Human Cardiac Development in the First Trimester

A High-Resolution Magnetic Resonance Imaging and Episcopic Fluorescence Image Capture Atlas

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With rapid advances in medical imaging, fetal diagnosis of human congenital heart disease is now technically feasible in the first trimester. Although the first human embryological studies were recorded by Hippocrates in 300 to 400 BC, present-day knowledge of normal human cardiac development in the first trimester is still limited. In 1886, 2 articles by Dr His described the development of the heart on the basis of dissections of young human embryos. Free-hand wax models were made that illustrated the external developmental anatomy. These wax plate reconstruction methods were used by many other investigators until the early 1900s. Subsequently, serial histological sections of human embryos have been used to further investigate human cardiac development. Using an analysis of histological sections and scaled reproductions of human embryos, Grant showed a large cushion in the developing heart at 6 6/7 weeks (Carnegie stage [CS] 14) and separate atrioventricular (AV) valves at 9 1/2 weeks (CS 22). At the end of 8 weeks (CS 8), separate aortic and pulmonary outflow tracts were observed. Orts-Llorca et al used 3-dimensional (3D) reconstructions of transverse sections of human embryos to define the development of the truncus arteriosus and described completion of septation of the truncus arteriosus in 14- to 16-mm embryos, equivalent to an estimated gestational age (EGA) of 8 weeks (CS 18).

Given the complex tissue remodeling associated with cardiac chamber formation and inflow/outflow tract and valvular morphogenesis, the plane of sectioning often limited the information that can be gathered on developing structures in the embryonic heart. These technical limitations, in conjunction with limited access to human embryo specimens, have meant that much of our understanding of early cardiac development in human embryos is necessary for clinical evaluation and diagnosis of congenital heart disease in the first trimester. This will be increasingly important as improvements in medical technology allow earlier access to first-trimester human fetal cardiac imaging and in utero intervention.

Recent studies have shown the feasibility of using magnetic resonance imaging (MRI) to obtain information on human embryo tissue structure. MRI data can be digitally resectioned for viewing of the specimen in any orientation, and 3D renderings can be obtained with ease. Similarly, episcopic fluorescence image capture (EFIC), a novel histological imaging technique, provides registered 2-dimensional (2D) image stacks that can be resectioned in arbitrary planes and rapidly 3D rendered. With EFIC imaging, tissue is embedded in paraffin and cut with a sledge microtome. Tissue autofluorescence at the block face is captured and used to generate registered serial 2D images of the specimen with better image resolution than MRI. Data obtained by MRI or EFIC imaging can be easily resectioned digitally or reconstructed in 3D to facilitate the analysis of complex morphological changes in the developing embryonic heart. In this manner, the developing heart in every embryo can be analyzed in its entirety with no loss of information resulting from the plane of sectioning.

Using MRI and EFIC imaging, we conducted a systematic analysis of human cardiovascular development in the first trimester. The 2D image stacks and 3D volumes were generated from 52 human embryos from 6 1/2 to 9 1/2 weeks of EGA, equivalent to CS 13 to 23. These stages encompass the developmental window during which all of the major milestones of cardiac morphogenesis can be observed. Using the MRI and EFIC data, we constructed a digital atlas of human heart development. Data from our atlas were used to generate charts summarizing the major milestones of normal human heart development through the first trimester. MRI and EFIC images obtained as part of this study can be viewed as part of the online Human Embryo Atlas. To view the Human Embryo Atlas content, visit http://apps.nhlbi.nih.gov/
Embryos from the Kyoto collection, at the Congenital Anomaly Research Center at the Kyoto University in Japan, were collected after termination of pregnancies for socioeconomic reasons under the Maternity Protection Law of Japan. Embryos were derived from normal pregnancies without any clinical presentations. The specimens were in fixative for an estimated duration of 30 to >40 years, making them unsuitable for immunohistochemistry or any molecular/cellular analysis. This collection represents a random sample of the total intrauterine population of Japan. During accessioning into the Kyoto collection, the embryos were examined and staged according to the CS criteria proposed by O’Rahilly. For this study, 52 embryos from the Kyoto collection (see Table I of the online-only Data Supplement) were donated to the Carnegie collection of normal human embryos archived at the National Museum of Health and Medicine of the Armed Forces Institute of Pathology (http://nmhm.washingtondc.museum/collections/hdac/Carnegie_collection.htm). Each embryo’s age was determined through the use of previously reported postconceptional ages, which were then converted to EGA or menstrual age by adding 14 days and reported in weeks.

