In Situ Morphometric Analysis of Left and Right Ventricles in Fetal Rats: Changes in Ventricular Volume, Mass, Wall Thickness, and Valvular Size

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ITO, T., HARADA, K. and TAKADA, G.  \textit{In Situ Morphometric Analysis of Left and Right Ventricles in Fetal Rats: Changes in Ventricular Volume, Mass, Wall Thickness, and Valvular Size}. Tohoku J. Exp. Med., 2001, \textbf{193} (1), 37-44 —— We studied morphological changes in the left and right ventricles of fetal rats in late-gestation using rapid whole-body freezing technique. Pregnant Wistar rats (term, 21.5 day) were immediately frozen in liquid nitrogen on 17-, 18-, 19-, 20-, and 21-day of gestation. The frozen fetal hearts were serially sectioned with a sliding microtome and photographed. The ventricular volume, mass, wall thickness, and area of valvular orifice were measured on the photographs. During the study period, the left and right ventricular volumes increased very rapidly (9.9-fold and 7.6-fold, respectively) compared with the increase in the body weight (4.0-fold); the volumes divided by body weight increased linearly. Left and right ventricular masses also rapidly increased (5.9-fold and 5.0-fold, respectively). Mass/volume ratios for the two ventricles rapidly decreased. The wall thicknesses divided by body weights rapidly decreased with the progression of the gestational age. The left and right ventricles at 17 day of gestation have relative hypertrophy and relatively large valvular orifices as compared with those in terminal gestation. The improvement of the relative hypertrophy of the ventricles may indicate the morphological and functional maturation of the fetal heart.

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Fetal echocardiography has become an established method for evaluating cardiac morphology in human fetus (Allan et al. 1980; Kleinman et al. 1980; Lange et al. 1980; Silverman and Golbus 1985; Schmidt et al. 1988), and serial changes in fetal left and right ventricular dimensions and diameters of great vessels has been reported (Azancot et al. 1983; St. John Sutton et al. 1984; Shime et al. 1986; Tan et al. 1992; Hornberger et al. 1995). Such quantitative evaluation is useful for diagnosis of fetal cardiac diseases. An accurate in situ assessment of ventricular volume and mass, however, is still difficult even by fetal echocardiography. Using rapid whole-body freezing technique (Momma et al. 1987, 1992a, b, c), we studied serial changes in left and right ventricular morphology including volume, mass, wall thickness, and valvular size in fetal rats in late-gestation, intending to elucidate the process of morphological maturation of ventricles in fetal heart. Our findings obtained by such method can be applied to evaluating in detail the changes in cardiac morphology by fetal echocardiography in human fetus.

MATERIALS AND METHODS

Changes in morphological features of the left and right ventricles in fetal rats were studied using the rapid whole-body freezing technique. Pregnant Wistar rats (pregnant period, 21.5 days) were killed on 17-, 18-, 19-, 20-, and 21-day of gestation by cervical dislocation under general anesthesia by etherization and frozen immediately in liquid nitrogen. Frozen fetuses were taken out, the thoraxes were trimmed, and the hearts were serially sectioned in the frontal or short axis plane using an electro-freezer (Komatsu Solidate Co., Tokyo) and a sliding microtome (Yamato Kohki Solidate, Saitama). The thickness sectioned was 300 μm in the fetuses of 20- and 21-day of gestation and 100 μm in those of 17-, 18-, and 19-day of gestation. These cross-sections were phoned with a binocular stereoscopic microscope (Wild M 10 Photomacroscope, Wild Heerbrugg, Switzerland) using color film. A magnification of 12.5× or 20× was used for observation. Numbered section paper (1×1 mm) was also photographed and used for the scale.

