the spontaneous uterine contractions that interfere with image acquisition. In staging embryos, gestational day 0.5 (E0.5) was defined as noon of the day a vaginal plug was found after overnight mating. Preparation and imaging of the pregnant mouse occurred in a closely regulated thermal environment to maintain mouse and embryo temperatures at 37 ± 1°C (36). A focused 40-MHz UBM transducer was used, with a measured lateral resolution of 90 µm and axial resolution of 30 µm at −6 dB, and depth of penetration of 7–10 mm.

Fig. 1. Diagram of the ultrasound bimicroscopy (UBM) Doppler system showing “semi-invasive” embryos in utero. The embryos are bathed in 37°C phosphate-buffered saline (PBS), which acts as both a normothermic environment as well as an acoustic coupling medium. The pregnant mouse is positioned on a heating pad within the two-level holding stage. Both the PBS and the maternal rectal temperatures are maintained at 37 ± 1°C.
well as at the proximal dorsal aorta (just beyond the aortic arch). Blood imaged at 40 MHz is highly echogenic, imparting a speckle movement in the heart and blood vessels. Therefore, the diameter of the aorta was judged by the width of the blood column or by the clear demarcation of the aortic wall typically present at older stages (E13.5–E14.5). Aortic curvature was determined using a best-circle fit method. A UBM image of the dorsal aorta was digitized from a VHS videotape using DV Rex Video 2.52 (Canopus; San Jose, CA), compressed into MPEG format using Amber version 2.0 (Canopus), and then imported into PowerPoint (Microsoft; Redmond, WA). The radius of curvature of the aorta was determined offline by superimposing a circle with a curvature that best fit the aortic curvature onto the UBM image, and by measuring its radius. To obtain an accurate radius of curvature, only dorsal aortas imaged in a strictly sagittal plane were analyzed. “Inlet length,” the distance required for Newtonian flow to become fully developed (see Inlet length and fully developed flow), was measured from the proximal dorsal aorta just beyond the aortic arch to the middorsal aorta. Finally, the cross-sectional aortic geometry was assessed by imaging a cross section of the embryo perpendicular to its long axis.

The nonfocused PW Doppler transducer operates at a center frequency of 43 MHz, with a lateral beam width of 1.24 mm. The sample volume of the PW Doppler was placed over the region of interest (middorsal aorta) using UBM guidance. The Doppler frequency shifts were processed and displayed within 5–10 s using a LabView virtual instrument (National Instruments; Austin, TX); the complex Doppler signal was analyzed via a short-time window, Fourier transform approach, resulting in a spectrogram representation of the Doppler waveform (Fig. 4). The Doppler signals were recorded on digital audiotape for detailed offline analysis. Although velocities were not corrected for Doppler incident angle, the optimal angle and highest velocities in the middorsal embryonic aorta were always sought (36).

It should be noted that measurements of dorsal aortic curvature, aortic diameter, and inlet length were performed in semi-invasively imaged embryos, whereas blood flow velocity and heart rate data were obtained noninvasively in a previous study (36). Although it is not an ideal situation, we felt that parameters such as aortic diameter, curvature, and inlet length could be more precisely measured semi-invasively, whereas velocity and heart rate are more physiologically when measured noninvasively. Thus the calculations of fluid mechanical indexes such as the Reynolds number (Re), Womersley parameter (α), and Dean number (Nd) are derived from pooled data and not the averages of data from individual embryos. However, as will be seen, the values fall well within a range that allows for unambiguous interpretation.

All animals used in this study were maintained according to protocols approved by the Institutional Animal Care and Use Committee at New York University School of Medicine.

Statistics. All results are expressed as means ± SD. Differences between the diameters of the proximal and middorsal aorta were analyzed using a paired t-test.

