Embryonic Heart Failure in NFATc1−/− Mice
Novel Mechanistic Insights From In Utero Ultrasound Biomicroscopy

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Abstract—Gene targeting in the mouse has become a standard approach, yielding important new insights into the genetic factors underlying cardiovascular development and disease. However, we still have very limited understanding of how mutations affect developing cardiovascular function, and few studies have been performed to measure altered physiological parameters in mouse mutant embryos. Indeed, although in utero lethality due to embryonic heart failure is one of the most common results of gene targeting experiments in the mouse, the underlying physiological mechanisms responsible for embryonic demise remain elusive. Using in utero ultrasound biomicroscopy (UBM), we studied embryonic day (E) 10.5 to 14.5 NFATc1−/− embryos and control littermates. NFATc1−/− mice, which lack outflow valves, die at mid-late gestation from presumed defects in forward blood flow with resultant heart failure. UBM showed increasing abnormal regurgitant flow in the aorta and extending into the embryonal–placental circulation, which was evident after E12.5 when outflow valves normally first develop. Reduced NFATc1−/− net volume flow and diastolic dysfunction contributed to heart failure, but contractile function remained unexpectedly normal. Among 107 NFATc1−/− embryos imaged, only 2 were observed to be in acute decline with progressive bradyarrhythmia, indicating that heart failure occurs rapidly in individual NFATc1−/− embryos. This study is among the first linking a specific physiological phenotype with a defined genotype, and demonstrates that NFATc1−/− embryonic heart failure is a complex phenomenon not simply attributable to contractile dysfunction. (Circ Res. 2004;95:92-99.)

Key Words: cardiac development ■ embryonic circulation ■ heart failure ■ NFAT ■ ultrasound biomicroscopy

Despite the proliferation of mouse models with aberrant cardiovascular development and subsequent in utero demise, the inability to characterize embryonic circulatory physiology has severely limited our mechanistic understanding of their targeted gene defects. In numerous such models, “heart failure” is the presumed cause of embryonic lethality (see Copp1 and Rossant2), but mechanisms remain largely unknown. Characterization of hemodynamic changes would provide a powerful link between a given genotype, its associated structural phenotype, and its physiological phenotype.

Null mutant mice lacking the nuclear factor of activated T cells c1 gene (NFATc1−/−) do not form aortic and pulmonary (outflow) valves and die in utero between embryonic days (E) 13.5 to 17.5.3,4 Notably, they demonstrate no morphological outflow tract abnormalities before E12.5, the approximate initial stage of outflow valve development.5,6 Moreover, there are no associated problems in the formation of ventricular myocardium (trabeculation or compaction), coronary vascularization, vascular endothelium, or extracellular matrix, nor is there evidence of ventricular or atrial chamber dilatation or unusual hypertrophy.3,4 The valvular problems in NFATc1−/− mice presumably lead to severe defects in forward blood flow with congestive heart failure.3,4 Therefore, it has been assumed that NFATc1−/− mice, similar to other models of cardiac disease and heart failure, die of progressive contractile failure as the heart fails to pump adequate blood to support the embryo.

We hypothesized that NFATc1−/− mouse embryos might exhibit multiple mechanisms of heart failure, not appreciated on postmortem histology. We sought to characterize blood flow in this model before and after the time of valve formation, in order to achieve a better mechanistic understanding of embryonic heart failure, by using detailed multiparameter analyses of in utero embryos with ultrasound biomicroscopy (UBM)-Doppler (see review7). Our results demonstrate that embryonic heart failure is a complex phenomenon, not simply due to contractile failure, and that different mechanisms may underlie even contractile failure itself.

Materials and Methods

Animals
All animals were maintained according to protocols approved by the Institutional Animal Care and Use Committee at New York Univer-
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University School of Medicine, Children’s Hospital of Philadelphia, and Vanderbilt University School of Medicine. The NFATc1 gene was disrupted in embryonic stem cells (ES) of balbc mice as previously described; the ES clone line (114) used in this study exhibits 100% impaired aortic and pulmonary valve development.4 The mice were rederived in the Transgenic Mouse/ES Cell Chimera Facility within the Skirball Institute of Biomolecular Medicine at New York University School of Medicine (New York). Embryos staged between E10.5 and 14.5 were obtained from heterozygous mutant intercrosses (note: E0.5 was defined as noon of the day vaginal plugs were detected after overnight mating).