In situ left and right ventricular volumes and masses of fetal rats were measured as follows. The cross-sectional areas of left ventricular cavities and muscles traced on the color photographs were measured using an offline computed digitizer (Cardio 500, Kontron Medical System, Euchung, Germany), and the areas multiplied by the thickness (100 or 300 μm) were summed up. According to Emery and McDonald (1960), the ventricular septum was divided into the left and right ventricles in the same proportion as the left and right ventricular free wall thickness. Left and right ventricular wall thicknesses were measured at the level of the two papillary muscles of the left ventricle. The mitral and tricuspid valvular orifices were traced in the short axis plane on the color photographs, and their areas were measured using the digitizer. The pulmonary and aortic valvular orifices were assumed to be circular shapes and their areas were calculated from the internal diameters of their valvular ring. One major problem in our study is the freezing-timing in a cardiac cycle. Cardiac arrest is known to occur following ventricular fibrillation or sinus bradycardia in deep hypothermia (Barratt-Boyes et al. 1971; Stephenson 1974), therefore, in our experiments, either ventricular fibrillation or sinus bradycardia was assumed to be a preceding condition. We judged the freezing-timing from the ventricular figures and the status of the four valves. In the fetuses of 18-, 19-, 20-, and 21-day of gestation, most of the hearts seemed to be frozen at end-diastole or near end-diastole, because the atrioventricular valves were largely open and the pulmonary and aortic valves were closed. However, only in the 17-day fetuses, the freezing-timing of hearts were variable, some
hearts being frozen at systole. Therefore, we selected only the hearts frozen at end-diastole or near end-diastole for measurement and Excepted ones frozen at other cardiac cycles.

Morphometric data are presented as mean ± standard error of mean (s.e.m). Comparisons between the 2 groups were submitted to Student t-test.

RESULTS

The body weights of fetal rats rapidly increased during the fetal life from 17th day to 21st day (4.0-fold). The shapes of the left and right ventricle were characteristic. The two ventricles had the same free wall thickness as did the ventricular septum, which was straight. During the study period, the volumes of the left and right ventricle increased rapidly (9.9-fold and 7.6-fold, respectively) (Table 1). Although the right ventricular volume was 1.3-fold larger than the left one in the 17-day fetuses, the two volumes became almost equal after 18-day of gestation (Table 1). Therefore, only the 17-day fetuses showed the dominance of the right ventricle. The two volumes divided by body weight also increased linearly (Fig. 1). The left ventricular volume per body weight increased more rapidly than that of the right ventricular volume (Fig. 1).

The masses of the left and right ventricle

<table>
<thead>
<tr>
<th>Gestational day (n)</th>
<th>17 (18)</th>
<th>18 (15)</th>
<th>19 (16)</th>
<th>20 (18)</th>
<th>21 (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>1.2 ± 0.1</td>
<td>1.7 ± 0.1*</td>
<td>2.3 ± 0.1*</td>
<td>3.5 ± 0.1*</td>
<td>5.1 ± 0.1*</td>
</tr>
<tr>
<td>LV volume (mm³)</td>
<td>0.68 ± 0.08</td>
<td>1.65 ± 0.22*</td>
<td>2.63 ± 0.27*</td>
<td>4.14 ± 0.28*</td>
<td>6.73 ± 0.62*</td>
</tr>
<tr>
<td>RV volume (mm³)</td>
<td>0.92 ± 0.11</td>
<td>1.75 ± 0.23*</td>
<td>2.57 ± 0.25*</td>
<td>4.07 ± 0.26*</td>
<td>7.01 ± 0.58*</td>
</tr>
<tr>
<td>LV mass (mm³)</td>
<td>1.62 ± 0.09</td>
<td>3.01 ± 0.20*</td>
<td>4.08 ± 0.18*</td>
<td>5.80 ± 0.21*</td>
<td>9.00 ± 0.51*</td>
</tr>
<tr>
<td>RV mass (mm³)</td>
<td>1.80 ± 0.10</td>
<td>2.76 ± 0.18*</td>
<td>4.00 ± 0.18*</td>
<td>5.70 ± 0.20*</td>
<td>9.07 ± 0.50*</td>
</tr>
<tr>
<td>LVWT (mm)</td>
<td>0.30 ± 0.01</td>
<td>0.35 ± 0.02*</td>
<td>0.43 ± 0.03*</td>
<td>0.45 ± 0.02*</td>
<td>0.49 ± 0.04*</td>
</tr>
<tr>
<td>RVWT (mm)</td>
<td>0.28 ± 0.01</td>
<td>0.35 ± 0.02*</td>
<td>0.39 ± 0.02*</td>
<td>0.44 ± 0.03*</td>
<td>0.45 ± 0.05*</td>
</tr>
</tbody>
</table>

LV, left ventricular; RV, right ventricular; WT, wall thickness.