RESULTS

Embryonic blood characteristics and rheology. Plasma behaves as a Newtonian fluid: homogeneous and incompressible (32). Blood, however, is a suspension of cells (primarily erythrocytes) in plasma and is a non-Newtonian fluid, although under certain circumstances it could be treated essentially as a Newtonian fluid (for reviews, see Refs. 14 and 32). Embryonic erythrocyte counts, and therefore hematocrit, increase progressively with maturation. Published erythrocyte counts in normal mouse embryos are \(-4 \times 10^6\) per mm\(^3\) at E12 and \(6–10 \times 10^5\) per mm\(^3\) at E14 compared with \(-4–5 \times 10^6\) per mm\(^3\) in the very early newborn period (38). Erythrocyte counts are therefore \(-10%\) of early postnatal levels at E12, increasing to \(-20%\) of early postnatal levels at E14–E15. Because hematocrit is the product of mean corpuscular volume and erythrocyte count, and because neonatal mean corpuscular volume is \(\sim 100 \mu m^3\), then the hematocrit of a young newborn mouse is 0.50 (38). Because erythrocyte sizes are similar in embryos, the hematocrit at our embry-
Onic stages is therefore 10–20% of early postnatal levels, or 0.05–0.10. At these hematocrits, blood viscosity is 1.2–1.3 times that of plasma (3), so that kinematic viscosity (ν, defined as ρ/μ = viscosity/density) is 0.016 stokes at E11.5 and 0.017 stokes at E14.5. Non-Newtonian properties of blood could clearly influence the spatial velocity profile. The rheologic properties of blood are determined by particle-particle and particle-wall interactions; at hematocrit <0.20, they have classically been felt to be insignificant (17). A recent review (30) stated that “in vessels >0.2 mm (in diameter), the blood flow can be treated as a homogeneous suspension with little error,” and this, with “normal” hematocrits closer to 0.40. Because the embryonic dorsal aorta is closer to 0.35 mm in diameter (see below) and the hematocrit is far <0.40, this statement suggests that embryonic blood can be treatable as a homogeneous suspension with essentially no confounding effects of red blood cell aggregation. However, recent data (26) have suggested that early mouse embryonic blood exhibits non-Rayleigh scattering, a finding that raises the possibility of rheologic properties of embryonic blood that would result in non-Newtonian flow, possibly due to red blood cell aggregation or turbulent flow. Such a conclusion, however plausible, is hampered by several problems. First, the investigators’ calculation of the frequency dependence of the backscatter coefficient relied on measurements that exhibited large standard deviations at only two frequencies. Second, frequency-dependent attenuation of the backscatter signal may have also contributed to the variance from the “usual” fourth power relationship between frequency and backscatter intensity observed in Rayleigh scattering (14, 28, 42). The experiments of Kuo and Shung (25) show that stirred erythrocyte suspensions at hematocrit of 0.06 exhibited Rayleigh scattering, although these were nonaggregating suspensions in saline. Moreover, an ultrasound frequency of 40 MHz corresponds to a wavelength of ~33 μm compared with erythrocyte diameters of ~15 μm (38); thus erythrocytes would not act as scatterers, but rather as reflectors, and the fourth power-frequency relationship of the backscatter intensity would no longer hold. In fact, this relationship, or the “spectral slope,” is likely to be underestimated because of such factors (42). Indeed, Lockwood et al. (28) showed that at frequencies >35 MHz, “Rayleigh scattering in blood cannot be expected,” finding a frequency-backscatter intensity relationship proportional to the 1.3–1.4 power. Still, it should be noted that other studies have indicated the possibility of significant red blood cell aggregation even in larger arteries, although aggregation tended to occur in diseased arteries or under abnormal flow conditions, such as aortic dissection (see Ref. 11 for a review). Even if some red blood cell aggregation occurs in the embryonic aorta, it is not clear what effect this will have on the spatial velocity profile; however, significant aggregation (large aggregates) would almost certainly disturb flow as an inhomogeneous suspension. Given the information currently available, we believe that early embryonic blood behaves essentially as plasma.

Is flow laminar? Whether flow is laminar depends on the nondimensional Re for steady flows and the nondimensional α for oscillating flows (14, 31, 32, 44). Although strictly applicable only to steady flows, Re, given by Refs. 14, 32, and 44, is

\[ \text{Re} = \left( \frac{V_{\text{mean}} \times D}{\nu} \right) \]

Table 1. **UBM-Doppler hemodynamic flow data**

<table>
<thead>
<tr>
<th>Stage</th>
<th>DAO Diameter, mm</th>
<th>HR, beats/min</th>
<th>( \omega ), beats/s</th>
<th>Velocity, mm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>E11.5</td>
<td>0.33 ± 0.04</td>
<td>227 ± 30 (151–264)</td>
<td>23.8 ± 3.1 (15.8–27.6)</td>
<td>76 ± 30 (58–127)</td>
</tr>
<tr>
<td>E12.5</td>
<td>0.36 ± 0.05</td>
<td>244 ± 18 (217–276)</td>
<td>25.6 ± 1.9 (22.7–28.9)</td>
<td>89 ± 31 (70–158)</td>
</tr>
<tr>
<td>E13.5</td>
<td>0.35 ± 0.03</td>
<td>264 ± 33 (181–308)</td>
<td>27.6 ± 3.5 (19.0–32.3)</td>
<td>139 ± 22 (105–173)</td>
</tr>
<tr>
<td>E14.5</td>
<td>0.34 ± 0.03</td>
<td>261 ± 44 (171–318)</td>
<td>27.3 ± 4.7 (17.9–33.3)</td>
<td>161 ± 38 (119–226)</td>
</tr>
</tbody>
</table>

