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Research article

Echocardiographic parameters in different age and sex of Sprague-Dawley rats under isoflurane anesthesia

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Abstract

Echocardiography is a useful technique for diagnosing cardiovascular disease that is safe, reproducible and accurate. A comprehensive understanding of echocardiographic parameters in different age and sex is useful for cardiovascular study. Thirty Sprague-Dawley rats of both sexes at different age underwent repetitive echocardiography. The characteristics of early and late diastolic waves through the mitral inflow depend on the heart rate. The rats had fast heart rates, with early and late diastolic Doppler flows commonly fused. Several parameters in male rats were higher than in females except for ejection fraction, fractional shortening, isovolumetric relaxation time, pre-ejection fraction and ejection time that did not differ. Different age, sex, breed and anesthesia protocol can all cause diverse results. Rat echocardiography can be potentially used as a model for human cardiovascular research. Results revealed changes in echocardiographic parameters in different age and sex to better understand normal cardiovascular functions in rat model.

Keywords: Age, Echocardiography, Sex, Sprague-Dawley

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INTRODUCTION

Cardiovascular disease is a major cause of morbidity worldwide. Evaluation of cardiovascular functions requires information concerning blood pressure, blood profile and medical imaging such as chest radiography and echocardiography. Non-invasive echocardiography is a powerful technique for evaluating heart structures and functions. Echocardiography has been established as a safe, reproducible and accurate examination technique. Recently, the use of rodents as a model in cardiovascular research has been widely accepted but some parameters differ with diverse age, sex, breed and anesthesia protocol (Oláh et al., 2019; Walsh-Wilkinson et al., 2019; Watson et al., 2004).

A comprehensive understanding of echocardiographic parameters in rats is important to provide information for researching the cardiovascular system. Therefore, the aim of this study was to determine cardiac changes of Sprague-Dawley rats measured by the echocardiography technique in different age and sex under isoflurane anesthesia.

MATERIALS AND METHODS

Animals

Two-week-old Sprague-Dawley rats (Charles River Laboratories, Japan), weighing from 70 to 100 grams, fifteen of each sex, were randomly enrolled. They were housed under controlled temperature at 25 ± 0.5 °C with a 12-h light/dark cycle ventilated room and fed with standard food and water ad libitum. All procedures and protocols were performed and approved following the standard guidelines described in "Guide for the Care and Use of Laboratory Animals" of Tokyo University of Agriculture and Technology (Approval number: R02-24).

Experimental protocol

Two groups of rats were studied (n = 15/group) as male and female groups. Two weeks after acclimatization, each group was measured for body weight, blood pressure and echocardiography, repeated seven times throughout the entire experiment at 4, 6, 8, 10, 12, 16 and 20 weeks of age.

Blood pressure measurement

Each rat was placed inside a restraining cage and a blood pressure cuff was placed firmly over the base of the rat's tail. A BP Monitor for Rats and Mice Model ML-2000 (Muromachi Kikai Co., Ltd., Japan) was used to measure blood pressure, according to the company guidelines. Under a quiet atmosphere, all rats were trained to be familiar with the restraining procedure before conducting the experiment because excitation and excessive movement caused fluctuation of the heart rate (HR) and blood pressure. All data were recorded five times.

Echocardiography

All rats underwent weighing and echocardiography at 4, 6, 8, 10, 12, 16 and 20 weeks of age. Isoflurane was used for restraint because of its minimal effect on heart rate and smooth recovery. Mask inhalation of 5% isoflurane with 2.5 L/min oxygen was performed for induction of general anesthesia. Rats were allowed to breathe spontaneously after reducing isoflurane to 2.5% for maintenance. The rats were shaved over the chest area, positioned laterally and equipped with ECG limb leads. An echocardiography machine (Hitachi Aloka Medical ProSound Premier CV, Japan) with a 12-MHz probe was used and performed according to the American Society of Echocardiography guidelines (Schiller et al., 1989). The Doppler was recorded at the tip of the mitral valve in parasternal long-axis apical 4-chamber view, placed parallel to the blood flow to obtain maximum velocities. Pulmonary venous flow was measured by placing the Doppler at the pulmonary vein opening into the left atrium (LA). Pulmonary venous flows were depicted in three major wave reflections as systolic wave (Sm), diastolic wave (Dm) and end-diastolic flow (AR). Tissue Doppler imaging (TDI) was measured at septal and lateral mitral annuli. In this view, 1) early (E wave) and late (A wave) diastole from mitral inflow, 2) systolic wave (Sm), diastolic wave (Dm), retrograde end-diastolic flow (AR), and AR wave duration (ARdur) from pulmonary venous flow, and 3) tissue Doppler imaging (TDI) measured at septal and lateral mitral annuli were recorded.