*p < 0.05. vs. 17-day of gestation.

![Graph](image)

Fig. 1. Changes in left and right ventricular volumes per body weights in fetal rats. ———, left ventricle; ———, right ventricle. *p < 0.05. vs. day 17.
also increased rapidly during the study period (5.6-fold and 5.0-fold, respectively) (Table 1). Although the masses divided by body weight also increased, the rates of their increments were much slower than those of the ventricular volumes divided by body weight (Fig. 2). The left and right ventricles had similar mass throughout the study period (Table 1). The wall thicknesses of the two ventricles also increased as the gestational age progressed (Table 1), although the wall thicknesses divided by body weight rapidly decreased (Fig. 3). The differences between the two thicknesses were minimal during the study period (Table 1).
Fig. 4. Changes in the ratios of left and right ventricular masses to their volumes in fetal rats.

---, left ventricle; -----, right ventricle. *p<0.05. vs. day 17.

**Table 2. Areas of mitral, tricuspid, aortic, and pulmonary valvular orifices in fetal rats**

<table>
<thead>
<tr>
<th>Gestational day (n)</th>
<th>17 (18)</th>
<th>18 (15)</th>
<th>19 (16)</th>
<th>20 (18)</th>
<th>21 (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of VO (mm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral valve</td>
<td>0.14±0.01</td>
<td>0.26±0.02*</td>
<td>0.34±0.04*</td>
<td>0.38±0.03*</td>
<td>0.49±0.02*</td>
</tr>
<tr>
<td>Tricuspid valve</td>
<td>0.20±0.02</td>
<td>0.32±0.03</td>
<td>0.40±0.04*</td>
<td>0.43±0.03*</td>
<td>0.59±0.04*</td>
</tr>
<tr>
<td>Aortic valve</td>
<td>0.05±0.01</td>
<td>0.09±0.01*</td>
<td>0.12±0.01*</td>
<td>0.21±0.02*</td>
<td>0.26±0.02*</td>
</tr>
<tr>
<td>Pulmonary valve</td>
<td>0.11±0.01</td>
<td>0.13±0.01</td>
<td>0.18±0.01*</td>
<td>0.20±0.02*</td>
<td>0.27±0.02*</td>
</tr>
</tbody>
</table>

VO, valvular orifice. *p<0.05. vs. 17-day of gestation.

The ratios of the masses to volumes in the left and right ventricles at 17-day of gestation were significantly larger than those after 18-day of gestation (Fig. 4). Therefore, the two ventricles, particularly the left ventricle have relative hypertrophy at 17-day of gestation.

The areas of the tricuspid, mitral, pulmonary, and aortic valvular orifices showed linear and rapid increase during the study period (Table 2). The areas of the orifices of the tricuspid and pulmonary valves were significantly larger than those of the mitral and aortic valves, respectively, throughout the study period (Table 2). The ratios of the areas of the tricuspid and mitral valvular orifices to the right and left ventricular volumes significantly decreased as the gestational age progressed (Fig. 5), suggesting relatively large atrioventricular orifices for ventricular volumes at 17-day of gestation as compared with those in terminal gestation. The ratios of the areas of pulmonary and aortic valvular orifices to the volumes of their own ventricles also decreased, however, in much slower rate than those of mitral and tricuspid valvular orifices (Fig. 6).

