

Morphological Adaptation of the Cardiovascular System in Fetal Rats during Late Gestation

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TOYONO, M., ITO, T., HARADA, K., TAMURA, M. and TAKADA, G. *Morphological Adaptation of the Cardiovascular System in Fetal Rats during Late Gestation.* Tohoku J. Exp. Med., 1999, 188 (4), 299-309 — The aim of this study was to evaluate morphological changes of the cardiovascular system in fetal rats during late gestation. We used the rapid whole-body freezing technique for rats of day 17 through 21 of gestation. The right and left ventricular volumes increased markedly and significantly during this period by about 11- and 24-fold, respectively. Although the right ventricular volume was 108% larger with statistical significance than the left ventricular volume on day 17, they were almost equal after day 19. The length of the primum septum of the atrium significantly increased by 92% within 4 days, but the opening distance of foramen ovale significantly decreased by 14%. The ratio of the inner diameter (the sum of right and left pulmonary arteries to ductus arteriosus) significantly increased from 0.72 ± 0.03 on day 17 to 1.17 ± 0.07 on day 21. There was also a significant increase in the ratio of the inner diameters of the ascending to descending aorta. These observations suggest that the reduction of the opening distance of foramen ovale reflect the growth of pulmonary arteries. ————— whole-body rapid freezing technique; fetal rats; cardiovascular system; morphological changes. © 1999 Tohoku University Medical Press

Recent advances in cross-sectional and Doppler echocardiography have facilitated the observation of gestational age-related changes in both the cardiovascular system and hemodynamic state in the human fetus (Sahn et al. 1980; Allan et al. 1982; Sutton et al. 1984, 1994; Tan et al. 1992; Schmidt et al. 1995). However, anatomical details of foramen ovale and peripheral pulmonary arteries remain obscure because of difficulties encountered in human studies. Pulmonary blood flow increases in late gestation (Rudolph 1974), which could be related to morphological changes in the peripheral pulmonary arteries or foramen ovale or both. The rapid whole body freezing technique developed by Momma et

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al. (1987, 1992a, b, c) provides an excellent method for in situ studies of morphological changes in cardiac chambers and great vessels during the transition from fetal to neonatal circulation in the rat. However, little is known about the development of the cardiovascular system during gestation in the rat. The purpose of this study was to demonstrate gestational age-related changes in the cardiac chambers, great vessels, primum septum of the atrium, and foramen ovale of the rat.

MATERIALS AND METHODS

Freezing, cutting, and photographing

Morphological changes of cardiac chambers and great vessels in fetal rats were studied using the rapid whole-body freezing technique established by Momma et al. (1987, 1992a, b, c). Wistar rats (21.5 days pregnant) were used. In this study, day 0 of gestation was defined by the presence of sperm in the vaginal smear. Pregnant rats were killed on day 17, 18, 19, 20 and 21 of gestation by cervical dislocation and frozen immediately in liquid nitrogen. Frozen fetuses were removed, and their thoraxes were trimmed and sectioned in the frontal plane of the heart using an electro-freezer (Komatsu Solidate Co., Tokyo) and a sliding microtome (Yamamoto Solidate Co., Ohmiya), with which the minimum possible slice thickness was $5\ \mu\text{m}$. Cross sections were photographed with a binocular stereoscopic microscope (Wild M 10 Photo-microscope, Wild Heerbrugg, Switzerland) using color films. Ventricular cross sections were photographed serially every $300\ \mu\text{m}$ in fetuses of day 20 and 21 of gestation, and every $100\ \mu\text{m}$ in those of days 17, 18 and 19 of gestation. Cross sections in view of vessels and foramen ovale were photographed serially every $50\ \mu\text{m}$. A magnification of $10\times$ or $12.5\times$ was used for observation of ventricle and that of $12.5\times$ or $20\times$ was used for observation of vessels and foramen ovale. Section paper ($1\times 1\ \text{mm}$) was photographed and used as a scale (Fig. 1).