In parasternal long-axis apical 5-chamber view, 1) left ventricular outflow tract (LVOT) velocity, and 2) isovolumetric relaxation time (IVRT) were recorded. In parasternal short-axis at papillary muscle level, left ventricular geometry was measured as 1) interventricular septum (IVS), 2) left ventricular internal diameter (LVID), 3) left ventricular posterior wall (LVPW), 4) end diastolic and systolic volume (EDV and ESV), 5) ejection fraction (EF) and 6) fractional shortening (FS). Pombo's method was used to measure ventricular dimension using M-mode (Pombo et al., 1971). IVS, LVID and LVPW parameters were recorded at end systole and end diastole. Left ventricular (LV) length measured from apex to closed mitral valve at early systole and left ventricular mass index (LVM) were also calculated using the following equation (Ono et al., 2002) ;

$$LVM = 1.4 \times [(LVIDd + LVPWd + IVSd)3 - LVIDd3] \times 0.8 + 0.14.$$

At aorta-main pulmonary artery level, 1) LA diameter, 2) aorta (Ao) diameter and 3) right ventricular outflow tract (RVOT) velocity were recorded. Echocardiography took approximately 15 min to measure on each rat to limit the anesthesia side effects. The diameters of Ao and LA were measured in this view. The proper view must clearly display the aortic and pulmonary valves, and only three cusps of the aortic valve can be seen in this view.

All parameters were categorized into specific groups as 1) LV chamber dimension or geometric parameters including IVS, LVID, LVPW, LVM, LA, Ao, LA/Ao ratio and LV, 2) Cardiac function parameters including EDV, ESV, EF, FS, IVRT, CO, SV, PEP and ET, 3) Pulsed-wave Doppler parameters including E, A, RVOT, LVOT, Sm, Dm, AR and ARdur, and 4) Tissue Doppler imaging (TDI) parameters including septal and lateral s', e' and a'.

Statistical analysis

Data were presented as minimum, maximum, mean and median ± standard deviation and the Mann-Whitney test was used to compare differences between repeated measurements. All statistical analyses were computerized using the commercial software package Prism 7.0a (GraphPad Inc., La Jolla, USA) for Macintosh. A difference of $P < 0.05$ was considered statistically significant.

RESULTS

Body weight and blood pressure

Changing body weight and blood pressure parameters in different age of male and female rats are shown in Table 1. Male and female rats measured from 4 to 20 weeks old displayed as minimum, maximum, mean, median and standard deviation values are shown in. Body weight of male rats appeared higher than females and was significantly different from 6 weeks of age to the end of the study period (Figure 1a). At 4 weeks of age, body weight of male and female rats (140-180 g) was not significantly different but after that body weight of male rats increased more than females. HR was not significantly different throughout the study period at about 420 bpm, sometimes increasing to over 500 bpm, and calculated using R-R interval of ECG under isoflurane anesthesia. Systolic, mean and diastolic blood pressures averaged 110 mm Hg, 75 mm Hg and 60 mm Hg, respectively with a significant difference in systolic blood pressure between male and female rats but mean and diastolic blood pressures did not differ throughout the study period.

Left heart geometry and functions

Left ventricular internal diameter end diastole (LVIDd) was a significant parameter at every age of male and female rats (Table 2 and Figure 1b) which was measured in M-mode (Figure 2). Other geometric parameters were not significantly different at some age points but the overall trend in male rats showed a significant increase in geometric parameters compared to females. Similarly, LVM (Figure 1c) and LV dimensions in male rats also increased significantly when compared to females. LA and Ao diameters increased significantly throughout the study period, while the LA/Ao ratio remained stable at around 1.0 (ranging from 0.7 to 1.4 mm). The Doppler over RVOT (Figure 3) in male rats showed a significant increase in RVOT velocity compared to females, ranging from 58 to141 cm/s, with mean values 95 cm/s in males and 104 cm/s in females. Table 3 shows that cardiac functions representing EDV, CO and SV in male rats were higher than in female rats but EF, FS, PEP, ET and IVRT were not significantly different. These data showed EF ranging from 70.0 to 54.0% and FS ranging from 34.0 to 45.0%.

