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Cardiovascular assessment of fetal mice by in utero echocardiography

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Abstract

To establish a developmental profile of fetal mouse cardiovascular parameters, we analyzed a large body of ultrasound measurements obtained by in utero echocardiography of C57BL/6J fetal mice from embryonic day 12.5 to 19.5 (term). Measurements were obtained using 2D, spectral Doppler and M-mode imaging with standard clinical cardiac ultrasound imaging planes. As these studies were conducted as part of a large scale mouse mutagenesis screen, stringent filtering criteria were used to eliminate potentially abnormal fetuses. Our analysis showed heart rate increased from 190 to 245 bpm as the mouse fetus grew from 8 mm at embryonic day 12.5 to 18.7 mm at term. This was accompanied by increases in peak outflow velocity, E-wave, E/A ratio and ventricular dimensions. In contrast, the A-wave, myocardial performance index and isovolemic contraction time decreased gradually. Systolic function remained remarkably stable at 80% ejection fraction. Analysis of intra and interobserver variabilities showed these parameters were reproducible, with most comparing favorably to clinical ultrasound measurements in human fetuses. A comprehensive database was generated comprising 23 echocardiographic parameters delineating fetal mouse cardiovascular function from embryonic day 12.5 to term. This database can serve as a standard for evaluating cardiovascular pathophysiology in genetically altered and mutant mouse models.

Keywords

Fetal ultrasound; Image comparison; Mouse fetus; Development; Cardiovascular system

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INTRODUCTION

Mice are now widely used to model a variety of human heart diseases. As mice are air breathing mammals with four chamber hearts, they have proven to be an excellent model system for studying human congenital heart disease (Wessels and Sedmera 2003). In fact, most of the major congenital heart anomalies seen clinically can be found in mutant mouse models (Yu et al. 2004; Shen et al. 2005). Consequently the assessment of fetal mouse cardiac structure and function has become increasingly important for investigating the etiology of human congenital heart disease. Of critical importance in such studies is the availability of tools for fetal mouse cardiovascular phenotyping. Echocardiography is one imaging modality extensively used for cardiac assessments in the clinical setting. It has also become invaluable for cardiovascular assessments in mouse models. However, the very small size of the fetal mouse heart (less than 5 mm at birth) has made echocardiography of fetal mice technically challenging.

Mouse fetal echocardiography has been carried out using standard clinical ultrasound systems (Gui et al. 1996; Spurney et al. 2004), as well as ultra-high frequency ultrasound systems, also referred to as ultrasound biomicroscopes (Srinivasan et al. 1998; Phoon et al. 2000, 2004; Foster et al. 2002; Zhou et al. 2002, 2003). Ultra high frequency ultrasound systems equipped with 30–40 MHz transducers have greatly improved 2D spatial resolution that allow even very early mouse embryos (from embryonic day 5.5) to be imaged (Srinivasan et al. 1998; Phoon et al. 2000; Foster et al. 2002). However, the higher ultrasound frequencies cause reduced penetration depth, making it more difficult to obtain optimal ultrasound imaging planes for cardiac assessments (Zhou et al. 2003). To overcome this limitation in imaging depth, mouse fetuses examined by ultrasound biomicroscopy have been exteriorized (Phoon et al., 2000, 2004), which raises some concerns that hemodynamic function may be perturbed. In contrast, clinical ultrasound systems have higher penetration depth, and as result, the acquisition of images in the desired ultrasound imaging planes is greatly facilitated. This allows for in utero ultrasound interrogations, even in near term mouse fetuses. However, given the reduced 2D spatial resolution of clinical ultrasound systems, cardiac assessments can only be made in later stages of mouse fetal development.

Echocardiography of fetal mice has been reported in a number of studies, with most using ultrasound biomicroscopy systems. In this study, we evaluated a large body of echocardiographic data obtained as part of a large scale mouse mutagenesis screen focused on cardiovascular phenotyping (Yu et al. 2004; Shen et al. 2005). The echocardiographic measurements were acquired by noninvasive in utero ultrasound scanning of mouse fetuses conducted serially using a clinical ultrasound system equipped with a 15 MHz transducer. For this analysis, we first eliminated any potentially abnormal fetuses using stringent filtering criteria similar to those employed clinically for evaluating human fetal echocardiographic measurements (Bronstein et al. 1992; Tan et al. 1992). The data from the remaining fetuses were evaluated statistically, with remeasurements made to assess intra and interobserver variabilities. We found 23 of the echocardiographic parameters can be reliably obtained for mouse fetal cardiac assessments. Overall, the developmental profile of mouse fetal cardiac function is similar to that of human fetuses. The echocardiographic

measurements obtained in this study should be a valuable resource for future prenatal studies of disease and mutant mouse models.

METHODS

Ultrasound imaging and Doppler echocardiography

Ultrasound scanning was conducted as previously described using an Acuson Sequoia C256 ultrasound system with a 15 MHz L8 linear phased array transducer (Acuson Corp., Atlanta, GA, USA) on 2500 fetal mice derived from N-ethyl-N-nitrosurea mutagenized mice (Yu et al. 2004; Shen et al. 2005). Further information on the mutagenesis and breeding protocols of C57BL6/J mice also can be found at URL: <http://pga.jax.org/protocols.html>. The mother was sedated using 1.5% isoflurane, with her body temperature maintained at 36–37°C, and heart rate at 450–550 beats per minute (bpm). B mode and M-mode axial resolution was 0.05–0.1 mm, and B-mode lateral resolution was 0.2–0.5 mm (Zhou et al. 2002). Temporal frame rate in echo-mode was set at 60 Hz. A 1.0 mm sampling gate was used to obtain inflow and outflow velocities, and the maximal sweep speed was 200 mm/sec. The spectral Doppler and M-mode images were recorded in multiple screen shots, with the Doppler and M-mode measurements obtained from individual heart beats. Ultrasound parameters acquired from spectral Doppler, 2D and M-mode imaging will be introduced in the results. All experiments were conducted in accordance with an approved animal protocol of the National Heart Lung Blood Institute (Protocol Number H-0086).