Measurements

Morphological changes in the ventricular cardiac chambers and great vessels in situ were studied on the color prints. The diameters of the ascending and descending thoracic aortae were measured at the middle part of longitudinal direction. The diameter of the right superior (actually the anterior in rat) vena cava was measured at the middle between the confluence of the azygos vein and the junction to the right atrium. As this study aimed to supplement information obtained in human, the left superior vena cava, which is always present in rat, was not observed. The diameter of the inferior vena cava was measured at the supradiaphragmatic portion. The diameter of the main pulmonary artery was measured at the middle between the pulmonary valve and the bifurcation, and that of the ductus arteriosus was measured at the middle between the bifurcation and junction with the descending aorta. The diameters of the right and left

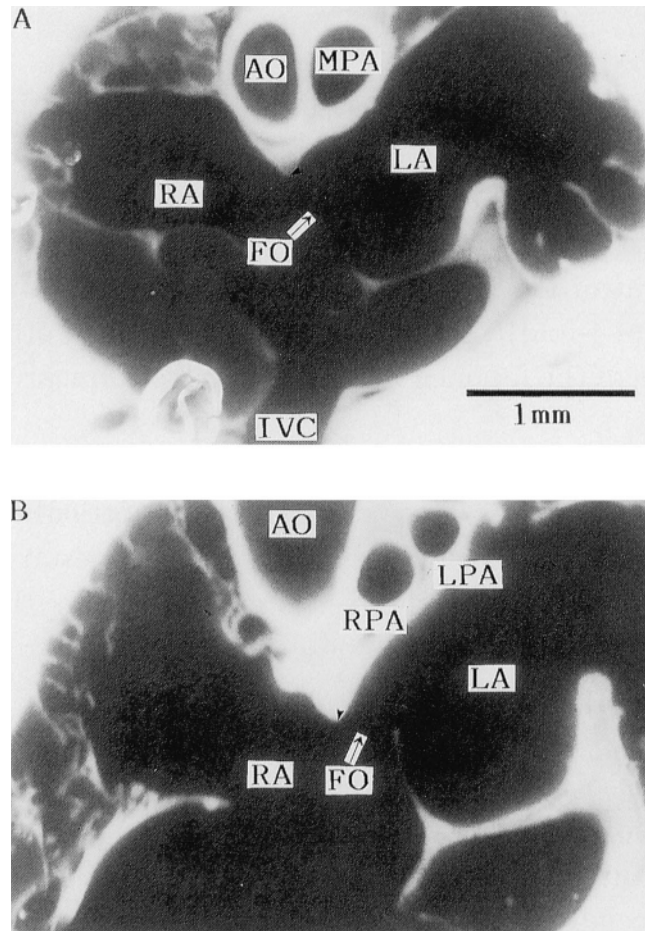


Fig. 1. Photographs of cross sections of the heart through the short axis at the level of the foramen ovale. At day 17 (A) and 21 (B) of gestation. The opening distance of foramen ovale became relatively smaller. RA, right atrium; LA, left atrium; FO, foramen ovale; MPA, main pulmonary artery; AO, ascending aorta; IVC, inferior vena cava. LPA, left pulmonary artery; RPA, right pulmonary artery.

pulmonary arteries were measured at a point on the pulmonary hilus, proximal to the first branching. The diameter of the right upper pulmonary vein was measured at a point just distal to the junction to the left atrium. Ventricular volumes of fetal rats were measured in situ as follows. The cross sectional areas of ventricular cavities traced on the color photographs were measured using an off-line computer digitizer (Cardio 500, Kontron Medical System, Euechung, Germany) and the areas were multiplied by the thickness of sections (100 or 300 μm). The length of the septum primum of the atrium was measured from the cranial rim of the septum primum of the atrium to its caudal end. The opening distance of the foramen ovale was measured in the craniocaudal direction from the cranial rim of the septum primum of the atrium to the caudal end of the septum secundum of the atrium (Fig. 1). All fetuses were fixed at diastole because the semilunar valve was open and the atrioventricular valve was closed.

Statistical analysis

Morphometric data are presented as mean \pm standard error of mean (s.e.m.). The two groups were compared by Student's *t*-test.

RESULT

The body weight of the fetal rats rapidly and linearly increased during the observation period (4.4-fold) (Table 1). Likewise, all the other data except for the opening distance of the foramen ovale also increased linearly and significantly.

Ventricular volumes

There were rapid increases in the right and left ventricular volumes by about 11- and 24-fold, respectively, from day 17 to day 21 of gestation. Although the right ventricular volumes were significantly larger than the left ventricular volumes on days 17 and 18, both volumes were equivalent from day 19 (Fig. 2).