Image Acquisition and Doppler Interrogation

The mouse preparation and imaging platform, and acquisition and processing of UBM-Doppler data have been described previously, modified for semiinvasive imaging to readily identify embryos for genotype-phenotype correlation.6–10 Our imaging technique allowed embryos to remain in their normal in utero environment at physiological temperatures and yields physiological data.10 In order to avoid observer bias, all measurements of Doppler parameters and UBM dimensions (see next section) were made before any knowledge of the genotype data. Data from any obviously injured embryos were excluded.

Imaging Protocols and Doppler Waveform and UBM Image Analysis

We first sought to characterize middorsal aortic blood flow from E10.5 to E13.5 and collected data from 383 embryos. As we developed facility with imaging litters, we also undertook, in a second protocol, to concurrently characterize dorsal aortic blood flow, cardiac ventricular size and function, and umbilical arterial/venous blood flow, in order to characterize changes in cardiac function as well as distribution of blood flow. For the second protocol, we obtained data from an additional 205 embryos from E12.5 to E14.5, because our data from the first protocol indicated that flows were essentially normal at E10.5 to E11.5. Thus, dorsal aortic data were available from both protocols (n = 588), spanning stages E10.5 to E14.5.

For each embryo, three consecutive Doppler waveforms were analyzed and the data averaged. Various flow parameters were measured in the mid-dorsal aorta and umbilical vessels, as previously described (see Phoon et al11). UBM images of the heart in a four-chamber view allowed assessment of ventricular size and function11 (see also Figure 3). Because the UBM maximal frame rate is 8/second and embryonic heart rate is 150 to 250/minute, consecutive cycles do not show the heart at maximal end-diastolic and minimal end-systolic volumes. Therefore, to improve assessment of ventricular function by UBM, we recorded diastolic and systolic ventricular dimensions for at least 10 consecutive cycles, and used the maximal and minimal values to obtain the end-diastolic area and end-systolic area, respectively, and to derive the fractional area shortening (= [end-diastolic area − end-systolic area]/end-diastolic area). Because blood is highly echogenic at the ultrasound frequencies used,12 endocardial borders cannot be delineated. Therefore, and because cardiac septation is not yet complete at the stages studied, epicardial biventricular areas were used as surrogates for ventricular volumes.

The limitations of small embryonic heart size (approximately 1 to 2 mm in any one dimension), low temporal resolution of UBM, and blood echogenicity precluded certain refined functional analyses often used in clinical cardiology, such as assessment of regional wall motion abnormalities.

Dorsal aortic diameter (middorsal aorta) and umbilical artery/vein diameters were measured from 3 separate frames, and cross-sectional areas calculated from the average diameter. Blood volume flow was calculated according to a recently developed model.9

Genotyping

Genotyping of the yolk sac or tail DNA of each embryo was performed using standard polymerase chain reaction techniques.4

Histology

Paraffin-embedded embryos were sectioned (6 µm thick) and stained with hematoxylin and eosin (H&E) as previously described.4

Statistical Analysis

All data are expressed as mean±SD. Comparisons between two groups were analyzed with the two-tailed Student’s t test, for parametric distributions with equal variances; or Mann Whitney U test, for nonparametric distributions with unequal variances. Because we did not rely on any single statistical difference in our data interpretation but rather looked for trends across stages, we did not use techniques such as Bonferroni’s correction for large numbers of comparisons, but instead set statistical significance at P<0.01.

Results

Wild-Type and Heterozygote Embryos Exhibit a Normal Physiological Phenotype

Ratios of living wild-type (NFATc1+/+), heterozygote (NFATc1+/−), and null mutant (NFATc1−/−) embryos at E10.5 to E14.5 were consistent with previous data indicating in utero lethality occurring largely between E12.5 through E14.5 (data not shown).3,4 We first compared UBM-Doppler data between NFATc1+/+ (n=168) and NFATc1−/− (n=313) embryos. Because NFATc1−/− embryos are viable and fertile, and demonstrate no histological abnormalities,3,4 wild-type and heterozygote mutant embryos should exhibit similar hemodynamics, which was confirmed (data not shown). Therefore, we increased the power of the study by pooling NFATc1+/+ and NFATc1−/− data (control group, n=481), and compared them with their NFATc1−/− littermates (n=107).