Values are means ± SD. E11.5–E14.5, embryo at gestational days 11.5–14.5; DAO, middorsal aorta; HR, heart rate; \( \omega \), radial velocity, = \( 2\pi \times \text{HR} \). Velocity is peak spectral Doppler velocity. HR and velocity data are adapted from Ref. 36. Values in parentheses refer to range.
and is often used by investigators to determine arterial flow characteristics, but mean velocity \(V_{\text{mean}}\) is substituted with peak velocity. \(D\) refers to tube diameter. When \(R\) is calculated using this peak velocity, the assumption is that this \(R\) represents the “worst case scenario,” although turbulence under pulsatile conditions occurs during the deceleration of flow (44). Peak blood flow velocity in the dorsal aorta increases with gestation and averages \(\sim 75\) mm/s at E11.5 and \(\sim 160\) mm/s at E14.5, but velocities as high as \(226\) mm/s were obtained at E14.5 (Table 1). Using these velocities and our measured dorsal aortic diameters (Table 1), we obtain a maximal \(R\) of only 452, at E14.5 (Table 2). These values are far lower than the critical \(R\) (typically \(>1,000–2,000\)) needed for development of turbulent flow (2, 32, 44).

The critical \(R\) for laminar to turbulent transition in pulsatile flow also depends on the rate of change of the velocity field, as expressed by \(\alpha\) (14, 31, 32, 44). \(\alpha\) is a function of vessel radius \(R\), the circular frequency of the heartbeat \(\omega = 2\pi \times \text{heart rate in beats/s}\), and \(v\), such that

\[
\alpha = R \sqrt{\omega/v}
\]

Because heart rates are \(\sim 240\) beats/min or \(\sim 4\) beats/s (see Table 1), \(\alpha\) is \(\sim 0.70\) at E11.5–E14.5, with a maximal \(\alpha\) of only 0.76 (Table 2). For small arteries with parabolic spatial velocity profiles, \(\alpha\) is typically in the range of 0.5–3.0 (4). Evans (14) has also stated that for very low values of \(\alpha\) (about \(<2\)), the velocity profile is parabolic. Thus our low \(\alpha\) also indicates laminar flow with a parabolic spatial velocity profile (39).

**Does aortic tapering affect flow?** Although taper would impact flow velocities and pressure gradients (mean velocity increases as dorsal aorta tapers) and impedance (wave reflection node locations change), taper by itself would not impact the spatial velocity profile geometry significantly (32), although it may exert indirect effects by increasing or decreasing the velocity of the fluid column. Regardless, UBM imaging of the proximal and middorsal aorta reveals no measurable or visible taper (Fig. 2). Pooled data from 65 embryos aged E11.5 through E14.5 revealed no significant difference between proximal and middorsal aortic diameters (\(P > 0.290\)).

**Is the cross-sectional shape of the aorta circular?** Unless there is extrinsic compression of the dorsal aorta, the shape of the aortic cross section should be circular under the influence of an internally distending and pulsating fluid column. We were able to qualitatively determine the cross-sectional shape of the dorsal aorta in 45 of 68 embryos (66%); in the remaining embryos, image quality was inadequate to judge arterial geometry. In every case, and at all stages from E11.5 through E14.5, the aorta appeared circular in cross section (Fig. 5).

**Effect of aortic curvature.** Tube curvature generates secondary flow phenomena (vortices) and skews the spatial velocity profile (2, 8, 9, 34). The magnitude of both the secondary flow and skewing of an axisymmetric spatial velocity profile is a function of the nondimensional \(N_d\) given by (8, 9)

\[
N_d = \frac{R e (\alpha/v)}{R}
\]

where \(R\) refers to tube (aortic) radius and \(r\) is the radius of curvature, which was calculated by UBM using a best circle-fit method. \(r\) is large, so that \(R/r\) is low and decreases with gestational age, being maximal at E11.5 (Table 2). Thus \(N_d\) is \(\sim 40–50\) at all stages, with a maximal \(N_d\) of only \(\sim 100\) (Table 2). Empirical data show that for \(N_d = 1,140\) and \(R/r = 0.10\), there is only mild skewing of the velocity profile that occurs only after the tube has curved \(>90^\circ\); secondary flow phenomena are considered small (8). Because \(N_d\) and \(R/r\) in the mouse embryo are much lower than these values, and because dorsal aortic curvature is \(<90^\circ\) (Fig 2), our results indicate a minimally skewed axisymmetric spatial velocity profile. Thus the flow behaves as if in a straight tube.