MRI and EFIC

High-resolution MRI and EFIC images were obtained from 52 human embryos from 6½ to 9½ weeks of gestation (CS 13 to 23). These specimens from the Kyoto collection were imaged by MRI and EFIC during preparation for accessioning into the Carnegie collection (Table I of the online-only Data Supplement). Human embryos in formalin were treated with 1:20 Magnevist (Berlex, Montville, NJ)/10% formalin solution for 3 days and then rinsed and prepared in 5- to 30-mm tubes, depending on embryo size, with fixative or low-melting agar. Samples that diffused gadolinium into the media were further soaked in plain fixative for ≥2 days and reimaged. Imaging was performed at the National Institutes of Health Mouse Imaging Facility on a 7.0-T Bruker vertical-bore MRI system with 150-G/cm gradients (Bruker, Billerica, Mass) and 5- to 30-mm microimaging birdcage coils (Bruker). Some larger samples also were imaged on a 7.0-T, 16-mm horizontal-bore Bruker Paravision system with 39-G/cm gradients and a 38-mm birdcage coil. MRI was acquired with Paravision 3.0.2 operating systems. Samples were imaged with a 3D rapid gradient echo (SNAP) sequence with a repetition time of 30 to 40 ms, an echo time of 3.3 to 4.0 ms, 20 to 90 averages, an acquisition time of ~12 to 50 hours, and matrixes of 256×128×128 to 512×512×512 (see Table II of the online-only Data Supplement). Over the whole collection, MRI resolution ranged from 29×35×35 to 117×105×105 μm³. Resolution was proportionate to the sample size, with the smallest embryos having the highest-resolution data sets. Individual image data sets are 3D and nearly isotropic, with all 3 voxel dimensions being within 10 μm of each other in an individual data set. Most data sets are in the range of 35×35×35 to 60×60×60 μm³. The resolution of each data set is listed in Table II of the online-only Data Supplement.

In preparation for EFIC, embryos stored in 10% phosphate-buffered formalin were dehydrated and embedded in a mixture of paraffin wax (70.4%), Vybar (24.9%), steearic acid (4.4%), and red aniline dye Sudan IV (0.4%) using techniques previously described. The embedded embryos were then sectioned with a sliding microtome (Leica SM 2500) to obtain 5- to 8-μm-thick sections. The block face was sequentially photographed using epifluorescent illumination with a 100-W mercury lamp and a Leica MZ16A stereomicroscope equipped with 425/480-nm excitation/emission filters. Images were captured with an ORCA-ER digital camera (Hamamatsu, Shizuoka, Japan).

Offline Image Processing and Data Analysis

MRI images originally recorded in DICOM were converted into TIFF format with ImageJ (http://rsb.info.nih.gov/ij/). The EFIC 2D image stacks were captured and exported as TIFF files. Both the EFIC and MRI data were processed with OpenLab (Improvison Inc, Waltham, Mass). We generated 3D reconstructions and QuickTime virtual-reality movies with Volocity (Improvison Inc). The 2D image stacks also were digitally resectioned with Volocity to view internal and external cardiac structures in planes similar to standard echocardiographic imaging planes used clinically. In EFIC images, each pixel was a square with length dimensions ranging from 2.34 to 13.4 μm per pixel edge. Thus, pixel dimensions ranged from 5.48 to 179.56 μm². For each embryo, we generated serial 2D image stacks and 3D reconstructions. From this analysis, we were able to delineate all of the major milestones of human heart development, including...
chamber formation; septation of the atria, ventricles, and truncus arteriosus; and valvular morphogenesis.