Ascending and descending aorta

The diameters of both the ascending and descending aorta increased linearly during the observation period. However, this increase was greater in the ascending aorta (2.0-fold) than the descending aorta (1.8-fold), and therefore the ratio of diameter of the ascending aorta to that of the descending aorta significantly increased from 0.73 on day 17 to 0.83 on day 21 ($p < 0.05$, Fig. 3).

Right superior and inferior vena cava

There was also a linear increase in the diameters of both the right superior vena cava and the inferior vena cava during gestation. This increase was greater in the right superior vena cava (1.6-fold) than the inferior vena cava (1.4-fold).

Pulmonary vessels and ductus arteriosus

The diameters of both the main pulmonary artery and the ductus arteriosus increased by 81% and 72%, respectively, during the 4-day study period. The diameters of the right and left pulmonary arteries and the right upper pulmonary vein rapidly increased. The proximal and distal portions of the right pulmonary artery increased by 138% and 158%, respectively. Those of the left pulmonary artery increased by 136% and 145%, respectively. The right upper pulmonary vein increased by 78% within the 4 days. The ratio of the sum of diameters of the proximal right and left pulmonary arteries to diameter of the ductus arteriosus increased by 49% (Fig. 4), and the same ratio of the distal pulmonary arteries increased by 63%.

Primum septum of the atrium and foramen ovale

Between gestational day 17 to day 21, the primum septum of the atrium grew

TABLE 1. *Morphologic adaptation of cardiovascular system in the late gestation in fetal rat*

Measurement	day 17	day 18	day 19	day 20	day 21	S.S.
Body weight (g)	1.15 ± 0.04 (13)	—	2.70 ± 0.06 (16)	—	5.04 ± 0.08 (14)	*
Right ventricular volume (ml)	0.57 ± 0.09 (13)	2.62 ± 0.41 (8)	2.90 ± 0.22 (21)	4.07 ± 0.33 (18)	6.36 ± 0.83 (14)	*
Left ventricular volume (ml)	0.28 ± 0.06 (13)	1.65 ± 0.22 (8)	2.99 ± 0.27 (21)	4.14 ± 0.28 (18)	6.73 ± 0.62 (14)	*
(diameter, × 10 μm)						
Inferior vena cava	68 ± 3 (9)	72 ± 2 (10)	78 ± 2 (20)	83 ± 3 (14)	97 ± 3 (14)	*
Right superior vena cava	54 ± 2 (8)	65 ± 2 (8)	73 ± 2 (20)	76 ± 3 (16)	84 ± 3 (15)	*
Main pulmonary artery	31 ± 1 (12)	39 ± 1 (11)	49 ± 1 (24)	53 ± 1 (16)	56 ± 2 (14)	*
Right pulmonary artery, proximal	13 ± 1 (13)	20 ± 1 (11)	25 ± 1 (23)	27 ± 1 (17)	31 ± 1 (14)	*
distal	12 ± 1 (12)	17 ± 1 (11)	25 ± 1 (20)	25 ± 1 (13)	31 ± 1 (14)	*
Left pulmonary artery, proximal	11 ± 1 (13)	16 ± 1 (11)	21 ± 1 (22)	21 ± 1 (17)	26 ± 1 (14)	*
distal	11 ± 1 (12)	16 ± 1 (11)	21 ± 0.4 (21)	21 ± 1 (15)	27 ± 1 (14)	*
Right upper pulmonary vein	23 ± 1 (10)	26 ± 1 (11)	32 ± 1 (20)	31 ± 2 (4)	41 ± 2 (13)	*
Ductus arteriosus	32 ± 1 (13)	40 ± 1 (11)	49 ± 1 (22)	51 ± 2 (15)	55 ± 3 (14)	*
Ascending aorta	30 ± 1 (12)	37 ± 1 (11)	47 ± 1 (24)	55 ± 1 (18)	61 ± 2 (15)	*
Descending aorta	41 ± 1 (10)	50 ± 1 (10)	59 ± 1 (21)	71 ± 2 (14)	77 ± 2 (15)	*

Mean ± s.e.m. (); number of rats.

S.S., statistical significance. * $p < 0.05$. day 17 vs. day 21.