Ultrasound data analysis and statistics

Mean and standard deviation were calculated for all ultrasound parameters at each gestational age, and statistical analysis was carried out using the analysis of variance (ANOVA) and Tukey's multiple comparisons. Correlations between different echo parameters, or between individual echo parameter and gestational age were studied by linear and quadratic regression models. Measurements from individual echocardiographic parameters were plotted with the regression estimates set at the 95% confidence interval. The significance of the regression models was tested by the likelihood ratio test, and the squared term was dropped if its coefficient was not significantly different from 0 by the Wald Test. The differences between inflow A wave velocity and outflow velocity at the same developmental stage were tested by two sample t test. The statistical significance was set at $P < 0.05$. Analyses were performed using SAS 9.0 (SAS Institute Inc., Cary, NC, USA) and S-PLUS 7.0 (Insightful Corp., Seattle, WA, USA).

Analysis of Inter and Intraobserver Variability

Ultrasound data from 10 to 35 fetuses selected at random from each development stage was remeasured using the Acuson KinetDx workstation (Acuson Corp., Atlanta, GA, USA). Ultrasound measurements remeasured by the initial sonographer was used to assess intra-observer variability, and measurement by a second observer provided interobserver variability. Intra- and interobserver variabilities were presented as percentages and calculated as the difference between two measurements divided by the mean of the measurements.

RESULTS

Echocardiographic measurements were previously obtained by serial echocardiography from mouse embryonic day (E) 12.5 to E19.5 (term), with each litter usually scanned three times (Yu et al. 2004; Shen et al 2005). The C57BL6/J female mouse usually carried out 5 to 7 fetuses per litter, which is easy to be identified by the ultrasound scanning. We used standard cardiac imaging planes established for human echocardiography to facilitate comparison of the mouse fetal echocardiographic measurements with clinical data obtained for human fetuses (Hess et al. 2004; Rychik et al. 2004). We note that echocardiographic measurements are greatly dependent on the imaging planes used for ultrasound data acquisition. Not every echocardiographic parameter was acquired for each fetus, as the mouse fetal position in utero dictated the imaging planes that can be acquired. We used a combination of 2D imaging, spectral Doppler, and M-mode analysis. 2D imaging was used to assess fetal growth parameters, while flow velocity and time intervals obtained from spectral Doppler were used to assess hemodynamic function. M-mode measurements provided ventricular chamber wall and lumen dimensions. Fetuses (53 of 1055 fetuses scanned) exhibiting echocardiographic measurements more than 2 standard deviations from the mean were eliminated. Also eliminated from the analysis were fetuses shown from the ultrasound imaging to have pericardial effusion, hydrops, regurgitant flow or high velocity inflow/outflow as detected by color flow imaging. Data from the remaining fetuses were statistically analyzed, and remeasurements were used to evaluate inter and intraobserver variabilities.

Growth Parameters

Crown to rump length (CRL), fetus area, and heart area measurements were obtained using 2D images acquired in the frontal imaging plane (Fig. 1a). These measurements provided a growth profile of the fetus. Fetuses with CRL more than 2 standard deviations below the mean for its gestational age were eliminated. Measurements from the remaining fetuses were used to calculate the mean and standard deviation for these growth parameters. From E12.5 to E19.5, the CRL increased from 7.9 ± 0.8 to 18.7 ± 0.7 mm and fetus area from 23.2 ± 5 to 127 ± 8 mm². The area of the heart, due to its small size, could be measured only from E14.5, and it increased from 2.9 ± 0.3 to 7.7 ± 0.7 mm² at E19.5 (Table 1). The ratio of heart area over fetus area showed a gradual increase from 0.050 to 0.062 (Table 1). A quadratic relationship was noted between these variables and gestation age (Fig. 1d–g). Plot of the fetus area and heart relative to the CRL showed a concordant increase (Fig. 1c). Remeasurements of these growth parameters by the same and different observers showed intra and interobserver variabilities ranging from 2–12%, with CRL being the most robust, with a 2% intraobserver and 4% interobserver variability (Table 2).

Ultrasound Imaging Planes for Echocardiographic Measurements

At E12.5 and E13.5, the very small size of the fetal mouse heart dictated the use of nonconventional frontal/sagittal/transverse ultrasound imaging planes defined by the embryo's body axes (Shen et al. 2005). With the aid of color-flow imaging, inflow/outflow tracts and ventricular chambers could be detected. At these early stages, only spectral Doppler measurements were reliably acquired. The outflow measurements were not

assigned as pulmonary or aortic velocities at these stages, as 2D spatial resolution was not sufficient to detect the two separate outflows (Table 3). At E14.5, ultrasound scanning was reliably conducted using six standard clinical cardiac imaging planes, which included apical 3/4/5 chamber views (Fig. 2a–c; Supplemental movie 1), left ventricular (LV) long axis view (Fig. 2d; Supplemental movie 2), ventricular short axis view (Fig. 2e; Supplemental movie 3) and great vessel short axis view (Fig. 2f). The apical 3/4/5 chamber views can be alternately obtained by angling the transducer with guidance from color flow imaging. Together these six imaging planes allowed cardiac anatomy to be examined. For example, from the LV long axis view, LV inflow and aortic outflow (Fig. 2d) could be readily identified.

Spectral Doppler Assessment of Inflow and Outflow Velocities

Spectral Doppler analysis carried in the apical 3/5 chamber and LV long axis views was used to obtain aortic velocity from the ascending aorta. Aortic velocity showed increases from 0.33 ± 0.07 m/sec at E14.5 to 0.38 ± 0.08 m/sec at E19.5 (Table 3). The pulmonary outflow velocity was obtained using the short axis view of the great vessels. It increased from 0.34 ± 0.06 m/sec at E14.5 to 0.40 ± 0.08 m/sec at E19.5 (Table 3). Left ventricular inflow velocity was obtained from the mitral valves in apical 3/5 chamber and LV long axis views (Table 3; Fig. 4). From E12.5 to E19.5, the active filling inflow “A-wave” decreased linearly from 0.37 ± 0.06 m/sec to 0.34 ± 0.05 m/sec, while the passive filling “E-wave” increased from 0.06 ± 0.02 m/sec to 0.15 ± 0.03 m/sec, with the E/A ratio increasing from 0.19 ± 0.05 to 0.44 ± 0.06 (Table 3; Fig. 4). There was significant A-wave dominance between E12.5 and E14.5, with the A-wave velocity greater than the outflow velocity at these stages (Table 3; Fig. 3b and Fig. 4). At E12.5, A-wave was seen without detection of the E-wave in 60% of the fetuses (Table 3; Fig. 3a, compare to Fig. 3b). Overall there was good reproducibility in these spectral Doppler measurements, with remeasurements showing intra/interobserver variabilities ranging between 4 and 12% (Table 2).