**Cardiac Loop**

The cardiac loop or looped heart tube is observed from EGA 6½ to 7½ weeks (CS 13 to 17). A 3D reconstruction of the heart at 7½ weeks (CS 17) reveals internal structures of the cardiac loop (Figure 1). The only exit for blood from the left-sided inflow limb, consisting of the atrial cavity, AV junction, and the presumptive left ventricle, is the interventricular foramen (also known as primary foramen, primary interventricular foramen, bulboventricular foramen, or embryonic interventricular foramen; double arrow in Figure 1); the only exit for blood from the right-sided outflow limb, consisting of the presumptive right ventricle, is the truncus arteriosus (arrowhead in Figure 2). Also of note, the AV junction (Figure 1) is surrounded by endocardial cushion tissue, which is contiguous with the truncus arteriosus.

The developmental changes seen in the cardiac loop are shown in more detail in Figure 2, with images from embryos at 6½ weeks (CS 13) (Figure 2A through 2E) and 7½ weeks (CS 17) (Figure 2F through 2I). As the looped heart tube matures, the atrial and ventricular chambers expand in size, giving rise to distinct subdivisions recognizable as the primitive left and right atria and presumptive left and right ventricles (Figure 2H). At 6½ weeks (CS 13), the endocardial cushions seen lining the AV junction appear thin with little apparent cellular content. As development progresses, they become filled with dense material (Figure 2B, 2E, and 2G and Movie VIIIa and VIIIb of the online-only Data Supplement).
The interventricular foramen also shows striking changes during this developmental period. It is a wide and open communication at 6½ weeks (CS 13) (asterisk in Figure 2C), but as the chambers grow, it becomes a narrow and more distinct opening (foramen) by 7½ to 7¾ weeks (CS 16 to 17) (asterisk in Figures 2H and 3C). The superior AV cushion can be seen (Figure 2G). The inflow, consisting of the venous confluence or primitive atrium (Figure 2A), is observed to communicate with the ventricular chamber via the AV junction (Figure 2B, 2E, and 2G). The presumptive left ventricle communicates with the presumptive right ventricle via the interventricular foramen (asterisk in Figure 2C and 2H). The outflow from the cardiac loop comprises the as-yet undivided truncus arteriosus (T in Figure 2D and 2I) arising from the presumptive right ventricle.

**Atrial Septation (EGA 6½ to 8 Weeks)**

The process of atrial septation is thought to begin with a thin septum primum growing from the posterior wall of the atrium, from a location cranial to the pulmonary vein orifice. It grows toward and eventually fuses with the endocardial cushions. At 6½ weeks of gestation (CS 14), the mesenchymal cap of the primary atrial septum can be seen in contact with the superior AV cushion. The atrial spine, a mesenchymal structure, also was observed. The atrial spine fuses with the inferior AV cushion (6½ weeks [CS 14]) (Figure 3A and 3B and Movie VIIIa and VIIIb) and plays an important role in closure of the primary foramen. Although the pulmonary vein orifice was not seen by our imaging, we can infer from previous studies that it lies to the left of the atrial spine. Septum primum can be observed at 6½ weeks of gestation, and its developmental progression through 7½ weeks can be seen in Figures 2H, 3A, 3B, and 3E and Movies I, VIIIa, and VIIIb). Later, septum secundum develops as an infolding of the dorsal wall of the right atrium, completing atrial septation with fenestrations forming the foramen ovale. Both atrial septum primum and secundum were present by 8 weeks (CS 18) (Movie II of the online-only Data Supplement). At this stage, the mesenchymal cap can be seen fused with the now divided superior AV cushion (Figure 3F). This is consistent with developmental timing suggested by others.