Distinctive Abnormal Blood Flow Is Observed in NFATc1−/− Embryos

To maintain efficient, unidirectional, forward blood flow to the body, the aortic and pulmonary valves prevent regurgitation of flow back into the heart during diastole. Therefore, their absence should result in distinctive, diastolic flow reversal (regurgitation) evident on the aortic blood flow Doppler waveform; this regurgitant flow would represent an abnormal volume load to the embryonic heart. Indeed, this hallmark finding (Figure 1A and 1B) was seen in >80% of E13.5 to E14.5 embryos but only 19% of E12.5 and 3% of E10.5 to 11.5 embryos. Because true valve formation normally occurs at approximately E13,6 the outflow tract endocardial cushions, which appose dynamically in a valve-like manner before true valve development, appear to function normally in NFATc1−/−, as expected from previous histological data.4 After E12.5, NFATc1−/− regurgitant flow worsened with advancing gestation (Figure 1C and 1D). Because the flow reversal waveform returns to baseline by the end of diastole (that is, there is no flow between the aorta and the ventricles), equalization of pressures has occurred by the end of diastole, evidence of “free” (unrestricted) regurgitation. Aortic blood flow patterns correlated with the histological phenotype (Figure 1E through 1G).

Cardiac Contractile Function Is Preserved in NFATc1−/− Embryos

Despite the abnormal flow in NFATc1−/− embryos, UBM-Doppler parameters of systolic function showed no consistent differences between control and NFATc1−/− mouse embryos,
Figure 1. NFATc1<sup>-/-</sup> embryos displayed a hallmark physiological phenotype, reversed diastolic aortic flow, that did not occur in the presence of aortic and pulmonary valves. A, Normal dorsal aortic Doppler flow waveform in a wild-type E13.5 embryo contrasted with B, representative E13.5 NFATc1<sup>-/-</sup> Doppler waveform showing characteristic regurgitant flow (asterisks). C, Abnormal flow reversal commenced at approximately E13 in NFATc1<sup>-/-</sup> embryos, the stage at which outflow valves should normally develop. Regurgitant flow volume, as indicated by the reverse velocity-time integral (VTI) as a percentage of forward VTI, worsened with advancing gestation, as expected. D, Increasing peak regurgitant flow velocities (REV) in the dorsal aorta also indicated worsening flow reversal with advancing gestation (*P<0.0001, NFATc1<sup>-/-</sup>, open circles, compared with control data, filled circles). Notably, peak forward arterial flow velocity (FWD) increased normally in NFATc1<sup>-/-</sup>, without any increase in variances. E, Representative H&E sections in the sagittal plane show normal aortic valve development (arrow) in a wild-type E13.5 embryo, whereas the littermate NFATc1<sup>-/-</sup> embryo with retrograde aortic flow shows absence of valve formation (F). NFATc1<sup>-/-</sup> embryos appear to develop normal myocardium and, histologically, fail to exhibit either ventricular or atrial chamber dilatation. The occasional NFATc1<sup>-/-</sup> embryo that exhibited unexpectedly normal aortic flow (without retrograde flow) appeared slightly less mature, with the outflow tract guarded still by endocardial cushions (arrow) (G); these embryos therefore behaved similarly to those at E12.5. a indicates atria; v, ventricles. Scale bar in E=500 μm. Data are presented as mean±SD.
including heart rate, UBM-derived fractional area shortening, and multiple Doppler parameters of contractility and systolic stroke work, even at E13.5 and E14.5 (Figures 1D and 2C through 2F).

In the mature human heart, the volume load imposed on the left ventricle by aortic regurgitation often results first in augmented systolic function, followed by progressive systolic deterioration. Therefore, it is possible that systolic dysfunction in NFATc1−/− embryos might be obscured by hyperdynamic systolic function during data averaging. Arguing against this was the absence of increased variance in any of our averaged data (Figures 1D and 2A through 2D).

Embryonic Blood Flow Is Reduced in NFATc1−/− Embryos, Despite Preserved Contractile Cardiac Function

Although forward systolic flow [as measured by velocity-time integral (VTI), stroke volume, or cardiac output] is an indicator of contractile function, the embryo depends on the total, or net, amount of blood flow distributed around its body for nutrition and growth. We therefore characterized the net blood flow by subtracting retrograde flow from forward flow parameters, because reversed blood flow is of no apparent physiological benefit to the embryo. Adjusted mean flow indices were significantly lower in NFATc1−/− embryos than in controls from E11.5 through E14.5 (Figure 2E and 2F).

