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● *Original Contribution*

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 two-dimensional (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 beats per minute 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. (E-mail: loc@nhlbi.nih.gov) Published by Elsevier Inc. on behalf of the World Federation for Ultrasound in Medicine & Biology.

Key Words: **Fetal ultrasound, Image comparison, Mouse fetus, Development, Cardiovascular system.**

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 to 40 MHz transducers have greatly improved two-dimensional (2D) spatial resolution that allows 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

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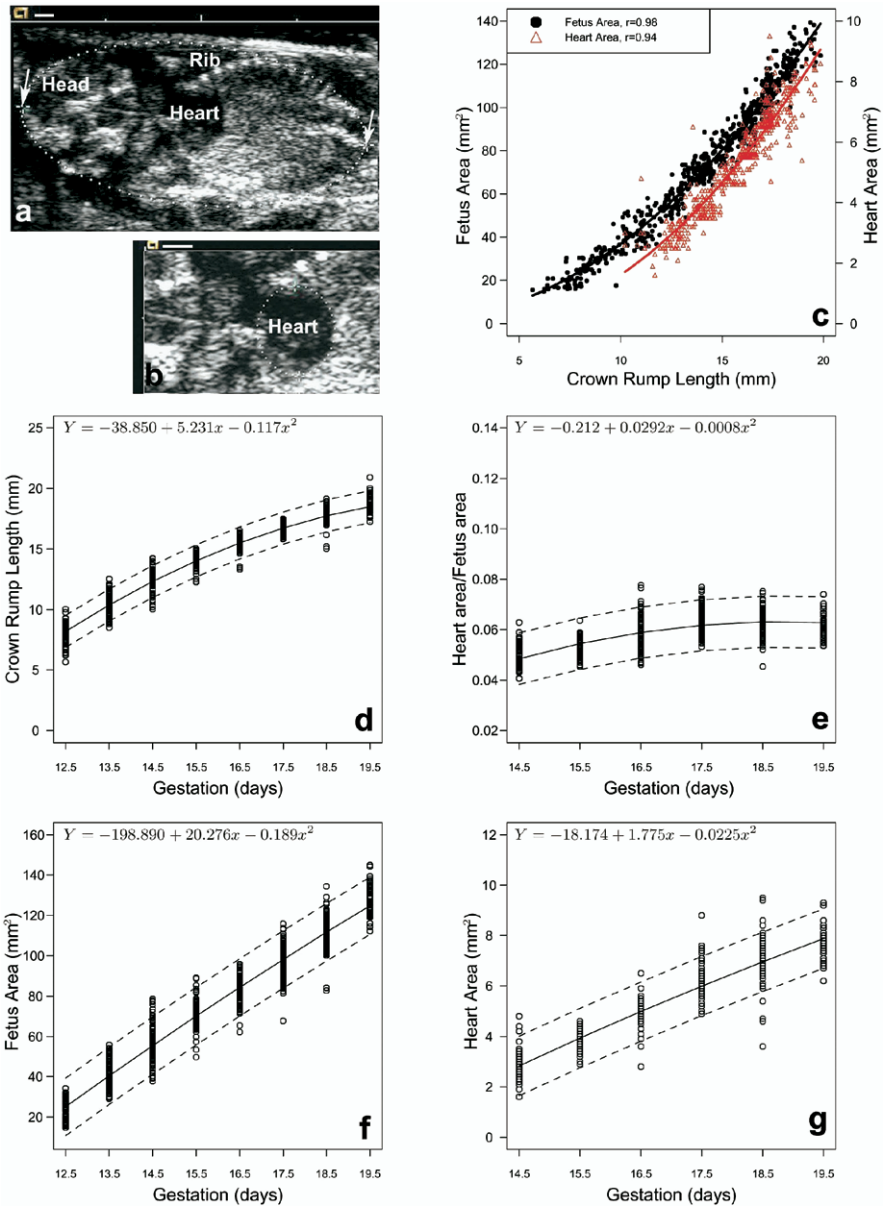

Fig. 1. Ultrasound imaging for profiling growth of mouse fetuses. (a) and (b) Two-dimensional ultrasound image in the frontal plane (a) was used to measure the crown-to-rump length (length between arrows) and fetus area (dotted outline). Magnified view shown in (b) was used to measure the heart area (dotted outline). Calibration bars = 1 mm. (c), (d), (e), (f) and (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.

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 sys-