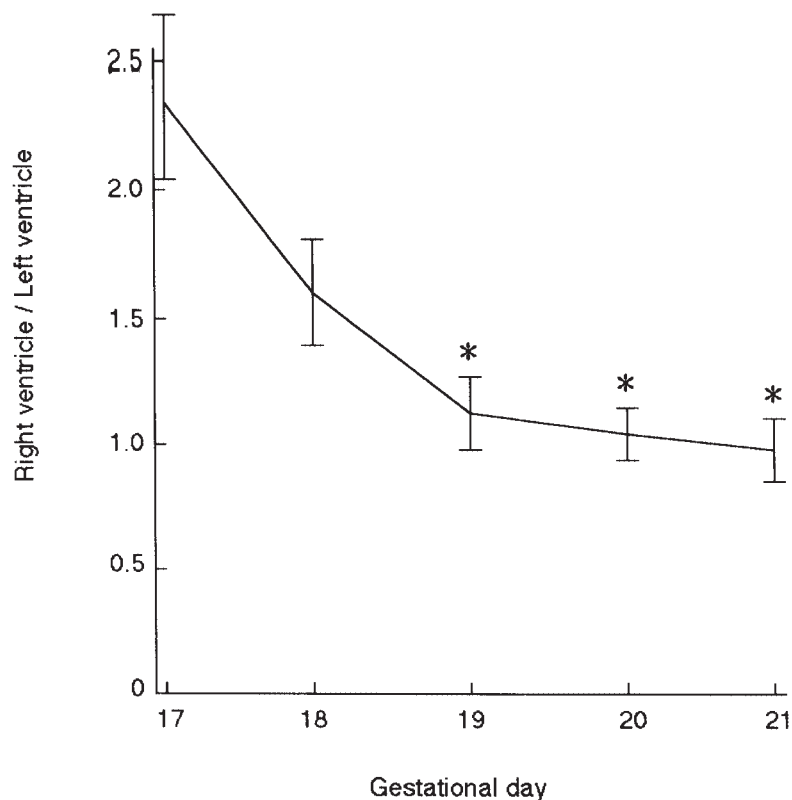


Fig. 2. Right to left ventricular volume ratios from day 17 to 21 of gestation in fetal rats. Mean \pm S.E.M. (Number of rats in parentheses): day 17 (13), day 18 (8), day 19 (21), day 20 (18), day 21 (14). * $p < 0.05$. vs. day 17.

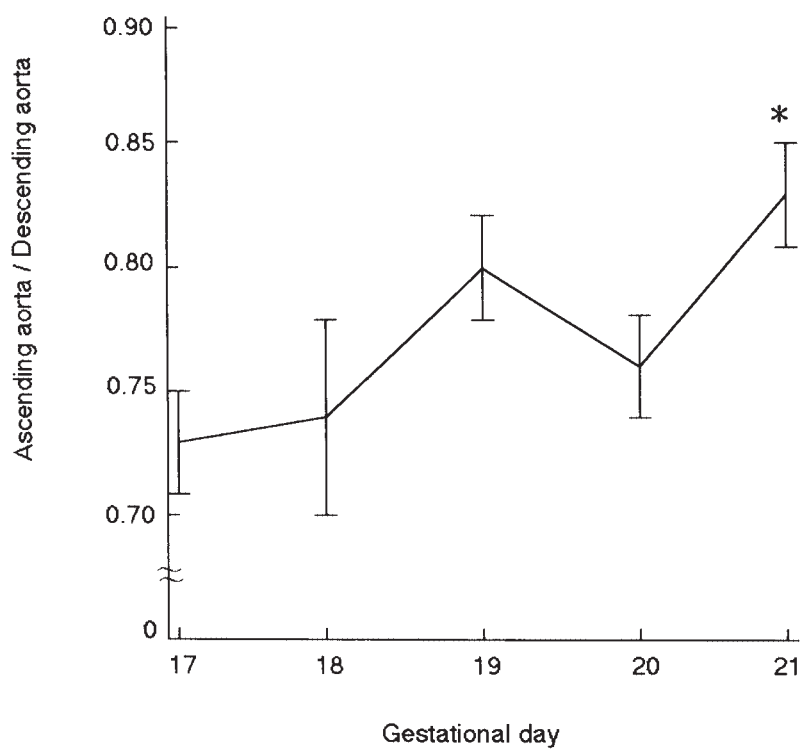


Fig. 3. Ratios of the inner diameter of ascending to descending aorta from day 17 to 21 of gestation in fetal rats. Mean \pm S.E.M. (Number of rats in parentheses): day 17 (9), day 18 (10), day 19 (21), day 20 (14), day 21 (10). * $p < 0.05$. vs. day 17.