Diastolic Dysfunction Is Rarely Observed in NFATc1−/− Embryos

Volume overload of the ventricles in the failing human heart often leads to elevated ventricular diastolic pressures, which are reflected in the atria and venous vascular bed. Because the resultant hydrostatic forces typically lead to edema, we would expect the NFATc1−/− embryo with regurgitant flow to exhibit pericardial effusions and generalized edema, such as seen in human fetuses with absent aortic and pulmonary valves. Pericardial effusions are frequently seen in NFATc1−/− mouse embryos after death (Figure 3), and such effusions have been the basis for the presumption of heart failure in this and many other mouse models. However, in vivo UBM revealed that NFATc1−/− ventricles were not abnormally dilated (Figure 2), consistent with prior histological data. Interestingly, we observed abnormal pericardial effusions in only 3 of 107 living NFATc1−/− embryos in...
which we obtained Doppler data (Figure 3); a fourth exhibited a moderate pericardial effusion, but with extremely poor contractions on UBM and no blood flow discernible by Doppler (data not shown). That we observed so few embryos with effusions suggests that they are late, not early, conditions. Thus, the presence of frequent pericardial effusions postmortem may be secondary to cellular deterioration and endothelial leakage after death; they do not truly reflect in vivo hemodynamics. Our results suggest a noncompliant myocardium (which does not dilate in the face of a volume load) but with normal relaxation properties (that allows normal or near-normal filling pressures). Taken together, our data argue that, although diastolic dysfunction contributes to embryonic demise, it is not as severe as postmortem histology has suggested.

**Embryonal–Placental Blood Flow Is Altered in NFATc1−/− Embryos**

We next investigated the hypothesis that alterations in dorsal aortic flow in NFATc1−/− embryos also alter umbilical flows and the distribution of blood flow between embryo and placenta. Alterations in embryonal–placental flow would potentially influence embryonic oxygenation status and might therefore contribute to embryonic lethality.

First, we observed NFATc1−/− reversed diastolic flow extending into the umbilical artery (Figure 4A). As in dorsal aorta, Doppler time intervals in the umbilical artery appeared normal in NFATc1−/− (data not shown). In control embryos, the proportion of blood in the dorsal aorta that flowed to the placenta decreased from ≈90% at E12.5 to ≈70% at E14.5. However, in NFATc1−/− embryos, the proportion of aortic flow to the placenta increased slightly from E12.5 through E14.5 (Figure 4B).

In the midgestation mouse embryo, umbilical venous blood flow exhibits continuous and undulating flow with a nonzero diastolic velocity, because of a relatively high placental impedance with low capacitance that allows partial transmission of the umbilical arterial pulse; the venous pulsatility (magnitude of undulation) decreases with advancing gestation as impedance falls.8 As expected at E12.5, umbilical venous pulsatility was normal, in the context of normal umbilical arterial flow without retrograde diastolic flow. However, in E13.5 to E14.5 NFATc1−/− embryos, umbilical venous pulsatility was higher than normal (Figure 4C), due to a reduced diastolic velocity that would be predicted by the diastolic flow reversal in the umbilical artery. Thus, NFATc1−/− embryos failed to exhibit the normal decrease in umbilical venous pulsatility with advancing gestation.

**Abnormal NFATc1−/− Hemodynamics Do Not Appear Secondary to an Abnormal Peripheral Vascular Bed**

It is possible that an abnormally developed vascular bed contributes to abnormal blood flow parameters and embryonic demise. However, the intraembryonic vascular system appeared normally developed, as demonstrated by normal dorsal aortic calibers in both control and NFATc1−/− embryos (data not shown) and by qualitative observations of all embryos, both in vivo with UBM and ex vivo after dissection. Similar analyses showed that the embryonal–placental vascular system, yolk sac formation, and placentation appeared normally developed (data not shown). Our observations are consistent with previous immunohistochemical analyses that vascular endothelium appears unaffected in NFATc1−/− embryos.3,4

**NFATc1−/− Embryos Die Acutely Without Chronic Progressive Deterioration**

Four NFATc1−/− embryos were excluded from the pooled analysis, because they displayed rapidly progressive deterioration or arrhythmia that prevented a valid analysis, which requires that embryos exhibit stable (unchanging) hemodynamics. Two E13.5 embryos displayed a rapidly progressive bradycardia and irregular rhythm that were immediately preceded by normal hemodynamics (Figure 5). The progressive bradyarrhythmia was not sudden in onset, but accompanied changes in the Doppler waveforms that indicated systolic failure; therefore, arrhythmia did not appear to be a primary event. These were the only two that exhibited arrhythmias. In two E14.5 embryos, UBM demonstrated very poor contractions, with minimal or no blood flow by Doppler. Neither exhibited obvious arrhythmias, and only one showed
a moderate pericardial effusion. That we observed so few NFATc1−/− embryos with poor hemodynamics also suggests that in most, death is not due to chronic, but more likely to acute heart failure.