**Inlet length and fully developed flow.** For flow to become fully developed, the inlet length \(L\) needs to be adequate enough to dissipate entrance effects that flatten the spatial velocity profile (14, 32). \(L\) is given by (30)

\[
L = \left( \frac{k VR^2}{v} \right)
\]

where \(k\) is an experimentally derived constant. For \(k = 0.06\) (5), and using averaged peak Doppler velocities from Table 2, \(L = 1.3\) mm at E11.5 and 2.4 mm at E14.5. Because the parameter \(V\) in the above equation should refer to the time- and space-averaged velocity, use of peak velocity would introduce error by overestimating the inlet length. Blood flow has its true “entrance” at the aortic valve and then develops as it courses around the arch. Therefore, flow is already

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### Table 2. Flow characteristics at various stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>(Re (R_{\text{max}}))</th>
<th>(\alpha (\alpha_{\text{max}}))</th>
<th>(R/r (R_{r \text{max}}))</th>
<th>(N_d (N_{d \text{max}}))</th>
<th>(L, \text{mm})</th>
</tr>
</thead>
<tbody>
<tr>
<td>E11.5</td>
<td>157 ± 62 (261)</td>
<td>0.63 ± 0.04 (0.69)</td>
<td>0.062 ± 0.019 (0.095)</td>
<td>39 ± 6 (80)</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>E12.5</td>
<td>199 ± 70 (356)</td>
<td>0.72 ± 0.03 (0.76)</td>
<td>0.052 ± 0.010 (0.069)</td>
<td>45 ± 4 (94)</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>E13.5</td>
<td>286 ± 45 (356)</td>
<td>0.71 ± 0.05 (0.76)</td>
<td>0.033 ± 0.003 (0.038)</td>
<td>52 ± 3 (69)</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>E14.5</td>
<td>323 ± 76 (452)</td>
<td>0.68 ± 0.06 (0.75)</td>
<td>0.026 ± 0.006 (0.033)</td>
<td>52 ± 7 (82)</td>
<td>2.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. \(\alpha\), Womersley parameter; \(\alpha_{\text{max}}\), Womersley parameter based on the maximal HR at each stage, as shown in Table 1; \(L\), “inlet length” measured by ultrasound biomicroscopy from just distal to the aortic arch to the middorsal aorta (∼15 measurements at each stage); \(N_d\), Dean number calculated using the average Reynolds number (Re) at each stage and the measured \(R/r\) for each embryo; \(N_{d \text{max}}\), maximal \(N_d\) calculated using \(R_{\text{max}}\) and \(R_{r \text{max}}\) at each stage; \(R_{\text{max}}\), Re based on the maximal velocity at each stage as shown in Table 1; \(R/r\), ratio of aortic radius to radius of curvature of the aorta (∼12 measurements at each stage). Values in parentheses refer to ranges.
Is the tube (aorta) rigid? Aortic wall pulsations would complicate the calculation of volumetric flow, because vessel cross-sectional area would then be a dynamically changing quantity. Although we saw no evidence of aortic pulsations by UBM even over several cardiac cycles, the axial resolution (see MATERIALS AND METHODS) and low frame rate of UBM (8/s) may reduce its sensitivity to small and/or rapid changes in cardiac and vascular geometry. However, it should be noted that even in embryos exhibiting heart rates of 100–120 beats/min (<2 beats/s), we failed to observe any aortic pulsations. Moreover, previous observations also confirm that the embryonic aorta (10, 45) and small arteries (32) do not pulsate. We believe, therefore, that the embryonic mouse dorsal aorta can be treated essentially as a rigid tube.

Spatial velocity profiles in small arteries and arterioles: experimental evidence. No experimental data on spatial velocity profiles exist in a system such as the embryonic mouse dorsal aorta. However, results of related experiments may be relevant to the embryonic dorsal aorta.