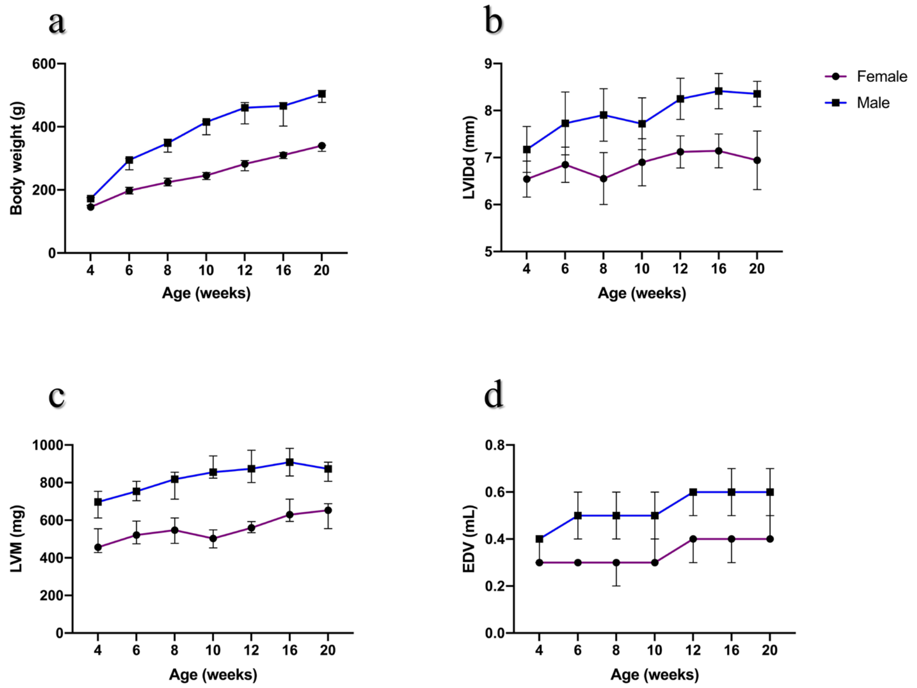

Figure 1 At different point of age, changing of (a) body weight, (b) LVIDd = left ventricular internal diameter at diastole, (c) LVM = left ventricular mass, and (d) EDV = end-diastolic volume in different age was different. Between male and female, body weight was significantly changed after 4-week of age, but LVIDd, LVM, and EDV were significantly changed from 4-week of age through the period of study.

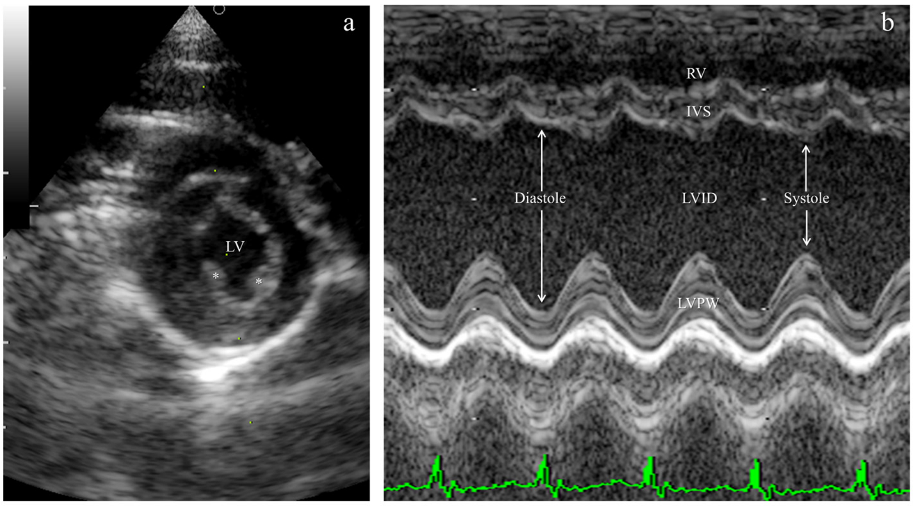

Figure 2 (a) Parasternal short-axis view at papillary muscle level; (b) Time-motion ™ mode (or M-mode) of LV; RV = right ventricle; LV = left ventricle; * (asterisk) = papillary muscles. In this view, ventricular geometry was measured with interventricular septum (IVS), left ventricular internal diameter (LVID), and left ventricular posterior wall (LVPW) during systole and diastole. The measured line must be placed between papillary muscles for better view and accurate results.