**Ventricular Septation (EGA 7½ to 9½ Weeks)**

Toward the end of the looped heart tube stages of development (7½ and 7¾ weeks, CS 16 and 17), distinct separation of presumptive left and right ventricular chambers is evident. The beginning of the muscular interventricular septum can be seen at these stages, but ventricular septation is not yet complete (Figures 2H, 3C, and 3D and Movie IVa and IVb of the online-only Data Supplement). By 8 weeks (CS 18), the muscular ventricular septum can be seen extending from the floor of the ventricular chamber toward the crux of the heart (Figure 3F). This leaves open a relatively large interventricular foramen that allows communication between the ventricles (Movies I and II of the online-only Data Supplement). Recent lineage tracing experiments in mice have suggested...
that the muscular interventricular septum is made up of cells originating from the ventral aspect of the primitive ventricle, with closure of the ventricular foramen mediated by dorsal migration of this precursor cell population; these cells likely represent a subpopulation of cells derived from the secondary heart field.23 Immunohistochemical analysis of human fetal cardiac tissue showed myocytes expressing the G1N2 antigen localized in a ring around the junction between the future right and left ventricles.24 In later developmental stages, G1N2-expressing cells are found in the area clinically called the inlet ventricular septum but not in the subaortic outflow septum.

At 8 weeks (CS 18), the ventricular septum at the level of the left ventricular outflow is closed (Figure 3G), but part of the inlet ventricular septum at the level of the AV valves remains open (arrowhead in Figure 3F and Movies III, IV, and VI of the online-only Data Supplement). The inlet and membranous portions of the ventricular septum are fully closed at 9½ weeks (CS 22), completing ventricular septation (Figure 3H and Movie V of the online-only Data Supplement). The area clinically called the inlet ventricular septum has been shown in prior studies to originate from the embryonic right ventricle.25 In agreement with previous reports on human development, our data showed that the final portion of the ventricular septum to close included what likely makes up a combination of the membranous and inlet ventricular septum. These findings suggest that an arrest in the development of the ventricular septum could result in ventricular septal defects similar to those observed clinically.

**Formation of the AV Valves**

(EGA 7 ½ to 8 Weeks)

AV valve morphogenesis begins at the looped heart tube stages, with large endocardial cushions seen prominently at the center of the cardiac loop (asterisks in Figures 3A and 4A). The AV canal is divided by the endocardial cushions, which form on the posterior (dorsal) and anterior (ventral) walls of the AV canal. These cushions eventually divide the AV canal into right and left AV orifices.2,19 A well-delineated AV junction can be seen at 7 ½ weeks of gestation (CS 16) (Figure 4B and 4C and Movie Xa and Xb of the online-only Data Supplement). At 7½ and 7 5/7 weeks (CS 16 and 17), the AV junction was still undivided. A few days later, by 8 weeks of gestation (CS 18), separate AV valves can be seen (arrowheads in Figure 4D and 4E), with left-sided mitral and right-sided tricuspid valves forming. An embryo at 8 weeks (CS 18) is ~10 mm in size, correlating well with the embryonic stage at which fusion of the endocardial cushions is thought to occur.26 The valve leaflets, however, appear thick at this stage (see Movie IV of the online-only Data Supplement). By 9½ weeks (CS 22), the AV valve leaflets are thinner and more mature in appearance (Figure 3H and Movie V of the online-only Data Supplement). At 7½ weeks (CS 16), distinct posterior and anterior cushions are not observed and the inferior AV cushion is
observed; this timing is consistent with previous reports of human embryonic development.²²

**Outflow Septation and Semilunar Valve Morphogenesis (EGA 7 ½ to 8 Weeks)**

The major developmental processes occurring at the level of the truncus arteriosus consist of septation into 2 separate arterial channels and semilunar valve morphogenesis. The truncus arteriosus is formed largely from cells derived from the secondary heart field.²⁷ Septation of the truncus arteriosus, which is dependent on activity of the secondary heart field and migrating neural crest cells,²⁸,²⁹ is achieved with in-growth of ridges. In the proximal truncus arteriosus, we observed truncal cushions in the form of swellings at 7 ½ weeks (CS 15) (arrowhead in Figure 5A). This forming aorticopulmonary septum undergoes a gradual spiraling course that ultimately completes truncus arteriosus septation into separate aorta and pulmonary arteries.²⁸ At 7 ½ weeks (CS 16), this spiraling course of the forming aorticopulmonary septum is evident as a spiraling in the orientation of the lumen (D and E) indicative of spiraling of the cushions (see arrowheads). F, The aorticopulmonary septum (arrowhead) has divided the distal portion of the truncus arteriosus into 2 separate arterial channels to the right and left of the aorticopulmonary septum. Scale bar = 0.9 mm. A (on compass) indicates anterior; P, posterior; R, right; L, left; Cr, cranial; Ca, caudal; A, primitive atrium/venous confluence; RA, right atrium; and LA, left atrium.