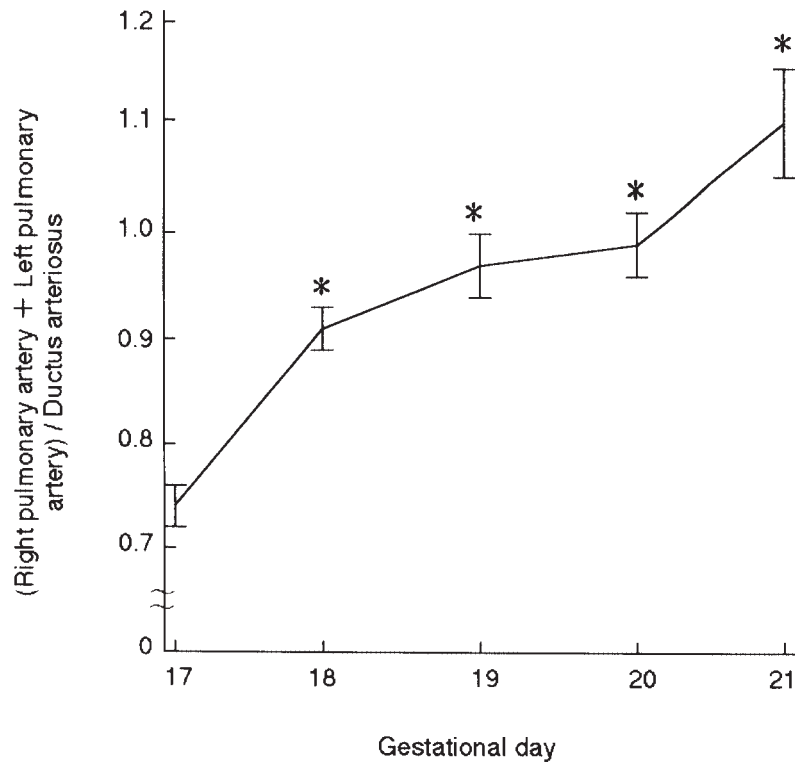


Fig. 4. Ratios of the sum of the inner diameter of the right and left pulmonary artery (proximal portion) to the inner diameter of the ductus arteriosus from day 17 to 21 of gestation in fetal rats. Mean \pm S.E.M. (Number of rats in parentheses): day 17 (13), day 18 (11), day 19 (22), day 20 (15), day 21 (13). * $p < 0.05$. vs. day 17.

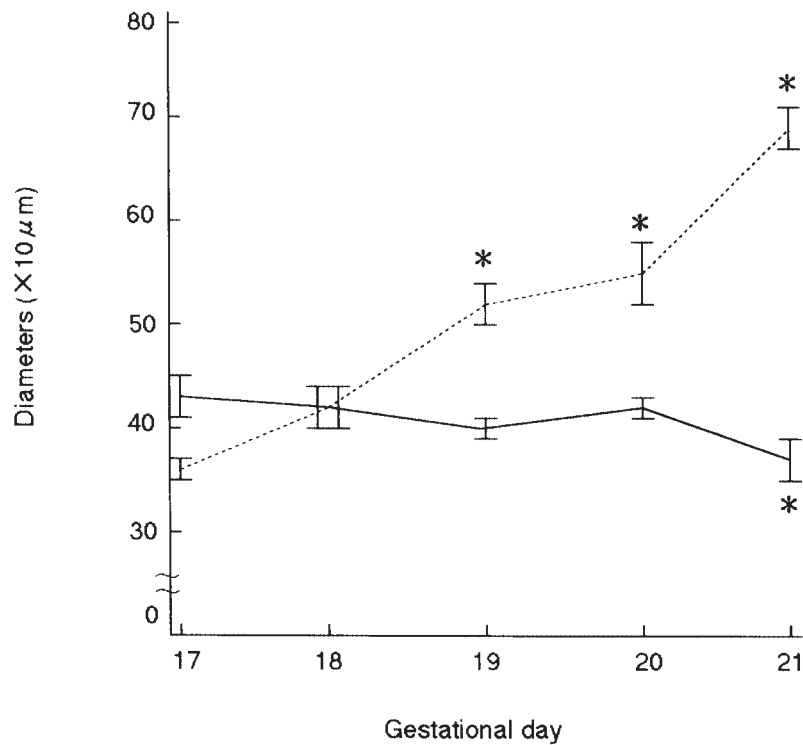


Fig. 5. Diameters of the foramen ovale and length of the primum septum of the atrium from day 17 to 21 of gestation in fetal rats. Mean \pm S.E.M. (Number of rats in parentheses): Primum septum of atrium (interrupted line); day 17 (12), day 18 (11), day 19 (21), day 20 (14), day 21 (14), Foramen ovale (solid line); day 17 (12), day 18 (11), day 19 (21), day 20 (17), day 21 (13). * $p < 0.05$. vs. day 17.