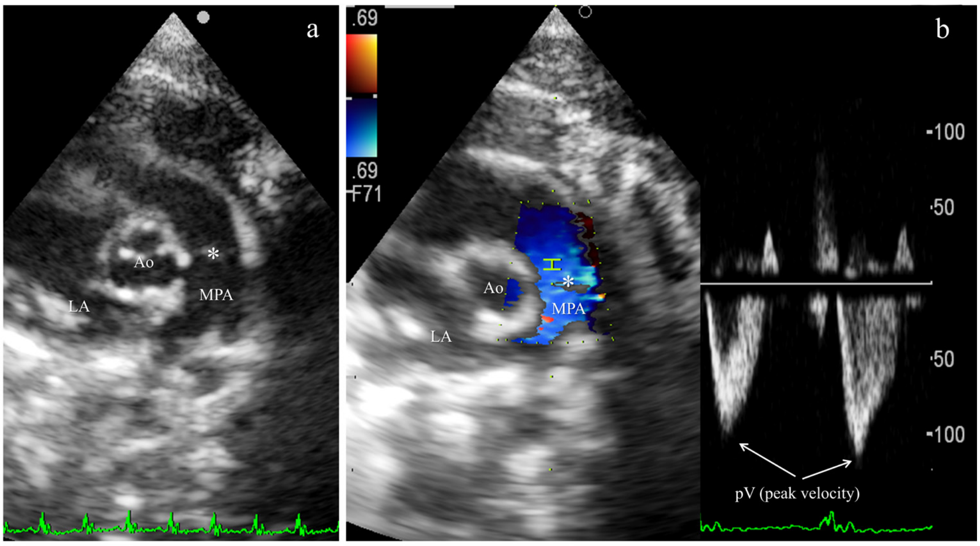

Figure 3 (a) Parasternal short-axis view at aorta-main pulmonary artery level; (b) indicating of the velocity of right ventricular outflow tract (RVOT) measured at pulmonic valve (asterisk), pV represents pulsed – wave Doppler velocity around 80.0 – 110.0 cm/s; LA/Ao ratio and pulmonic valve velocity are measured in this view; MPA = main pulmonary artery; Ao = aorta; * (asterisk) = pulmonary valve; LA = left atrium.

Doppler parameters

Results of pulsed wave Doppler at mitral valve are shown in Figure 4a, Table 4 and 5. Rats under general anesthesia still had very high heart rates at over 300 bpm. Early (E wave) and late (A wave) mitral inflow were commonly unseparated (fused E-A). Therefore, the representative E-A wave was recognized as fusion of E and A waves. Mitral inflow ranged from 70.2 to 132.2 cm/s in female rats and from 90.1 to 136.4 cm/s in male rats. Because of larger cardiac geometry, male rats recorded RVOT and LVOT Doppler at pulmonic valve and aortic valve faster than females. In this experiment, the AR wave occurred at the same time as the Dm wave and just after the P wave (Figure 4b). Median values of Sm were 19.0 and 20.0 cm/s in female and male rats, respectively and significantly different throughout the study. By contrast, Dm in female rats (27.0 cm/s) was higher than in male rats (24.5 cm/s). AR was not significantly different between female and male rats at 13.0 cm/s; however, ARdur in male rats at 33.0 ms was significantly longer than in female rats at 31.0 ms. In this study, s' at septal and posterior walls was significantly higher in male than female rats. Septal and posterior e'-a' velocities were not different between male and female rats, while the posterior velocity showed higher values than the septal velocity. Early mitral inflow in male rats was higher than in female rats. Subsequently, E/e'-a' was higher in both septal and lateral annuli.

Table 1 Body weight and blood pressure parameters.