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**Figure 5. Septation of the truncus arteriosus.** A, EFIC image of an embryo at EGA 7 ½ weeks (CS 15) in the sagittal plane shows a single orifice of the truncus arteriosus with inward swelling of the aorticopulmonary septum (arrowhead) that precedes septation of the truncus arteriosus. Scale bar = 0.622 mm. B and C, EFIC image of an embryo at EGA of 8 (CS 18) (B) shows a distinct pulmonary artery (PA) emerging from the right ventricle (RV) and a left ventricular outflow tract (LVOT) or aorta emerging from the left ventricle (LV). Three-dimensional volume of the same embryo (C) shows crossing of the great arteries. Scale bar = 1.35 mm (B and C). D through F, EFIC images of an embryo at EGA 7 ½ weeks (CS 16) in oblique transverse planes showing the truncus arteriosus. Note the changing orientation of the lumen (D and E) indicative of spiraling of the cushions (see arrowheads). F, The aorticopulmonary septum (arrowhead) has divided the distal portion of the truncus arteriosus into 2 separate arterial channels to the right and left of the aorticopulmonary septum. Scale bar = 0.9 mm. A (on compass) indicates anterior; P, posterior; R, right; L, left; Cr, cranial; Ca, caudal; A, primitive atrium/venous confluence; RA, right atrium; and LA, left atrium.

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**Figure 6. Developmental time course of human cardiac morphogenesis.** Outlined in the chart is the timing for major cardiac morphogenetic events and the presence of various cardiac structures in the human embryo. The timeline indicated for AV junction valve formation (green bar) refers to when a distinct AV junction is observed before AV valve leaflets are evident. The timeline indicated for semilunar valve formation (orange bar) refers to when distinct truncal cushion tissue is observed and before semilunar valve leaflets are evident. The demarcation of mitral valve, tricuspid valve, aortic valve, and pulmonary valve delineates the developmental stages when distinct valve leaflets are observed and the stages when the valve leaflets continue to undergo maturation and thinning. The timeline indicated for interventricular foramen refers to when any communication is present between the right and left ventricular chambers.
Bartelings and Gittenberger-de Groot suggested that in 7½-week (CS 16) embryos, septation begins at the ventriculoarterial junction and progresses proximal to distal in the truncus arteriosus. However, our findings show septation of the truncus arteriosus occurring in the opposite direction, being complete distally in the 7½-week (CS 16) embryo, at a time when the proximal truncus arteriosus is still undivided. This would suggest that the direction of septation is distal to proximal. This is supported by Kirby, who described the proximal truncus arteriosus closing zipper-like from distal to proximal toward the ventricles. Our data also support both the timing and direction of septation proposed by Anderson et al. They described septation of the truncus arteriosus initiating distally and progressing proximally with the presence of distal septation and the absence of proximal septation at 7½ weeks (CS 16). Moore and Persaud described bulbar ridges at the fifth week after conception equivalent to 7 weeks of gestation. Assuming that the bulbar and truncal cushions are forming at the same time, our finding of truncal cushions in the outflow at 7½ weeks (CS 15) also corroborates these investigators’ timeframe.

The process of semilunar valve morphogenesis, similar to AV valve morphogenesis, began earlier with the formation of truncal cushion tissue, which was observed in the outflow starting at 7½ weeks (CS 15). At 8 weeks (CS 18), distinct pulmonary and aortic valves can be seen (Figure 5B and 5C). These valve leaflets, as well as AV valve leaflets, are initially thick. They undergo a process of thinning as the valve leaflets continue to form and mature, a process that continues well after the formation of distinct valve leaflets (Figure 6). By 9½ weeks (CS 22), all of the major structures of the heart are formed, with the last developmental milestone being completion of the inlet ventricular septum (see above).