Time Interval Measurements from Spectral Doppler Tracings

Time interval measurements from the spectral Doppler tracings were used to obtain heart rate, isovolemic contraction time (IVCT), isovolemic relaxation time (IVRT), and ejection time (ET). The heart rate increased from 190 ± 12 bpm at E12.5 to 245 ± 20 bpm at E19.5 (Table 3, Fig. 4). IVCT, IVRT and ET decreased with development, with IVCT decreasing from 35 ± 14 to 15 ± 4 msec, IVRT from 44 ± 8 to 25 ± 5 msec and ET from 127 ± 11 to 98 ± 9 msec (Table 3, Fig. 5). Note IVRT was not obtained for E12.5 embryos that did not exhibit an E wave. The heart rate and ET showed excellent reproducibility, with intra/interobserver variabilities of 2–4% (Table 2). Using these time interval measurements, the myocardial performance index (MPI) was calculated. The MPI, defined as ET divided by the sum of IVCT and IVRT, is a composite measurement of diastolic and systolic function (Tsutsumi et al. 1999). MPI steadily decreased from 0.58 ± 0.04 at E12.5 to 0.43 ± 0.06 at E16.5, and remained stable thereafter to birth (Table 3, Fig. 5). Time interval measurements also showed good reproducibility, with intra/interobserver variability of 2–12% (Table 2).

Measurements of Systolic Heart Function

M-mode images obtained from the short axis view were used to measure ventricular wall thickness and chamber dimensions (Fig. 3c,d). Due to the difficulty of achieving a true short axis view, M-mode data were obtained for only 25% of the fetuses. A steady increase was observed for ventricular chamber dimensions from E14.5 to E19.5 (Table 4, Fig. 6). Ventricular contractility indices were derived from these measurements, including fractional shortening (FS), ejection fraction (EF), and heart rate corrected mean velocity of circumferential fiber shortening (MVCFc). These contractility indices showed cardiac systolic function remained fairly constant, with 80% EF, 44% Fs, and 9×10^{-4} circ/sec MVCFc from E14.5 to E19.5 (Table 5). Intra and interobserver variabilities for these M-mode parameters ranged from 3–12% (Table 2). An alternative means for measuring contractility entailed tracing the left ventricular area in diastole and systole using 2D images obtained in the apical three-chamber or LV long axis views (Fig. 3e–h). These imaging planes were relatively easy to acquire and were obtained for nearly 50% of the fetuses. EF was determined as 73% and fractional area change (FAC) as 57% (Table 5). Although measurements of the left ventricular area acquired from the 2D images showed high intra/interobserver variabilities (20–25%), the resulting derivative values for FAC and EF showed intra/interobserver variabilities of 5–12%, similar to that of M-mode derived EF measurements (Table 2). M-mode measurements showed thickness of the interventricular septum (IVS) increased between E14.5 and E19.5. In contrast, no significant change was detected in the thickness of the right ventricular (RV) and LV free wall, likely a reflection of the limited 2D spatial resolution of clinical ultrasound systems (Table 4).

DISCUSSION

Our analysis of a large body of ultrasound measurements obtained using a clinical ultrasound system for in utero echocardiography of fetal mice from E12.5 to term validated 23 echocardiographic parameters for fetal mouse cardiac assessment. This included flow velocities, time interval measurements, measurements of systolic function, and chamber dimensions. Our measurements were similar to those previously reported in other mouse fetal echocardiographic analyses carried out using clinical ultrasound systems (Gui et al. 1996; Spurney et al. 2004). Due to differences in experimental procedure and equipment design, it is difficult to make comparisons with ultrasound studies carried out with ultra-high frequency biomicroscopes. In some of the biomicroscope studies, a semi-invasive procedure involving exteriorizing the uterine horns was used, which can affect hemodynamic function (Phoon et al., 2000, 2004). The slower frame rates of biomicroscopes (8–30 Hz as compared to 60 Hz in clinical ultrasound systems) also made it difficult to obtain images of the heart at true end-diastole and end-systole. In addition, the use of pentobarbital and magnesium sulfate in the anesthetic mix in some studies is expected to depress cardiac function (Srinivasan et al., 1998). Together these factors likely contributed to the low FAC (34%) reported in one study using the biomicroscope (Srinivasan et al., 1998), as compared to the 57% FAC seen in our study. The acquisition of peak outflow velocities is also difficult with ultra-high frequency ultrasound systems, as the lack of phased array technology and color flow Doppler imaging would make it difficult to align the Doppler sample volume with the high resolution 2D images. We note only velocities from the dorsal aorta have been reported

in mouse fetal ultrasound studies carried out with the ultra-high frequency biomicroscope, as the dorsal aorta in the early embryos are highly echogenic and easily visualized by ultrasound (Srinivasan et al., 1998; Phoon et al., 2000, 2004). Nevertheless, with recent improvements, one study showed the feasibility of conducting noninvasive imaging of fetal mice in utero using an ultra-high frequency ultrasound system (Zhou et al., 2003). They reported heart rates, and E/A ratios similar to our study, but fetal mouse outflow velocities were not reported. With further improvements in frame rates and the future development of phased array transducers and color flow imaging, ultra high-frequency biomicroscopes will greatly enhance the analysis of cardiac structure and function in fetal mice. At present, commercially available ultra-high frequency ultrasound system can operate at 40 Hz with maximal imaging depth of 7–14 mm, with up to 60 μ m lateral and 50–100 μ m axial resolution (Vevo770, Visualsonics, Inc). This compares with 60 Hz and 20 mm imaging depth, with 50–100 μ m axial and 200–500 μ m lateral resolution with the clinical Acuson Sequoia ultrasound system (Zhou et al., 2002). The inherent limitation in penetration depth of ultra-high frequency ultrasound will make it difficult to interrogate pregnancies with a large number of fetuses, as the deeper fetuses will be inaccessible to ultrasound detection. These various considerations suggest there are trade offs that must be considered in selecting the optimal ultrasound system for a mouse fetal echocardiography study.