In the chick dorsal aorta (diameter 300–400 μm), recent Doppler power spectrum data indicate laminar flow, although the spatial velocity profile was not characterized (41). Greene et al. (19) showed that in human digital arteries of diameter ~1.0 mm, spatial velocity profiles were close to parabolic in both systole and diastole. Goldsmith et al. (17) showed that spatial velocity profiles become more parabolic when the hematocrit is reduced and velocity is increased; blunting of a parabolic profile becomes apparent when hematocrit is >0.20 in tubes of diameters 60–150 μm. Gahtgens et al. (15) found that blood, with hematocrit 0.305, maximal velocity 23 mm/s, and tube diameter 130 μm, showed parabolic flow profiles, but higher hematocrit, slower flow rates, or smaller tubes led to blunting of the profile. Thus hematocrits (0.05–0.10), flow velocities (~50–200 mm/s), and aortic dimensions (diameter ~300–350 μm) seen in early mouse embryos would be expected to result in parabolic spatial velocity profiles.

**Estimation of cardiac output in dorsal aorta: preliminary analysis.** The above results indicate that flow in the early mouse embryonic dorsal aorta is laminar and that the spatial velocity profile is parabolic. For any parabolic flow profile, the mean velocity is one-half the peak (centerline) velocity, so that Q is given by

\[
Q (\text{mm}^3/\text{s}) = \text{vessel cross-sectional area (mm}^2) \times \frac{1}{2} \times \text{time-averaged spectral Doppler peak velocity (mm/s)}
\]

or

**Stroke volume (mm}^3) = \text{vessel cross-sectional area (mm}^2) \times \frac{1}{2} \times \text{spectral Doppler velocity-time integral (mm)}

In this study, UBM-Doppler-derived velocity-time integrals at E13.5 averaged 10.1 ± 1.8 mm (n = 11) and at E14.5 averaged 11.7 ± 2.5 mm (n = 9) in noninvasively imaged (transabdominally imaged) embryos. The estimated average stroke volumes are then 0.52 mm}^3 (for average middorsal aortic diameter of 350 μm) and 0.53 mm}^3 (for average middorsal aortic diameter of 340 μm), respectively. These average values likely underestimate the true stroke volumes because the Doppler incident angles are not always optimal (36); indeed, on review of Doppler incident angles from UBM images, we estimate that the Doppler incident angles were <50° (thus velocity error due to angle <14%) and 10–15% of the time, the Doppler incident angles could not be accurately determined due to embryonic lie within the maternal abdomen. However, this error may be offset by intrinsic spectral broadening (see DISCUSSION). Still, our estimated stroke volumes are remarkably consistent with, and what we would expect based on, volumetric flows in the mouse obtained from video planimetry of the combined embryonic ventricular outputs (0.56–0.63 mm}^3) (24) and Doppler interrogation of umbilical arteries (0.37–0.42 mm}^3) (29). They are also remarkably consistent with values obtained in the chick dorsal aorta (~0.50 mm}^3) at similar stages of development (10), when chick dorsal aortic dimension and embryonic weight are comparable to those of the mouse (23, 29).

**DISCUSSION**

Our study, although basic in terms of fluid mechanics, strongly suggests that blood flow in the mouse embryonic dorsal aorta can be regarded as essentially Poiseuille flow in a straight, rigid tube, with a parabolic spatial velocity profile. This simple model appears to be an excellent first approximation, allowing for a reasonable determination of cardiac output in the middorsal aorta using peak spectral Doppler velocities.
The strength of this study lies in the assessment of arterial geometry and flow characteristics in the in utero early developing embryo, with preservation of intrinsic geometry and physiology of the cardiovascular system that would affect flow profiles. Such data have become available only through the relatively recent development of UBM and UBM image-guided PW Doppler and their application to the developing mammalian cardiovascular system in our laboratory (1, 36, 40). We believe ours is the first study to examine the physics of flow in the early developing embryonic circulation, where the dimensions and flow parameters are substantially different from the mature animal.