No obvious problems were noted with the coronary arteries or the myocardium, as previously reported.4

Discussion

This study definitively demonstrates that the lack of aortic and pulmonary valves in the NFATc1−/− mouse embryo results in abnormal circulatory flow patterns, not primary myocardial dysfunction. Using UBM-Doppler, we have now specifically linked the physiological phenotype (regurgitant diastolic arterial flow) with the structural phenotype (lack of outflow valves) and genotype (NFATc1−/−) in the in utero embryo. Although a few previous ultrasound studies have correlated abnormal blood flow patterns with an abnormal genotype, the limitations of poor spatial resolution in those studies precluded a mechanistic understanding of embryonic heart failure.15–17 In a recent study, diastolic dysfunction was linked to abnormal ventricular myoarchitecture in explanted FOG-2 mutant mouse embryos.18 Our data, for the first time, implicate multiple possible mechanisms for embryonic heart failure, even in a single model. Embryonic heart failure thus appears to be a complex phenomenon not simply attributable to contractile dysfunction or "pump failure."

Cardiovascular Hemodynamics

In human children and adults, severe aortic or pulmonary regurgitation frequently leads to chronic and progressive ventricular dilation, with myocardial fibrosis and progressive systolic failure.12,19 However, our multiparameter UBM-Doppler analysis surprisingly failed to reveal any chronic, progressive deterioration in contractile function, despite the abnormal volume load imposed on the developing heart.

Reduced cardiac output and diastolic dysfunction appeared to contribute to embryonic heart failure. It is notable that very few living embryos displayed pericardial effusions, so that the frequent effusions seen on histology are perhaps due to cellular deterioration and leakage postmortem. Interestingly, our data suggested that indices of net volume blood flow supplied to the embryo demonstrated abnormalities as early as E11.5 (Figure 2E and 2F), even though (lack of) proper valve formation does not occur until E13.5. This timing coincides with the stage at which a tissue boundary, normally established between invading neural crest–derived mesenchyme and transformed endocardial cell–derived mesen-

Figure 4. Abnormal hemodynamics were also present in the embryonal–placental circulation. A, NFATc1−/− embryos exhibited diastolic flow reversal in the umbilical artery in a temporal pattern very similar to that seen in the dorsal aorta. Similar to dorsal aorta, time intervals in the umbilical artery showed no differences between NFATc1−/− and control embryos at any stage (data not shown), thus further corroborating aortic data indicating preserved systolic function in NFATc1−/− embryos. B, However, the percentage of forward dorsal aortic flow coursing to the placenta was distinctly different in NFATc1−/− embryos (*P<0.01, at E12.5 and E13.5). Indeed, NFATc1−/− embryos failed to exhibit the normal decrease in the percentage of aortic flow coursing to the placenta as gestation advanced. C, Umbilical venous pulsatility index (ratio of [peak systolic−trough dia-

[stolic] to [peak systolic] flow velocities) was normal at E12.5, but increased at E13.5 (†P<0.001) and E14.5 (‡P<0.005), and therefore lacked the normal progression toward a less pulsatile waveform as observed in control embryos. Because umbilical venous flow represents the net amount of flow from the umbilical artery that courses through the placental circulation, this composite index of umbilical venous flow velocities provided further evidence for abnormal placental blood flow in NFATc1−/− mouse embryos. At E13.5, both peak systolic and trough diastolic velocities were lower, whereas at E14.5, peak systolic velocity was higher than control with a lower trough diastolic velocity (data not shown). Filled circles are normal data; open circles are NFATc1−/− data, for all panels.
speculate that any combination of these mechanisms may even minor perturbations in cardiac output, and we speculate that UBM-Doppler parameters such as acceleration time (53 ms), ejection time (140 ms), peak velocity (96 mm/second), aortic VTI (9.26 mm), stroke volume (0.37 mm³), and fractional area shortening (0.44) were all within the normal range. B. During the rapid deterioration phase approximately 5 to 10 minutes later, arrhythmia was evident, as was a worsening of parameters of contractile function, such as peak velocity, ejection time, and ejection time as a proportion of the cycle length. C. Within a few minutes of the onset of arrhythmia, the heart has now severely failed. For all panels, the velocity and time scales are identical.

chyme in the region of the semilunar valves, fails to develop in NFATc1−/− embryos (B. Zhou and H.S. Baldwin, unpublished data, 2004). Given evidence for subtle changes in mesenchymal differentiation, we speculate that UBM-Doppler may be detecting subtle changes in cardiovascular function before gross histological changes.