To compare our mouse fetal ultrasound findings with human echocardiographic measurements, it is important to consider differences in developmental timing of mouse vs. human embryos. The emergence of major developmental milestones (neural tube closure, somite development, fore and hindlimb development, retraction of the gut and ventral body wall closure), and the completion of key events in cardiac morphogenesis (outflow and chamber septation) would suggest a term mouse fetus may be equivalent to a first trimester human fetus at 10–12 weeks of gestation (Kaufman 1992; Abdulla et al. 2004). Within this developmental time frame, fetal mouse cardiovascular development and function compares favorably to that of human fetuses (Yagel et al. 2003). Thus we observed mouse fetal heart rate increasing from 190 bpm at E12.5 to 245 bpm at E19.5. In human fetuses, the embryonic pulse was first detected between days 26 and 32 at 80 bpm, then steadily increasing to 170 bpm at the end of the first trimester (Leiva et al. 1999; Howe et al. 1991). The increase in the ratio of heart and fetus areas with gestation age in mouse fetuses was similar to the increases in the ratio of heart/chest area and heart/chest diameter reported for human fetuses (Bronshtein et al. 1992; Gembruch et al. 2000). Mouse fetus area and heart area were also highly correlated to the crown-to-rump length as in human fetuses (Leiva et al. 1999; Zimmer et al. 1994). These parallel changes likely reflect the increasing physiological demands for blood circulation with fetal growth. Both human and mouse fetuses also showed increases in their aortic and pulmonary velocities, and decreases in IVCT and MPI (see below).

Our study showed at E12.5, some mice exhibited the A-wave but no E-wave, which was consistent with previous studies showing inflow changing from monophasic to biphasic after E11.5 in fetal mice (Gui et al. 1996). In human fetuses, this same transition was observed as early as 8 weeks of gestation (Yagel et al. 2003; van Splunder et al. 1996). As a small E-wave could be detected in mouse embryos as early as E9.5 using a 20-MHz zero-cross pulsed Doppler velocimeter (Ishiwata et al. 2003), it's likely the amplitude of the E-wave

normally ramps up at E11.5. The E/A ratio was observed to increase as development progressed, which may be indicative of an overall increase in ventricular compliance in fetal mice (Gui et al. 1996; Zhou et al. 2003; Spurney et al. 2004). This also has been observed in human fetuses from 20 weeks of gestation to term (van der Mooren et al. 1991; Chang et al. 2000; Abuamad et al. 2004). A-wave inflow velocities were observed to be greater than outflow velocities between E12.5 to E14.5, which would further suggest atrial systolic function may play a significant role in supporting early fetal development. Consistent with this, mice deficient in atrial myosin light chain 2 have been shown to die between E10.5 to 11.5 with diminished atrial contraction (Huang et al. 2003). A slow decrease in IVCT was found, likely attributable to increasing ventricular pressure. This also has been reported in human fetuses (Leiva et al. 1999; Chang et al. 2000). As seen in previous mouse and human fetal echocardiography studies, both peak aortic and pulmonary outflow velocities increased with development (Gui et al. 1996; Spurney et al. 2004; Phoon et al. 2000, 2004; Leiva et al. 1999; van der Mooren et al. 1991; Chang et al. 2000; Abuamad et al. 2004). MPI, an index that monitors systolic and diastolic function, showed decreases from E12.5 to E16.5, and remained steady thereafter. MPI also gradually decreased in human fetuses from 18 to 33 weeks of gestation (Tsutsumi et al. 1999).

Contractility indexes obtained from ventricular chamber dimensions derived from M-mode measurements were remarkably constant, remaining at 80%EF and 44%Fs from E14.5 to term. These values were similar to M-mode derived values in human fetuses, which are reported at 85% EF and 48% Fs between 10–40 weeks of gestation (Hsieh et al. 2000). Although 2D spatial resolution is limited with clinical ultrasound systems, 2D tracings of the ventricle area yielded 73% EF, surprisingly similar to the 80% EF obtained by M-mode. Ventricular chamber dimensions obtained by M-mode imaging were surprisingly robust despite the small size of the fetal mouse heart. Derivative values calculated from these measurements, i.e. LV stroke volume and cardiac output, were found to rise with gestation (data not shown). Although these values were significant when evaluated by analysis of variance and regression analyses, they exhibited wide intra/interobserver variability (data not shown). M-mode measurements showed significant increases in IVS thickness, but these measurements were just beyond the limit of resolution for M-mode imaging on the Acuson.