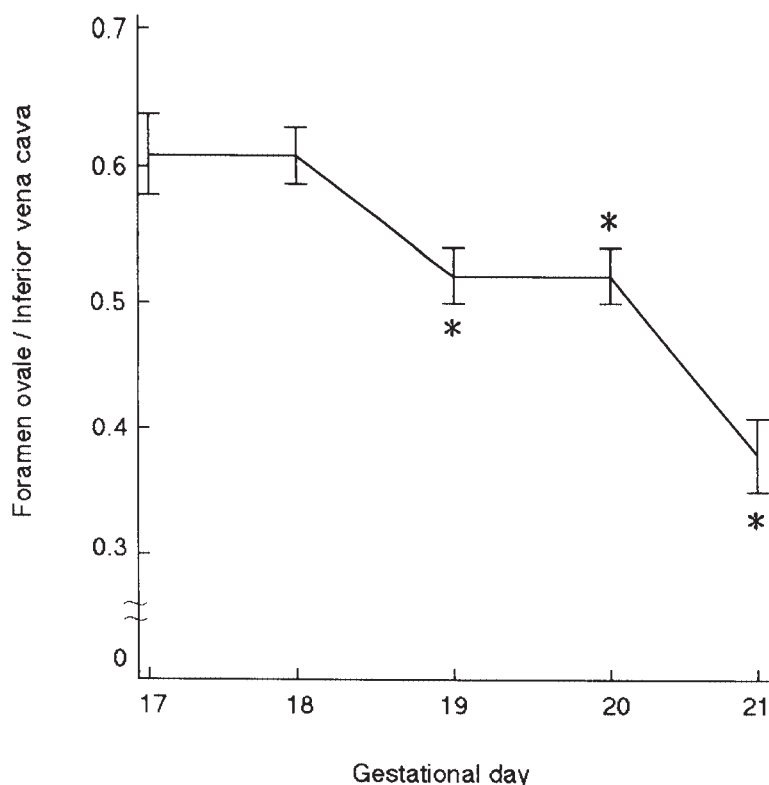


Fig. 6. Ratios of the diameter of the foramen ovale to the inner diameter of inferior vena cava. Mean \pm S.E.M. (Number of rats in parentheses): day 17 (8), day 18 (10), day 19 (20), day 20 (14), day 21 (13). * $p < 0.05$.

rapidly, and became 92% longer. However, there were no significant changes in the opening distance of the foramen ovale during this 4-day period and it became significantly smaller on day 21 of gestation. The ratio of the opening distance of the foramen ovale to the length of the primum septum of the atrium decreased by 50% in the same period ($p < 0.05$, Fig. 5). The ratio of the opening distance of the foramen ovale to the diameter of inferior vena cava also decreased by 14% ($p < 0.05$, Fig. 6).

DISCUSSION

This study demonstrated the age-related maturational changes in the cardiovascular system of the fetal rat. Although the hemodynamic changes during gestation are well known, little is known about the morphological changes in ventricular size, pulmonary artery and foramen ovale in the fetal rat. In this study, the left ventricular volume on day 17 of gestation was smaller than the right ventricular volume. However, the left ventricular volume progressively increased to approach that of the right ventricle by day 21. The sum of the diameters of the left and right pulmonary arteries to the ductus arteriosus, increased with gestational age. In contrast, the size of the foramen ovale did not change with gestational age. In our study, body weights of the fetal rats increased approximately 4-fold from day 17 to 21 of gestation. This period corresponds to the third trimester of gestation in human (Lubchenco et al. 1966).

Although we could not assess the hemodynamic changes, our results suggest that the morphological maturation of fetal pulmonary arteries plays an important role in left ventricular development.