Our data also support the possibility that alterations in placental blood flow, and therefore oxygenation, contribute to embryonic heart failure, although the exact physiological effects of the observed altered flow are unclear; we speculate that the abnormal umbilical venous flow patterns may alter cardiac filling as well. The embryo is exquisitely sensitive to even minor perturbations in cardiac output, and we speculate that any combination of these mechanisms may contribute to NFATc1−/− embryonic lethality. We did not find evidence of chronic arrhythmias as a cause of heart failure in this model.

The seemingly brief window of heart failure and demise limits the possibilities; these include acute electrical events (arrhythmias) that compromise cardiac output, and "catastrophic" coronary insufficiency causing acute myocardial failure. Because there were no indications of the former, we speculate that acute coronary insufficiency may account for at least some deaths. In the developing embryo, the difference between diastolic aortic and left ventricular pressures, which provides the necessary pressure gradient for coronary perfusion, is small, and as discussed, free outflow tract regurgitation would abolish the necessary coronary arterial perfusion pressure head, impairing coronary flow. It is possible that embryos exhibit regional wall motion abnormalities caused by myocardial ischemia that are not discernible by UBM before their acute demise. Why embryos should die at different time points despite similar hemodynamics is not known. Nevertheless, the timing suggested by our data is consistent with that observed in mouse models of aberrant epicardial and coronary arterial development, and may help to define, for the first time, the onset of coronary-dependent myocardial performance in utero. Although coronary vascular development begins with extracardiac investment of the epicardium derived from the proepicardial organ at E9.5, the myocardial requirement for coronary-derived circulation clearly occurs much later in development. These data suggest that such a requirement occurs between E13 and E14 in the mouse. However, further studies are needed to define the exact time point and molecular components of this critical transition during in utero cardiac development.

Insights Into Embryonic Myocardial Mechanics

The moderate degree of regurgitant flow and the lack of ventricular dilatation suggest a noncompliant embryonic myocardium. Other work in both mice and human fetuses has also suggested a relatively stiff myocardium early in development, which becomes more compliant as it matures. The NFATc1−/− embryonic heart appears to be remarkably robust, with death occurring more as an acute event (occurring over minutes), rather than secondary to chronic deterioration (occurring over hours to days). It is not at all clear how the embryonic heart is able to regulate blood flow in the presence of abnormal hemodynamics.

Relevance to Human Disease Conditions

Absence of either or both outflow tract valves is rare and is lethal. However, the condition is very likely more common than observed, because the observed incidence of congenital cardiac malformations is skewed by intrauterine death. It is not known at this time whether mutations in NFATc1−/− result in human cardiac disease. However, the NFATc1−/− model remains valuable because it presents the embryonic mammalian heart with abnormal volume loads that lead to heart failure, but does not appear to be complicated by coexistent multiple problems typical of other targeted genetic models.

Limitations

Extrapolation of these results to other mouse models of cardiac maldevelopment with heart failure must be made with caution. The NFATc1−/− mouse nevertheless has revealed the complexity of embryonic heart failure, a novel paradigm that now may be explored in other models as well. More detailed
data on the time course of death require serial, longitudinal evaluation of embryonic hemodynamics, techniques which are currently under development. Finally, anesthesia may affect embryonic cardiac function. However, the anesthetic choice does not likely influence our data interpretation, because we have an appropriate control group (wild-type and heterozygote littermates) and because UBM-Doppler data derived under similar anesthetic conditions appear more physiological than previous results obtained under invasive or less-well controlled conditions (see Ji et al10). Although UBM-Doppler has revealed some of the physiological mechanisms in play in this model of embryonic heart failure, clearly, the underlying mechanisms at the cellular level will require further elucidation.

Conclusions

UBM-Doppler is a powerful approach for characterizing cardiovascular physiology in the in utero mouse embryo, linking the physiological phenotype with genotype. Not only have we linked abnormal cardiovascular flow dynamics with the underlying genotype, but we have also moved toward a mechanistic understanding of embryonic heart failure. Our results indicate that, similar to human heart failure (see Auslender11), embryonic heart failure is a complex syndrome comprised of multiple mechanisms that require precise delineation, in different models, if optimal therapy is ultimately to be achieved.

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