Overall, the data generated in our study using a clinical ultrasound system provides a comprehensive database for assessing mouse fetal cardiovascular function. We note that given the small size of the fetal mouse heart, fetal echocardiography studies in mice are technically challenging. Human fetal echocardiography in the first trimester is also reported to be technically challenging, with only 20% success reported for human fetal echocardiography conducted at 11 weeks of gestation (Haak et al. 2002). This is despite the fact the human fetal heart is considerably larger, being more than 50 mm at 11 weeks. The echocardiographic data generated in our study provides a developmental profile of fetal mouse cardiac function that can be used for evaluating the pathophysiology of cardiovascular defects in genetically altered and mutant mouse models. The mouse fetal crown-to-rump length measurements also can be used for the noninvasive developmental staging of mouse embryos/fetuses in utero and for assessing fetal growth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Abdulla R, Blew GA, Hotlerman MJ. Cardiovascular embryology. *Pediatr Cardiol.* 2004; 25:191–200.
- Abuhamad A. Color and pulsed Doppler in fetal echocardiography. *Ultrasound Obstet Gynecol.* 2004; 24:1–9. [PubMed: 15229909]
- Bronshtein M, Siegler E, Eshcoli Z, Zimmer EZ. Transvaginal ultrasound measurements of the fetal heart at 11 to 17 weeks of gestation. *Amer J of Perinatol.* 1992; 9:38–42. [PubMed: 1550631]
- Chang CH, Chang FM, Yu CH, Liang RI, Ko HC, Chen HY. Systemic assessment of fetal hemodynamics by Doppler ultrasound. *Ultrasound Med Biol.* 2000; 26:777–85. [PubMed: 10942825]
- Foster FS, Zhang MY, Zhou YQ, Liu G, Mehi J, Cherin E, Harasiewicz KA, Starkoski BG, Zan L, Knapik DA, Adamson SL. A new ultrasound instrument for in vivo microimaging of mice. *Ultrasound in Med & Biol.* 2002; 28:1165–72. [PubMed: 12401387]
- Gembruch U, Shi CY, Smrcek JM. Biometry of the fetal heart between 10 and 17 weeks of gestation. *Fetal Diagn Ther.* 2000; 15:20–31. [PubMed: 10705210]
- Gui YH, Linask KK, Khowsathit P, Huhta JC. Doppler echocardiography of normal and abnormal embryonic mouse heart. *Pediatr Res.* 1996; 40:633–42. [PubMed: 8888295]
- Haak MC, Twisk JW, Van Vugt JM. How successful is fetal echocardiographic examination in the first trimester of pregnancy? *Ultrasound Obstet Gynecol.* 2002; 20:9–13. [PubMed: 12100411]
- Hess, DB.; Hess, LW. *Fetal Echocardiography.* Appleton & Lange; Stamford, CT: 2004.
- Howe RS, Isaacson KJ, Albert JL, Coutifaris CB. Embryonic heart rate in Human pregnancy. *Ultrasound Med.* 1991; 10:367–71.
- Hsieh YY, Chang FC, Tsai HD, Tsai CH. Longitudinal survey of fetal ventricular ejection and shortening fraction throughout pregnancy. *Ultrasound Obstet Gynecol.* 2000; 16:46–8. [PubMed: 11084964]
- Huang C, Sheikh F, Hollander M, Cai C, Becker D, Chu PH, Evans S, Chen J. Embryonic atrial function is essential for mouse embryogenesis, cardiac morphogenesis, and angiogenesis. *Development.* 2003; 130:6111–19. [PubMed: 14573518]
- Ishiwata T, Nakazawa M, Pu WT, Tevosian SG, Izumo S. Developmental changes in ventricular diastolic function correlate with changes in ventricular myoarchitecture in normal mouse embryos. *Circ Res.* 2003; 93:857–65. [PubMed: 14551244]
- Kaufman, MH. *The Atlas of Mouse Development.* San Deigo, CA: Academic Press; 1992. p. 383-88.
- Leiva MC, Tolosa JE, Binotto CN, Weiner S, Huppert L, Denis AL, Huhta JC. Fetal cardiac development and hemodynamics in the first trimester. *Ultrasound Obstet Gynecol.* 1999; 14:169–74. [PubMed: 10550875]
- Phoon CK, Aristizabal O, Turnbull DH. 40 MHz Doppler characterization of umbilical and dorsal aortic blood flow in the early mouse embryo. *Ultrasound in Med & Biol.* 2000; 26:1275–83. [PubMed: 11120365]
- Phoon CK, Ji RP, Aristizabal O, Worrad DM, Zhou B, Baldwin HS, Turnbull DH. Embryonic heart failure in NFATc1^{-/-} mice: novel mechanistic insights from in utero ultrasound biomicroscopy. *Circ Res.* 2004; 95:92–99. [PubMed: 15166096]
- Rychik J, Ayres N, Cuneo B, Gotteiner N, Hornberger L, Spevak PJ, Van Der Veld M. American Society of Echocardiography guidelines and standards for performance of the fetal echocardiogram. *J Am Soc Echocardiogr.* 2004; 17:803–10. [PubMed: 15220910]

- Shen Y, Leatherbury L, Rosenthal J, Yu Q, Pappas MA, Wessels A, Lucas J, Siegfried B, Chatterjee B, Svenson K, Lo CW. Cardiovascular phenotyping of fetal mice by noninvasive ultrasound and recovery of ENU induced mutations causing congenital cardiac and extracardiac defects. *Physiol Genomics*. 2005; 24:23–36. [PubMed: 16174781]
- Spurney CF, Leatherbury L, Lo CW. High-frequency ultrasound database profiling growth, development, and cardiovascular function in C57BL/6J mouse fetuses. *J Am Soc Echocardiogr*. 2004; 17:893–900. [PubMed: 15282495]
- Srinivasan S, Baldwin HS, Aristizabal O, Kwee L, Labow M, Artman M, Turnbull DH. Noninvasive in utero imaging of mouse embryonic heart development with 40-MHz echocardiography. *Circulation*. 1998; 98:912–18. [PubMed: 9738647]
- Tan J, Silverman NH, Hoffman JIE, Villegas M, Schmidt KG. Cardiac dimensions determined by cross-sectional echocardiography in the normal human fetus from 18 weeks to term. *Am J Cardiol*. 1992; 70:1459–67. [PubMed: 1442619]
- Tsutsumi T, Ishii M, Eto G, Hota M, Kato H. Serial evaluation for myocardial performance in fetuses and neonates using a new Doppler index. *Pediatr Int*. 1999; 41:722–27. [PubMed: 10618901]
- van der Mooren K, Barendregt LG, Wladimiroff JW. Fetal atrioventricular and outflow tract flow velocity waveforms during normal second half of pregnancy. *Am J Obstet Gynecol*. 1991; 165:668–74. [PubMed: 1892195]
- van Splunder P, Stijnen T, Wladimiroff JW. Fetal atrioventricular flow-velocity waveforms and their relation to arterial and venous flow-velocity waveforms at 8 to 20 weeks of gestation. *Circulation*. 1996; 94:1372–78. [PubMed: 8822995]
- Wessels A, Sedmera D. Developmental anatomy of the heart: a tale of mice and man. *Physiol Genomics*. 2003; 15:165–76. [PubMed: 14612588]
- Yagel, S.; Silverman, NH.; Gembruch, U. *Fetal Cardiology. Embryology, Genetics, Physiology, Echocardiographic Evaluation, Diagnosis and Perinatal Management of Cardiac Diseases*. London and New York: Taylor and Francis; 2003.
- Yu Q, Shen Y, Chatterjee B, Siegfried BH, Leatherbury L, Rosenthal J, Lucas JF, Wessels A, Spurney CF, Wu YJ, Kirby ML, Svenson K, Lo CW. ENU induced mutations causing congenital cardiovascular anomalies. *Development*. 2004; 131:6211–23. [PubMed: 15548583]
- Zhou YQ, Foster FS, Qu DW, Zhang M, Harasiewicz KA, Adamson SL. Applications for multifrequency ultrasound biomicroscopy in mice from implantation to adulthood. *Physiol Genomics*. 2002; 10:113–26. [PubMed: 12181368]
- Zhou YQ, Foster FS, Parkes R, Adamson SL. Developmental changes in left and right ventricular diastolic filling patterns in mice. *Am J Physiol Heart Circ Physiol*. 2003; 285:H1563–75.
- Zimmer EZ, Chao CR, Santos R. Amniotic sac, fetal heart area, fetal curvature, and other morphometrics using first Trimester vaginal ultrasonography and color Doppler imaging. *Ultrasound Med*. 1994; 13:685–90.