### Conclusions

As rapid advances in technology provide first-trimester human fetal cardiac imaging and opportunities for in utero intervention continue to advance, there is increasing need for data documenting human cardiac development in the first trimester. Using a large data set generated by MRI and EFIC imaging, we delineated the major developmental milestones of human cardiac morphogenesis spanning EGA 6½ to 9½ weeks.
weeks. A summary timeline is provided in Figure 6 for the temporal profile of atrial and ventricular septation, outflow septation, and valvular morphogenesis. In addition, Figures 7 and 8 were generated as reference guides to aid clinical practice. They contain thumbnail images of cardiac structures seen at each developmental milestone of cardiac morphogenesis. Full-size images and QuickTime movies of the 2D serial image stacks of these embryos are available in the online-only Data Supplement (Movies I through VII) and the Web-based Human Embryo Atlas (http://apps.nhlbi.nih.gov/HumanAtlas/). A deeper understanding of human cardiovascular development, including this large data set and the reference guides generated, may ultimately aid in clinical practice and facilitate prenatal diagnosis of congenital heart disease and appropriate counseling of families.

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**Disclosures**

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**References**


KEY WORDS: embryology ■ fetal development ■ human development ■ pregnancy trimester, first
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Human Cardiac Development in the First Trimester:
A High Resolution MRI and Episcopic Fluorescence Image Capture Atlas

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Table S1. Human Embryo Imaging by MRI and Episcopic Fluorescence Image Capture

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<th>Estimated Gestational Age (weeks)</th>
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<th>Imaging by EFIC*</th>
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*Number of specimen yielding good imaging data indicated in parenthesis.
## Table S2. MRI Acquisition Resolution

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QUICKTIME MOVIE LEGENDS

A. Quicktime Movies of 2D Image Stacks

Movie 1: companion to Figure 2F, EGA 7 5/7 (CS 17)
This image stack shows embryo at an estimated gestational age (EGA) 7 5/7 week, Carnegie Stage (CS) 17 embryo. Imaging is in an off-axis sagittal plane. *= interventricular foramen, also known as the primary foramen, primary interventricular foramen, and bulboventricular foramen. Arrowhead designates septum primum, seen extending from cranial aspect of atrial roof and extending caudally to septate the atria. RA = right atrium; LA = left atrium; V = ventricular mass; RV = presumptive right ventricle; LV = presumptive left ventricle.

Movie 2: companion to Figure 3F, EGA 8 weeks (CS 18)
This video stack represents EFIC imaging of an EGA 8 weeks (CS 18) gestation fetal heart. Imaging is in an off-axis coronal plane. Right and left sided ventricular chambers can be seen, connected by an interventricular foramen. Atrial septation is complete, and the septum primum is seen fused with the atroventricular cushions. The inlet component of the ventricular septum is still open. *= septum primum; arrowhead indicates the still open inlet ventricular septum; RA = right atrium; RV = right ventricle; LV = left ventricle.

Movie 3: companion to Figure 3G, EGA 8 weeks (CS 18)
This video stack shows EFIC imaging of an EGA 8 weeks (CS 18) human embryo. Imaging is in a coronal plane. This clearly delineates that the ventricular outflow tract septum is closed while a portion of the inlet ventricular septum is open. *= complete atrial septum; first arrowhead indicates the left ventricular outflow tract; second arrowhead indicates the right ventricular outflow tract; RA = right atrium; LV = left ventricle; RV = right ventricle.

Movie 4: companion to Figure 4D, EGA 8 weeks (CS 18)
This 2D image stack from MRI imaging of an EGA 8 weeks (CS 18) human fetus. This stack is shown in an off-axis coronal plane. Arrowheads indicate thick right and left atrioventricular valves; asterisks denotes open inlet ventricular septum; arrow points to closed outflow tract septum. On either side of arrow, right and left ventricular outflow tracts can be noted. RA = right atrium; RV = right ventricle.

