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Hemodynamic Parameters of Stage 20 to Stage 35 Chick Embryo

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ABSTRACT. Hemodynamic parameters of the chick embryo from stage 20 (3 d of a 21-d incubation) up to stage 35 (8 d) are described. Normal values of dorsal aortic flow velocity wave forms were measured with a 20-MHz directional-pulsed Doppler velocity meter that was validated to be accurate above 5 mm/s. An analysis of variance was carried out for each of the flow velocity parameters. The correlation coefficient that represents the reproducibility was satisfactory ($r > 0.90$). There was a 17-fold rise in mean dorsal aortic blood flow (mm^3/s). Heart rate doubled from 123 ± 12 to 239 ± 8 bpm, and stroke volume increased from 0.14 ± 0.08 to 1.28 ± 0.55 mm^3. A stage-related rise was seen in peak systolic and mean velocities and peak acceleration. These data may serve as a basis for flow velocity wave form investigation and interpretation in developmental stages of cardiac malformations. (*Pediatr Res* 34: 44–46, 1993)

High-resolution real-time ultrasound and Doppler techniques allow detailed analysis of human fetal cardiac anatomy and function during the second half of pregnancy. A reliable diagnosis of a wide range of cardiac anomalies can now be made (1–3), and as a result, a spectrum of cardiac pathology has emerged that appears to be different from that seen in postnatal life (4). Moreover, Doppler studies have demonstrated abnormal flow velocity patterns in the outflow tract in the presence of atrioventricular and semilunar valve pathology (5).

In the developing heart, morphogenesis and hemodynamic function are closely linked. Micro-Doppler and pressure studies in chick embryos have provided valuable information on this relationship in normal heart development (6, 7). To study the interaction between hemodynamics and morphology in abnormal heart development, an animal model with specific and reproducible cardiac malformations is needed. We recently developed a standardized method for inducing a spectrum of double-outlet right ventricle in the stage 35 chick embryo (8). Moreover, for future studies on hemodynamics associated with abnormal heart development, normal data on flow velocity parameters from stage 20 up to stage 35 must be collected.

In this article, we report on the validity, reproducibility, and normal values of dorsal aortic flow velocity wave forms in stage 20 to stage 35 chick embryos.

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MATERIALS AND METHODS

Fertilized White Leghorn chick eggs were incubated (blunt end up) at 38°C and staged according to Hamburger and Hamilton (9). The material was subdivided into stages 20, 24, 27, 29, 31, and 35 (d 3 to 8 of incubation). Stages 20 to 29 were chosen in view of earlier studies performed by Hu and Clark (6, 7).

For physiologic measurements, a 20-MHz directional pulsed Doppler velocity meter (model 545C-4 by Bioengineering, University of Iowa) was used (10, 11). The accuracy of this equipment was tested against a calibrated velocity. Anticoagulated pig blood was pumped at constant pressures to obtain constant flows. The blood was led through a 3-mm diameter channel, which was drilled into a perspex block. At an angle of 45° to the first channel, an additional channel was made in which the 750-μm Doppler probe was placed. At the outflow side of the first channel, a stretch of polyvinylchloride tubing (internal diameter 3 mm, length 100 cm) was connected and marked. Once a constant flow was acquired, a stopwatch was started at the first marking point, x = 0 cm, and stopped when blood reached x = 100 cm. The calibrated blood velocity was calculated from the time elapsed in crossing this trajectory of 100 cm. The time-average of the Doppler velocity V (mm/s) was determined by recording a voltage E supplied by the Doppler velocity meter, using the equation $V = 78.25 \cdot E/\cos 45°C$ (110 mm/s corresponds with 1 V). The relationship between Doppler velocity and calibrated velocity was determined from regression analysis.

Each embryo was exposed by creating a window in the shell and removing the overlying membranes. The embryo lay with its right side up and the dorsal aorta horizontal. Using a micromanipulator and projector jig, it was possible to position the Doppler probe consisting of a 750-μm piezoelectric crystal at a 45° angle to the dorsal aorta at the level of the sinus venosus (6, 7). A 45° angle was chosen for consistency in the velocity calculations.

The internal diameter of the dorsal aorta was measured at the same level with a filar micrometer eyepiece that was calibrated against a 10-μm scribed glass standard. The area was calculated from the equation area = $\pi d^2/4$, where d is the aortic diameter (mm).

Hemodynamic parameters. The analog wave forms were sampled at 300 Hz by a Lab Master data acquisition analog-digital board (Axon Instruments, Inc., Burlingame, CA) linked to a Commodore PC40 computer. The converter offered 12 bits at an input range of −10 to 10 V. Data were stored in a 5¼-inch 44-megabyte Bernoulli disk cartridge (Iomega Corp., Roy, UT).

A total of 10 embryos were studied in each stage. Only live embryos with the right side up and without any sign of bleeding were included in the final analysis. Within each embryo, a 2-min wave form recording was made. Three technically high-

quality wave form recordings of 5 s each were selected for analysis. Each 5-s recording contained 10 to 20 wave forms, depending on the stage. The mean value of a particular flow velocity parameter was first calculated for each 5-s recording and then averaged for that embryo. Finally, the mean value ± SD was calculated for all 10 embryos in each stage.