In human, the right ventricle is the larger ventricle at early gestation, and the size of the left ventricle gradually approaches that of the right ventricle in later gestation (Sahn et al. 1980; Schmidt et al. 1995), which is consistent with our results in fetal rats. Increased left ventricular volume and therefore increased left ventricular output may support the rapid growth of the head in the human fetus. Major determinants of increased left ventricular volume during gestation are pulmonary blood flow volume and blood flow volume through the foramen ovale (Rudolph 1974). Several studies have demonstrated the gestational age-related changes in cardiac output, pulmonary blood flow volume through the foramen ovale and left and right ventricular volume in normal human fetuses (Patten et al. 1929; Licata 1954; Rudolph 1974; Momma et al. 1992a, b, c; Sahn et al. 1980; Sutton et al. 1994; Schmidt et al. 1995). Sutton et al. (1984) estimated indirectly that the human fetal pulmonary blood volume represented 20% of the combined cardiac output during the second half of pregnancy and suggested that this proportion did not change with gestational age. However, other investigators have shown directly that the proportion of pulmonary blood flow volume of the combined cardiac output increased from 20 to 30 weeks of gestation (from 13 to 25%) (Rudolph 1974; Momma et al. 1987). Although there are few reports on the age-related changes in left and right pulmonary arterial size in the human fetus (Tan et al. 1992), our results demonstrated that the proportion of the left and right pulmonary arteries diameters to the ductus arteriosus diameter increased with gestational age. The findings obtained in our study may indicate that the blood flow through the main pulmonary artery shifted from the ductus arteriosus to peripheral pulmonary arteries in late gestation, supporting the findings of Rasanen et al. (1996). The increase in the proportion of pulmonary blood flow in human fetus and the increase in size of proportion of pulmonary artery to ductus arteriosus in fetal rats suggest that growth of the lungs and increase of the vasculature in lung tissue are associated with a decrease in the pulmonary vascular resistance and increased pulmonary blood flow during gestation (Rudolph 1974).

In this study, the size of foramen ovale did not change with gestational age and therefore the proportion of the foramen ovale to inferior vena cava decreased with gestational age. This result is similar to the findings that the ratios of the opening distance of the foramen ovale to the inferior vena cava decreased from 1.0 at 9 weeks of gestation to 0.39 at term (Patten et al. 1929; Licata 1954). Using Doppler echocardiography, the proportion of the blood flow volume through the foramen ovale of the combined cardiac output was observed to decrease from 34% at 20 weeks to 18% at 30 weeks of gestation (Sutton et al. 1994). These findings suggest that during late gestation, the foramen ovale may become restrictive, failing to increase in proportion to the combined cardiac output, supporting the

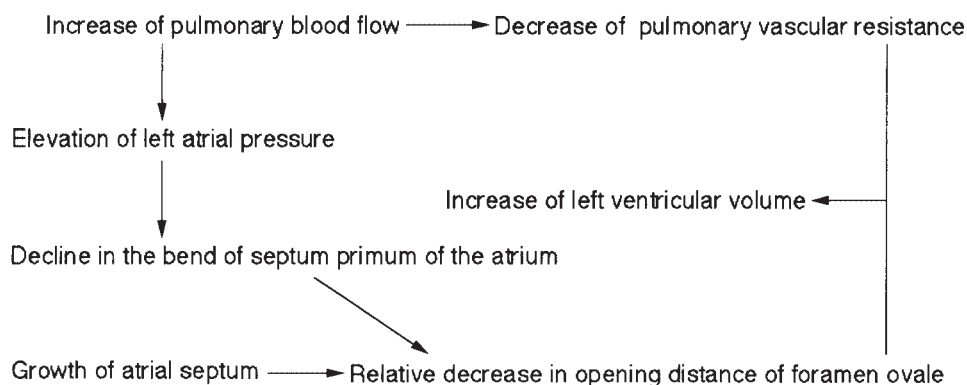


Fig. 7. Developmental changes in the cardiovascular system in fetal rats during late gestation.

age-related functional changes in the foramen ovale observed in human fetus.

Development of the cardiovascular system during late gestation may proceed as follows; 1) decreased pulmonary vascular resistance may result in an increased pulmonary blood flow volume, which induces the increase in peripheral pulmonary arterial size as shown in our study, 2) restrictive foramen ovale size causes reduction in the blood flow volume through the right atrium to the left atrium, 3) blood flow volume to the left ventricle shifts from the foramen ovale to the pulmonary artery toward late gestation. Thus, we have demonstrated that the cardiovascular system of the fetal rat effectively adapts to the postnatal, dynamic changes in circulation as shown in Fig. 7. These morphological changes observed in fetal rats may also be relevant to human fetal cardiovascular development.

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