Movie 5: companion to Figure 4F, EGA 9 3/7 (CS 23)
2D image stack from MRI scan of an EGA 9 3/7 week (CS 23) human fetus. The imaging plane is in an off-axis coronal plane. Thinner appearance of the atroventricular valves is seen. *= closed inlet septum; arrowheads point to the atroventricular valves. Two crossing, distinct outflow tracts can be seen. RV = right ventricle; LV = left ventricle.

Movie 6: companion to Figure 5B, EGA 8 (CS 18)
2D image stack from MRI scan of an EGA 8 weeks (CS 18) human fetus. The imaging plane is in an off-axis coronal plane. Asterisks denotes open inlet ventricular septum; long arrow points to closed outlet septum. To the left of the arrow is the pulmonary artery, and to the right is the aorta. RA = right atrium; LA = left atrium.

Movie 7: companion Figure 5D-F, EGA 7 3/7 (CS 16)
2D image stack shows EFIC imaging of an EGA 7 3/7 week (CS 16) human embryo. The stack is shown in the transverse plane. The movie plays in the same sequence as truncal septation, i.e. from distal to proximal (see Figure 5 panels 5 F,E,D). Initially, the distal truncal outflow tract is
seen with lumens that exhibit a superior/inferior position. As the movie progresses, moving more proximally along the outflow, the aorticopulmonary septum is observed (denoted by an asterisk), with channels that exhibit right/left orientation. Most proximally, the outflow tract or truncus arteriosus is not yet divided. Below the truncus arteriosus, the presumptive right ventricular outflow tract can be seen. ** = interventricular foramen; RA = right atrium; LA = left atrium; RV = presumptive right ventricle; LV = presumptive left ventricle.

**B. Quicktime Movies and QTVRs of 3D Reconstructions**

Movie “a” is Quicktime movie of 3D model, and Movie “b” is interactive QTVR of the same 3D model.

**Movies 8a,b: Companion Quicktime movie and QTVR to 3D model in Figure 3B (CS 14)**

3D reconstruction of an embryo at EGA 6 6/7 weeks, CS14, shown in the frontal plane. Note the septum primum, with mesenchymal cap attached to the superior endocardial cushion. A common ventricular mass is seen.

**Movies 9a,b: Companion Quicktime movie and QTVR to 3D model in Figure 3D (CS16)**

3D reconstruction using EFIC imaging in the transverse plane of an embryo at EGA 7 3/7 weeks (CS 16). The developing ventricles are profiled. The presumptive left ventricular cavity can been seen, with interventricular foramen opening into the presumptive right ventricular cavity.

**Movies 10a,b: Companion Quicktime movie and QTVR to 3D model in Figure 4C (CS16)**

3D reconstruction using EFIC imaging of an EGA 7 3/7 weeks (CS 16) embryo. Imaging is in the sagittal plane. The developing atrioventricular valve structure is clearly delineated. The atrioventricular orifice is primarily positioned over the presumptive left ventricle, with cushions and supporting structures well seen. The presumptive right ventricle and truncus arteriosus, with two outflow cushions, are also profiled.
SUPPLEMENTAL FIGURES

Ten Figures Containing Enlarged Images of Thumbnails in Figures 7 and 8
The FIGURE NUMBER and PANEL LABELS correspond to panel labels of the individual thumbnails in Figures 7 and 8.

Figure S7A-E: Inflow

Figure S7F-J: Atrioventricular Junction

Figure S7K-O: Ventricular Mass

Figure S7P-T: Outflow.

Figure S8A-E: Atrial septation.

Figure S8F-H: Ventricular septation: Outflow Septum

Figure S8I,J: Ventricular septation: Inlet Septum

Figure S8K-O: Atrioventricular valves

Figure S8P-U: Semilunar valves: Aortic Valves

Figure S8V-AA: Semilunar valves: Pulmonary Valve