Mean dorsal aortic blood flow (Q) was calculated from Q (mm^3/s) = V·area, where V is the mean dorsal aortic blood velocity. By measuring the cycle length between pulse waves and converting this to bpm, the heart rate was calculated. Stroke volume (mm^3) was determined from the quotient of mean dorsal aortic blood flow and heart rate. From the dorsal aortic dV/dt, the peak acceleration (mm/s^2) was derived.

Morphologic examination. After the wave form recordings were collected all embryos were removed from the egg. The embryos were processed for histologic sectioning in a routine way by fixing in Bouin and embedding in paraffin. Thereafter, the embryos, including the hearts, were serially sectioned. The sections were 5 μm thick and stained with hematoxylin/eosin.

Statistical analysis. The reproducibility (r) was defined according to the equation: $r = \sigma^2_B/[\sigma^2_B + (\sigma^2_W/N)]$, in which σ^2_B represents the between-embryo variance and σ^2_W the within-embryo variance of wave form parameters. These variances were estimated from an analysis of variance. The number of repeated 5-s recordings within one embryo is denoted by N, which in our study equals 3. The parameter r is the correlation coefficient between two "measurements" within one embryo, where a "measurement" is defined as the average of N repeated 5-s recordings. This clearly shows that $0 < r < 1$ and that r can be increased toward 1 by increasing the number N. The between-embryo variance σ^2_B also includes the effect of stage.

RESULTS

Figure 1 depicts the relationship between Doppler velocity and calibrated velocity ($y = 1.18x - 3.24$, $r^2 = 0.99$, standard error of the estimate = 1.38 mm/s).

Table 1 presents the results of the analysis of variance for each of the flow velocity parameters. The correlation coefficient that represents the reproducibility, using the average of three 5-s recordings as measurement outcome, was satisfactory.

Figure 2 gives an example of dorsal aortic flow velocity wave forms for each stage. It is clear from Table 2 that an increase was observed for each flow velocity parameter and vessel area with advancing stage.

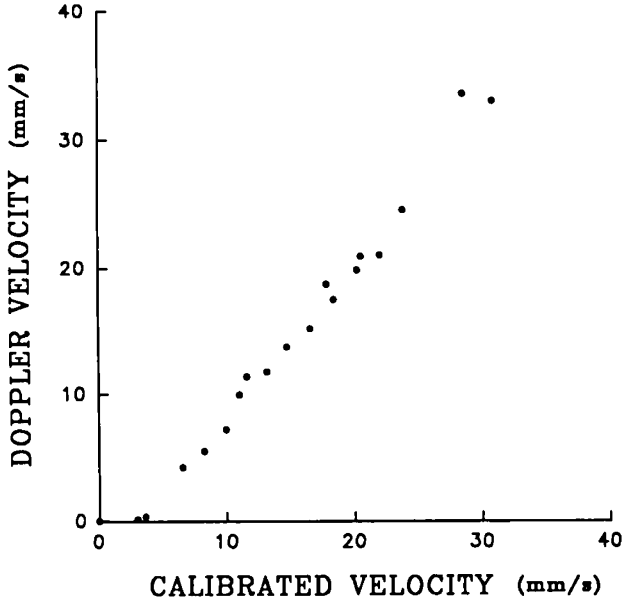

Fig. 1. Doppler velocity and calibrated velocity. Relationship between *in vitro* Doppler velocity and calibrated velocity from 0 to 35 mm/s.

Table 1. *Results of analysis of variance**

	σ^2_W	σ^2_B	r
Mean DAo velocity (mm/s)	2.63	32.38	0.97
Mean DAo blood flow (mm^3/s)	0.14	2.86	0.98
Heart rate (bpm)	35.74	1754.13	0.99
Stroke volume (mm^3)	0.02	0.44	0.98
Peak acceleration (mm/s^2)	35445	501193	0.98

* σ^2_W, variance within groups; σ^2_B, variance between groups (including stage effect); r = reproducibility (correlation coefficient between two "measurements," each calculated as the average of three 5-s recordings); DAo, dorsal aortic.

After histologic analysis, all hearts were diagnosed as normal.

DISCUSSION

The validation study of our 20-MHz directional-pulsed Doppler velocity meter showed that there is a close relationship between Doppler velocity and calibrated velocity. The regression line was found to be $y = 1.18x - 3.24$, $r^2 = 0.99$, standard error of the estimate = 1.38 mm/s. As can be seen in Figure 1, velocities below 5 mm/s will be underestimated due to a nonlinearity around zero, a finding that has already been discussed in the literature (12).

Flow velocity wave forms were collected up to stage 35 (d 8 of incubation). In previous studies, embryonic wave forms have been obtained up to stage 29 only (6, 7). The present results show a high reproducibility for all flow velocity parameters.