Figure 1. Ultrasound imaging for profiling growth of mouse fetuses

(a,b). 2D ultrasound image in the frontal plane (a) was used to measure the crown-to-rump length (length between arrows) and fetus area (dotted outline). Magnified view shown in (b) was used to measure the heart area (dotted outline). Calibration bars = 1 mm

(c-g). Fetus area and heart area were highly correlated to the crown-to-rump length (c). The crown-to-rump length (d), heart area/fetus area (e), fetus area (f), and heart area (g) were plotted against gestational age using regression analysis with the 95% confidence delimited.

Figure 2. Ultrasound imaging planes used for cardiovascular assessments of fetal mice

Diagnostic ultrasound imaging planes used for cardiovascular assessments are demonstrated with 2D ultrasound images and accompanying diagrams (below). Shown are apical 3 (a), 4 (b), and 5 (c) chamber views, and left ventricular long axis (d), ventricular short axis (e) and great vessels short axis (f) views. Calibration bars = 1 mm, with panels (a,b,d) and panels (e,f) at same magnification.

LA, left atrium; LV, left ventricle; Ao, aorta; RA, right atrium; RV, right ventricle; PA, pulmonary artery; DA, ductus arteriosus ; DAo, descending Aorta.

Figure 3. Spectral Doppler, M-mode and 2D imaging for cardiac assessments

(a,b) An E12.5 fetus exhibited monophasic inflow wave forms (absence of E-wave) (a), while another E14.5 fetus exhibited both E-wave and A-wave (b).

(c,d). M-mode images showed position of ventricular wall and septum in diastole and systole through multiple cardiac cycles. Wall thickness, and chamber dimensions in diastole (c) and systole (d) were obtained by measuring the distances between the respective peaks vs. troughs in the magnified M-mode images.

(e-h). 2D images in LV long axis view in diastole (e) and systole (g). Ejection fraction and fractional area change were calculated from the LV area tracing in diastole (f) and systole (h). Calibration bars = 1 mm

Figure 4. Spectral Doppler profile of fetal heart rate, E/A ratio and inflow velocities
Regression curves with 95% confidence intervals are plotted for fetal heart rate, E/A ratio, E-wave and A-wave vs. gestation days.

Figure 5. Spectral Doppler profile of fetal myocardial performance index

Regression curves with 95% confidence intervals were plotted for myocardial performance index (MPI), ejection time (ET), isovolemic contraction time (IVCT), isovolemic relaxation time (IVRT) vs. gestation days.

Figure 6. M-mode assessment of fetal cardiovascular development

M-mode measurements of ventricular dimensions in diastole (RV, d; LV, d) and systole (RV, s; LV, s), are plotted with 95% confidence intervals delineated. A slow increase in these echocardiographic parameters can be seen from E14.5 to E19.5.

Table 1

Developmental profile of fetal growth *

Stage	Crown-to-Rump Length [†] (mm)	Fetus Area [†] (mm ²)	Heart Area [†] (mm ²)	Heart Area/Fetus Area [†]
E12.5	7.9±0.8 ^A (n=77)	23.2±5 ^A (n=77)		
E13.5	10.5±0.9 ^B (n=92)	40.9±7 ^B (n=92)		
E14.5	12.5±0.9 ^C (n=101)	57.6±8 ^C (n=101)	2.9±0.5 ^A (n=70)	0.050±0.004 ^A (n=70)
E15.5	14.1±0.5 ^D (n=134)	71.4±6 ^D (n=134)	3.8±0.4 ^B (n=87)	0.053±0.004 ^B (n=87)
E16.5	15.4±0.6 ^E (n=112)	82.7±6 ^E (n=112)	4.9±0.5 ^C (n=87)	0.058±0.007 ^C (n=87)
E17.5	16.6±0.4 ^F (n=211)	96.9±7 ^F (n=211)	6.1±0.6 ^D (n=146)	0.063±0.004 ^D (n=146)
E18.5	17.7±0.6 ^G (n=139)	112.1±8 ^G (n=139)	7.1±0.8 ^E (n=93)	0.063±0.005 ^D (n=93)
E19.5	18.7±0.7 ^H (n=57)	126.7±8 ^H (n=57)	7.7±0.7 ^F (n=36)	0.062±0.005 ^D (n=36)

E: embryonic day

* Data in this table are also shown in Fig. 1.

[†] P<0.0001 when measurements from E12.5 to E19.5 were compared using ANOVA.^{A-H} Mean values with same letter not significantly different by multiple comparisons.