**Experimental evidence using peak versus mean Doppler velocities.** Our model is useful because only the peak (centerline) velocity, obtainable by spectral Doppler noninvasively (1, 36), is required to determine volumetric blood flow. Confounders of phasic mean velocity determinations are irrelevant. Several investigations (10, 23, 24) have processed the Doppler frequency shifts into phasic mean velocities, with the argument that such velocities may be easily converted into volumetric blood flow by multiplying by the cross-sectional area of the blood vessel. Importantly, such results rely on an accurate determination of the true mean blood flow velocity, however, which depends on proper weighting of the Doppler signal and assumptions that the reflected Doppler signal truly represents the velocities of the individual particles in the blood vessel. This approach is inherently inaccurate, because signal processing, extra-arterial motion, and nonuniform attenuation of the Doppler signal (due to depth, reflector characteristics, etc.) all contribute to errors in the Doppler-derived mean velocity (16, 32). Calibration of the system (10) does not necessarily obviate these confounding problems in determinations of phasic mean velocities because the phantoms are in isolation, not in the in vivo embryonic milieu where extra-arterial signals and nonuniform signal attenuation may occur. Moreover, in prior experiments (10, 22), the phantom was of substantially different caliber (3.0 mm diameter) than the aorta imaged (0.29–0.41 mm diameter), with a Doppler sample volume length of 1–2 mm (10, 22). This discrepancy between the phantom and the actual vessel sizes may also result in an inaccurate Doppler power spectrum for the blood vessel. Indeed, Hartley et al. (22) have stated that, ideally, such Doppler probes should be calibrated on the same vessel in which the flow is measured.

Recent evidence has also highlighted the inaccuracy of such systems processing mean phasic Doppler signals, favoring instead the use of spectral Doppler analysis (41). In contrast to the former, if the measured peak arterial velocity represents the highest particle velocity within the sample volume, then the recorded peak arterial velocity is an accurate measurement of the true peak arterial velocity. Needing only peak velocity to measure volumetric blood flow also provides the opportunity for the use of commercially available systems that display spectral (peak) Doppler-derived velocities, in the study of developmental cardiovascular physiology, such as in the study of Gui et al. (20). It should be noted that the accuracy of measuring maximum (peak) velocities with a system such as ours can be affected by the nature of the flow as well as instrumental factors. One important and well-recognized source of error is the phenomenon of intrinsic spectral broadening by which the maximal velocity observed on the Doppler waveform is in fact greater than the true maximal velocity of the blood flow (6, 7, 14). We do not believe that intrinsic spectral broadening poses a problem in this study for two reasons. First, our previous experiments have shown excellent agreement between the Doppler-derived velocities and the true velocities (1). Second, overestimation of velocities would simply overestimate such parameters as Re, α, Nd, and L. Our conclusions regarding laminar Poiseuille flow are in fact strengthened when these values are overestimated. It is recognized, however, that intrinsic spectral broadening may introduce error (velocity overestimation) into calculations of volumetric blood flow.

**Limitations and directions for future work.** We recognize that there is no currently available approach for fully validating the results of this study. Moreover, many of the parameters discussed (such as Re, α, Nd, and r) are close approximations and cannot be measured or calculated precisely. More research will also be necessary to determine whether red blood cell aggregation confounds this model in the embryonic mouse aorta. Nevertheless, we believe the combined theoretical and empirical analysis above argues strongly for Poiseuille flow in the mouse embryonic dorsal aorta.

This model is valid only where our assumptions have been validated in the dorsal aorta and probably in vessels in the relatively straight midportion of the umbilical cord. In contrast, the inflow and outflow tracts of the early developing heart do not lend themselves to our assumptions because there are dynamic changes of these regions during the cardiac cycle (20, 24, 33).

Further investigation is needed to gauge how accurately UBM can measure vessel diameter or cross-sectional area before a truly accurate model of embryonic volumetric blood flow can be fully developed. In this study, dorsal aortic diameter was ~300–350 μm at all stages; however, resolution may be inadequate for precise determination of dimensions at E11.5–E12.5 or earlier, although dorsal aortic boundaries appear distinct and appear to be accurately measured at older stages (E13.5 and E14.5). Moreover, despite limited evidence to the contrary (see **RESULTS**), there remains the possibility that aortic pulsations can confound precise estimates of volumetric flow, and this may require further investigation with newer imaging techniques. The preliminary values for stroke volume at E13.5 and E14.5 were obtained only to provide an idea of our model estimates. It is recognized that ultrasound measurement of dimensions may carry error, particularly of very small objects (18, 43). Finally, future experiments addressing volumetric flow will require closer attention to the Doppler incident angle.
In conclusion, from our experiments and others, it is clear that high-frequency ultrasound will play a critical role in the study of developmental cardiovascular physiology in both normal and abnormal animal models (functional genomics). This “first approximation” model of the spatial velocity profile is an important step toward precise in situ ultrasound characterization of cardiac output, blood flow distribution, and oxygen delivery in the developing mammalian cardiovascular system.

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