A marked increase in mean dorsal aortic velocity and vessel area was observed, resulting in a 17-fold rise in mean dorsal aortic blood flow. Heart rate increased 2-fold and stroke volume 9-fold. These data resemble those reported by Hu and Clark (6) and reflect rapid embryonic growth. Because the embryonic weight was not determined, the exact relationship between dorsal aortic blood flow and embryonic growth could not be established.

The observed increase in heart rate cannot be explained by parasympathetic or sympathetic neuron activity, because neither is functional until stage 42 (13, 14). However, circulating adrenergic and cholinergic agents, as well as other peptides, such as atrial natriuretic factor, may play a role in these heart rate changes (6). The embryonic heart rates observed in the present study are somewhat lower than those reported by Hu and Clark (6). One explanation may be a slight difference in environmental temperature at which the hemodynamic recordings were carried out; a reduction in the environmental temperature is associated with a decrease in heart rate (15). Dunnigan *et al.* (16) reported a heart rate of 210 bpm just before hatching. We found a heart rate of 239 bpm at stage 35, which suggests considerable plateauing of heart rate during remaining embryonic development.

The stage-related rise in peak systolic and mean velocities could be accounted for by increased volume flow, raised cardiac contractility, and a reduction in afterload. The latter could not be properly addressed because no pressure measurements were available. Our study shows that the stage-related rise in peak systolic and mean velocities is not coupled with major changes in the flow velocity wave form.

The observed stage-related rise in peak acceleration suggests an increase in cardiac contraction force with advancing embryonic development.

Normal cardiovascular development, as expressed by the relationship between form and function, is important for an understanding of congenital heart disease. The hemodynamic and morphologic data presented in this study may serve as a basis for flow velocity wave form investigation and interpretation in developmental stages of cardiac malformations.

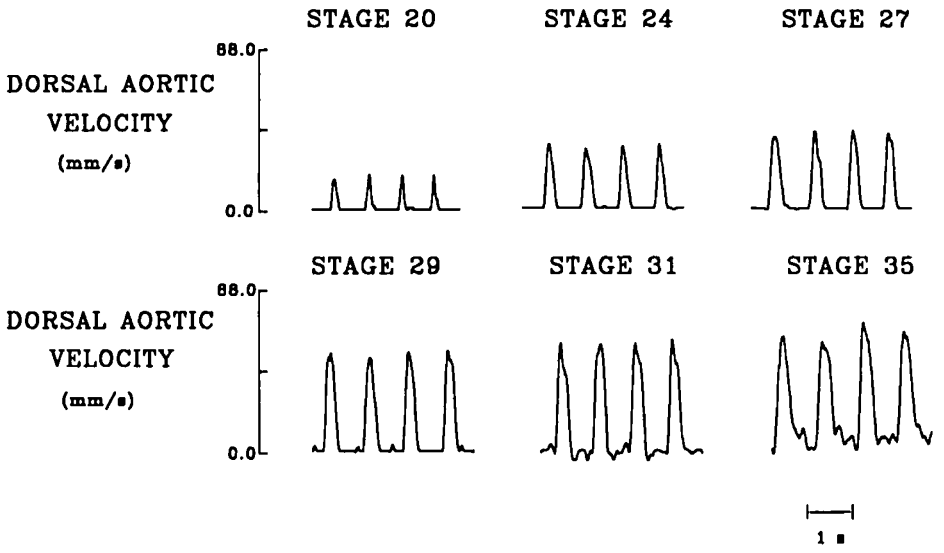

Fig. 2. Analog wave forms. Analog phasic dorsal aortic wave forms of stage 20, 24, 27, 29, 31, and 35 chick embryo.

Table 2. *Dorsal aortic wave form parameters at stages 20 to 35*

	Stage					
Hamburger-Hamilton incubation time	20 (3 d)	24 (4 d)	27 (5 d)	29 (6 d)	31 (7 d)	35 (8 d)
Mean dorsal aortic velocity (mm/s)	3.9 ± 0.8	6.1 ± 0.9	11.4 ± 0.7	11.9 ± 1.2	14.1 ± 3.5	19.9 ± 4.4
Aortic area (mm^2)	0.07 ± 0.02	0.14 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	0.21 ± 0.02	0.25 ± 0.02
Mean dorsal aortic blood flow (mm^3/s)	0.3 ± 0.1	0.9 ± 0.1	1.8 ± 0.2	2.0 ± 0.2	3.0 ± 0.8	5.1 ± 1.3
Heart rate (bpm)	123 ± 12	142 ± 10	177 ± 11	181 ± 6	221 ± 14	239 ± 8
Stroke volume (mm^3)	0.14 ± 0.08	0.35 ± 0.08	0.59 ± 0.11	0.66 ± 0.15	0.81 ± 0.39	1.28 ± 0.55
Peak acceleration (mm/s^2)	687 ± 90	867 ± 116	1464 ± 170	1283 ± 174	2288 ± 460	2506 ± 159
Peak systolic velocity (mm/s)	20.1 ± 1.0	26.2 ± 1.6	45.5 ± 2.9	49.7 ± 5.1	70.6 ± 14.0	82.1 ± 7.7

* Results are expressed as mean ± SD; $n = 10$ for all stages.

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