Table 2

Analysis of intraobserver and interobserver variability

Imaging Modality	Echo Parameter	Sample Size	Intraobserver Variability (%)	Interobserver Variability (%)
2D imaging	Crown rump length	188	2.2±1.9	3.5±2.9
	Fetus area	188	4.2±3.4	7.4±6.2
	Heart area	143	9.7±8.3	12.3±11.8
	Heart area/fetus area ratio	143	7.6±4.7	10.9±8.8
Spectral Doppler	Heart rate	200	2.3±3.4	2.7±2.9
	Isovolemic ventricular contraction time	200	7.2±6.7	9.2±8.3
	Isovolemic ventricular relaxation time	200	6.4±5.1	8.4±7.7
	Ejection time	200	2.5±1.7	4.2±3.3
	Myocardial performance index	200	7.4±5.3	8.9±7.7
	E-wave	184	10.2±7.8	11.8±10.5
	A-wave	200	4.1±5.8	5.4±3.6
	E/A ratio	184	7.5±6.3	8.5±8.0
	Peak aortic velocity	143	5.3±4.5	5.4±6.3
	Peak pulmonary arterial velocity	62	5.6±4.7	6.2±5.5
M-mode	RV dimension in diastole/systole	71	4.8/6.9±4.5/5.6	6.6/8.3±5.8/7.3
	LV dimension in diastole/systole	71	3.2/6±2.7/4.3	6.5/8.2±5.7/6.4
	LV % shortening fraction	71	7.1±5.2	12.2±10.9
	LV % ejection fraction	71	3.7±2.8	9.7±8.5
	Mean velocity of circumferential shortening	71	7.5±5.5	11.6±9.1
2D imaging	LV % ejection fraction	94	4.5±3.2	10.8±7.9
	LV % fractional area change	94	6.4±4.5	12.0±10.6
	LV area in diastole	94	20.7±16.1	24.0±16.8
	LV area in systole	94	21.2±16.8	24.6±16.7

Table 3

Ultrasound Measurement of Fetal Cardiac Function*

	Heart rate [†] (bpm)	IVCT (msec) [†]	IVRT (msec) [†]	Ejection time (msec) [†]	MPI [†]	E-wave [†] (m/sec)	A-wave [†] (m/sec)	E/A ratio [†]	Peak Ao [†] velocity (m/sec)	Peak PA velocity (m/sec)
5/a	190±12 ^A (n=58)	35±14 ^A (n=58)		127±11 ^A (n=58)			0.37±0.06 ^{A,2} (n=51)		0.29±0.10 ^{2,3} (n=47)	
5/b	191±19 ^A (n=39)	29±9 ^B (n=39)	44±8 ^A (n=39)	126±12 ^A (n=39)	0.58±0.04 ^A (n=39)	0.06±0.02 ^A (n=39)	0.37±0.06 ^{A,2} (n=39)	0.19±0.05 ^A (n=39)	0.29±0.07 ^{2,3} (n=34)	
5	197±14 ^{A,B} (n=78)	24±8 ^C (n=78)	42±9 ^A (n=78)	126±11 ^A (n=78)	0.52±0.09 ^B (n=78)	0.09±0.03 ^B (n=78)	0.37±0.09 ^{A,2} (n=78)	0.24±0.06 ^B (n=78)	0.32±0.05 ^{2,3} (n=69)	
5	203±17 ^B (n=172)	22±6 ^C (n=172)	37±7 ^B (n=172)	125±11 ^A (n=172)	0.48±0.07 ^C (n=172)	0.11±0.03 ^C (n=108)	0.38±0.09 ^{B,2} (n=108)	0.30±0.08 ^C (n=108)	0.33±0.07 ^{A,2} (n=97)	0.34±0.06 ² (n=12)
5	209±17 ^{B,C} (n=170)	19±5 ^D (n=170)	36±8 ^B (n=170)	119±11 ^B (n=170)	0.46±0.08 ^C (n=170)	0.12±0.03 ^{C,D} (n=127)	0.34±0.07 ^B (n=127)	0.35±0.07 ^D (n=127)	0.33±0.06 ^A (n=92)	0.34±0.07 (n=35)
5	216±20 ^C (n=164)	18±5 ^E (n=164)	31±5 ^C (n=164)	116±12 ^B (n=164)	0.43±0.06 ^{C,D} (n=164)	0.13±0.03 ^{C,D} (n=114)	0.33±0.06 ^B (n=114)	0.39±0.06 ^E (n=114)	0.33±0.08 ^A (n=101)	0.35±0.06 (n=40)
5	227±21 ^D (n=262)	17±5 ^{C,F} (n=262)	28±6 ^{C,D} (n=262)	108±12 ^C (n=262)	0.42±0.07 ^D (n=262)	0.13±0.03 ^{D,E} (n=201)	0.33±0.06 ^B (n=201)	0.41±0.07 ^{E,F} (n=201)	0.34±0.07 ^A (n=148)	0.36±0.09± (n=78)
5	236±23 ^D (n=172)	16±4 ^F (n=172)	27±6 ^D (n=172)	105±12 ^C (n=172)	0.42±0.07 ^D (n=172)	0.14±0.04 ^E (n=129)	0.33±0.07 ^B (n=129)	0.42±0.06 ^{F,G} (n=129)	0.37±0.09 ^B (n=96)	0.37±0.07 (n=37)
5	245±20 ^E (n=66)	15±4 ^F (n=66)	25±5 ^D (n=66)	98±9 ^D (n=66)	0.41±0.06 ^D (n=66)	0.15±0.03 ^E (n=48)	0.34±0.05 ^B (n=48)	0.44±0.06 ^G (n=48)	0.38±0.08 ^B (n=49)	0.40±0.08 (n=20)

IVCT=isovolumetric contraction time; IVRT=isovolumetric relaxation time; MPI=myocardial performance index; Ao=aortic; PA=pulmonary artery

Values in this table are also shown in Fig. 4 and 5

ANOVA.

Mean values with same letters are not significantly different by multiple comparisons.

Values at E12.5 without detectable E-wave. In absence of E-waves, IVRT and MPI were not calculated.

Values at E12.5 with both E-wave and A-wave.