**DISCUSSION**

Compared with human fetuses, fetal rats grow more rapidly and their body weights increase 4.0-fold during the fetal life from 17th day to 21st day (term). The morphogenesis of fundamental structure of the heart of rat is accomplished by 16-day of gestation, so that the
Fig. 5. Changes in the ratio of area of mitral valvular orifice to the left ventricular volume and the ratio of tricuspid valvular orifice to the right ventricular volume in fetal rats. 
VO, valvular orifice. ——, mitral valve; ———, tricuspid valve. *p < 0.05 vs. day 17.

Fig. 6. Changes in the ratio of area of aortic valvular orifice to the left ventricular volume and the ratio of pulmonary valvular orifice to the right ventricular volume in fetal rats. 
VO, valvular orifice. ——, aortic valve; ———, pulmonary valve. *p < 0.05 vs. day 17.

period from 17- to 21-day of gestation is important for the maturation of the heart, during which period the transition from the fetal to newborn circulation is prepared. This period in rats is thought to correspond to the period of late-gestation in human.

In human fetuses, cardiac output has been evaluated using Doppler echocardiography. It has been reported that the right ventricle ejects about 55% and the left ventricle ejects 45% of combined ventricular output (Kenny et al. 1986), indicating the right ventricular domi-
nance. In fetal lambs, Rudolph et al. (1985) described that the right ventricle ejects 60–65% of combined ventricular output. Therefore, the degree of right ventricular dominance in utero seems to be different among the species. In this study, the right ventricular volume was 1.3-fold larger than the left ventricular volume in fetuses of 17-day of gestation, indicating the right ventricular dominance in cardiac output, but there were no significant differences between the volumes of the two ventricles thereafter. We speculate that the degree of the right ventricular dominance gradually decreases with the progression of the gestational age. The pulmonary blood flow gradually increases with the progression of the gestational age, following the increase in left ventricular volume overload (Rudolph 1974; St. John Sutton et al. 1994). Our observation that the left ventricular volume divided by body weight increased may imply one of the adaptation mechanisms of fetal heart for the transition of circulation.

In this study, the volumes, masses, and wall thicknesses of the left and right ventricles increased rapidly and linearly during our study period. The rapid increases in these parameters are mainly related to the rapid increase in body weight and rapid growth in fetal rats. In addition, the volumes divided by body weights also increased linearly. We suppose that this fact indicates the morphological adaptation of both ventricles for the increase in cardiac output per body weight, supplying the increases in demands for blood flow volume in each organ with the progression of the gestational age.

The ratios of the masses to volumes in the left and right ventricles at 17-day of gestation were significantly larger than those after 18-day of gestation. From these results, we think that the left and right ventricles at 17-day of gestation have relative hypertrophy as compared with those in terminal gestation. The fetal myocardium is structurally and functionally immature as compared with that of the adult. The fetal heart has reduced myocardial contractility and low myocardial compliance, limiting its reserve for increase in output (Rudolph 1974). The mechanism of contraction is immature in fetal heart because of the reduction in the myofibrils in the ventricular myocytes (Nakanishi 1992). The exact cause of the relative hypertrophy of ventricles at 17-day of gestation and mechanism of the reducing of the hypertrophy thereafter can not be explained from only the results in this study. Some speculations such as the peculiarity of the species of rat, the structural immaturity of ventricular myocardium and the functional immaturity of ventricles may be associated with the relative hypertrophy.

It has been demonstrated by Doppler echocardiography in human fetuses that the left and right ventricular diastolic function is lower in early gestation than in late gestation (Kenny et al. 1986). We speculate that the relative hypertrophy of ventricles at 17-day of gestation may be associated with low myocardial compliance in fetal heart, although ventricular diastolic function can not be examine in this study.

Our study also demonstrated that the mitral and tricuspid valvular orifices were relatively large for the ventricular volumes at mid-gestational period, which findings may be associated with a result and compatible with the poor compliance of the left and right ventricles.

The serial changes in left and right ventricular morphology in fetal rats in this study might be closely related to the maturation of fetal heart or adaptation to change of output of ventricles. The findings obtained by in situ morphometric analysis in this study may be useful to elucidate the process of morphological maturation of normal heart in human fetuses and to diagnose fetal heart diseases using fetal echocardiography.

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References