Using two sample t test for comparing A-wave and outflow velocity at the same stage.

E12.5 and E13.5, only one outflow velocity is indicated, representing composite measurement from both outflows.

Table 4

M-mode measurement of fetal ventricular wall and dimensions*

Stage	RV anterior wall (mm)		RV dimension [†] (mm)		IVS [†] (mm)		LV dimension [†] (mm)		LV posterior wall (mm)	
	d	s	d	s	d	s	d	s	d	s
E14.5 n=21	0.30±0.05	0.32±0.04	0.70±0.08 ^A	0.41±0.07 ^A	0.31±0.04 ^A	0.34±0.06 ^A	0.75±0.06 ^A	0.44±0.05 ^A	0.31±0.03	0.32±0.04
E15.5 n=39	0.32±0.04	0.33±0.05	0.78±0.10 ^A	0.45±0.08 ^A	0.33±0.05 ^A	0.38±0.06 ^{A,B}	0.83±0.08 ^B	0.45±0.06 ^A	0.32±0.04	0.34±0.06
E16.5 n=30	0.32±0.04	0.36±0.05	0.90±0.09 ^B	0.52±0.08 ^B	0.34±0.04 ^A	0.39±0.06 ^{A,B}	0.93±0.07 ^C	0.53±0.07 ^C	0.34±0.04	0.37±0.06
E17.5 n=72	0.33±0.06	0.35±0.09	0.93±0.1 ^B	0.53±0.10 ^B	0.36±0.07 ^{A,B}	0.41±0.09 ^B	0.96±0.14 ^{C,D}	0.55±0.11 ^B	0.33±0.06	0.36±0.08
E18.5 n=48	0.33±0.06	0.35±0.07	0.97±0.14 ^{B,C}	0.55±0.10 ^B	0.37±0.05 ^B	0.41±0.07 ^B	0.99±0.13 ^{C,D}	0.56±0.10 ^B	0.34±0.05	0.36±0.06
E19.5 n=20	0.33±0.03	0.36±0.07	1.01±0.12 ^C	0.59±0.14 ^B	0.38±0.07 ^B	0.41±0.06 ^B	1.02±0.11 ^D	0.60±0.12 ^B	0.34±0.06	0.36±0.06

E=embryonic day; RV=right ventricular; IVS=interventricular septum; LV=left ventricular; d=diastole; s=systole.

* Data in this table are shown in graphs in Fig. 6

[†] P<0.001 when comparing measurements from E14.5 to E19.5 using ANOVA.

A–D Mean values with the same letter are not significantly different by multiple comparisons.

Table 5

Quantitative assessment of LV function[†]

Stage	%FAC		%EF		%EFs		MVCFc($\times 10^{-4}$ circ/sec)		LV area, diastole ^{‡I} (mm ²)		LV area, systole ^{‡I} (mm ²)	
	2D		2D	M-mode	M-mode		M-mode		2D		2D	
E14.5	57 $\pm$ 5 (n=20)	73 $\pm$ 5 (n=20)	80 $\pm$ 6 (n=21)	80 $\pm$ 6 (n=21)	40 $\pm$ 6 (n=21)	8.9 $\pm$ 1.5 (n=21)	8.9 $\pm$ 1.5 (n=21)	0.96 $\pm$ 0.25 ^A (n=20)			0.40 $\pm$ 0.09 ^A (n=20)	
E15.5	56 $\pm$ 6 (n=43)	72 $\pm$ 7 (n=43)	82 $\pm$ 5 (n=39)	82 $\pm$ 5 (n=39)	44 $\pm$ 6 (n=39)	9.2 $\pm$ 1.5 (n=39)	9.2 $\pm$ 1.5 (n=39)	1.34 $\pm$ 0.31 ^B (n=43)			0.60 $\pm$ 0.18 ^B (n=43)	
E16.5	57 $\pm$ 6 (n=46)	74 $\pm$ 6 (n=46)	80 $\pm$ 5 (n=30)	80 $\pm$ 5 (n=30)	42 $\pm$ 5 (n=30)	8.9 $\pm$ 1.5 (n=30)	8.9 $\pm$ 1.5 (n=30)	1.55 $\pm$ 0.38 ^C (n=46)			0.64 $\pm$ 0.15 ^B (n=46)	
E17.5	57 $\pm$ 6 (n=82)	73 $\pm$ 6 (n=82)	81 $\pm$ 7 (n=72)	81 $\pm$ 7 (n=72)	44 $\pm$ 7 (n=72)	9.1 $\pm$ 1.5 (n=72)	9.1 $\pm$ 1.5 (n=72)	1.86 $\pm$ 0.50 ^D (n=82)			0.81 $\pm$ 0.25 ^C (n=82)	
E18.5	55 $\pm$ 6 (n=43)	71 $\pm$ 6 (n=43)	82 $\pm$ 6 (n=48)	82 $\pm$ 6 (n=48)	43 $\pm$ 6 (n=48)	9.1 $\pm$ 1.3 (n=48)	9.1 $\pm$ 1.3 (n=48)	2.08 $\pm$ 0.57 ^E (n=43)			0.91 $\pm$ 0.30 ^C (n=43)	
E19.5	57 $\pm$ 4 (n=20)	73 $\pm$ 4 (n=20)	80 $\pm$ 8 (n=20)	80 $\pm$ 8 (n=20)	42 $\pm$ 9 (n=20)	8.9 $\pm$ 2.0 (n=20)	8.9 $\pm$ 2.0 (n=20)	2.11 $\pm$ 0.30 ^E (n=20)			0.88 $\pm$ 0.15 ^C (n=20)	

E=embryonic day; FAC=fractional area change; EF=ejection fraction; FS=fractional shortening; MVCFc=heart rate corrected mean velocity of circumferential fiber shortening; LV=left ventricular; d=diastole; s=systole.

[†]Imaging modalities used in acquiring measurements are as indicated (2D/M-mode).

^{‡I} P<0.0001 when measurements from E14.5 to E19.5 are analyzed by ANOVA.

A–E Mean values with the same letter are not significantly different by multiple comparisons.