Parameters	Age 4 weeks		Age 6 weeks		Age 8 weeks		Age 10 weeks		Age 12 weeks		Age 16 weeks		Age 20 weeks	
	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)
BW (g)	145.6±8.1	172.0±16.1	197.8±12.6	294.5±24.9$^{\#}$	224.0±14.8	348.0±28.2$^{\#}$	245.7±13.0	415.0±31.5$^{\#}$	282.2±17.1	460.0±49.1$^{\#}$	310.0±10.0	466.0±47.0$^{\#}$	340.0±13.0	504.0±21.0$^{\#}$
HR (bpm)	422.0±39.0	464.0±33.0$^{\#}$	405.8±45.0	367.5±29.0†	422.0±90.0	432.5±54.0	433.0±56.0	438.0±42.0	436.0±50.0	397.0±32.0$^{\#}$	427.0±95.0	449.0±42.0	417.0±50.0	416.0±35.0
SBP (mmHg)	100.0±10.0	118.0±12.0$^{\#}$	129.0±13.0	117.5±12.0$^{\#}$	115.0±10.0	109.0±9.0$^{\#}$	108.0±23.0	107.0±6.0	101.0±8.0	97.0±15.0	104.0±10.0	111.0±7.0$^{\#}$	97.0±6.0	109.0±10.0$^{\#}$
MBP (mmHg)	62.0±11.0	82.0±6.0$^{\#}$	90.0±10.0	82.5±11.0*	73.5±11.0	76.5±9.0	76.0±9.0	64.0±10.0$^{\#}$	71.0±10.0	72.0±16.0	79.0±8.0	73.0±12.0*	75.0±6.0	80.0±13.0
DBP (mmHg)	44.0±11.0	61.0±7.0$^{\#}$	69.0±11.0	63.5±13.0	50.5±14.0	59.5±13.0*	62.0±13.0	45.0±15.0$^{\#}$	57.0±11.0	60.0±16.0	68.0±11.0	58.0±14.0$^{\#}$	67.0±9.0	64.0±15.0

All parameters show in median ± standard deviation
BW = body weight; HR = heart rate; SBP = systolic blood pressure; MBP = mean blood pressure; DBP = diastolic blood pressure.
* $P < 0.01$ and $^{\#}$ $P < 0.005$ compared to Females at the same time-point

Table 2 Chamber dimension or geometric parameters.

Parameters	Age 4 weeks		Age 6 weeks		Age 8 weeks		Age 10 weeks		Age 12 weeks		Age 16 weeks		Age 20 weeks	
	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)
IVSd (mm)	1.4±0.2	1.6±0.2$^{\#}$	1.5±0.2	1.5±0.2	1.4±0.2	1.5±0.2	1.4±0.2	1.7±0.2$^{\#}$	1.4±0.2	1.7±0.1$^{\#}$	1.6±1.1	1.6±0.2	1.7±0.1	1.6±0.2
LVIDd (mm)	6.6±0.4	7.4±0.5$^{\#}$	6.8±0.4	8.0±0.7$^{\#}$	6.4±0.6	7.8±0.6$^{\#}$	6.9±0.5	7.9±0.6$^{\#}$	7.1±0.3	8.3±0.4$^{\#}$	7.1±0.4	8.4±0.4$^{\#}$	7.2±0.6	8.3±0.3$^{\#}$
LVPWd (mm)	1.7±0.2	1.9±0.2$^{\#}$	1.6±0.2	1.8±0.2*	1.8±0.2	1.9±0.3	1.5±0.1	2.1±0.2$^{\#}$	1.6±0.2	1.8±0.2$^{\#}$	1.7±0.2	1.8±0.1	1.7±0.2	1.7±0.2
IVSs (mm)	2.1±0.2	2.1±0.4	1.8±0.2	2.3±0.1$^{\#}$	2.2±0.2	2.2±0.2	2.0±0.2	2.5±0.3$^{\#}$	2.1±0.4	2.4±0.3*	2.3±0.3	2.4±0.3	2.4±0.3	2.4±0.3
LVIDs (mm)	3.6±0.2	4.0±0.5*	4.5±0.6	4.6±0.7	3.8±0.4	4.7±0.6$^{\#}$	4.1±0.4	4.6±0.6$^{\#}$	4.2±0.4	4.8±0.3$^{\#}$	4.1±0.5	4.9±0.4$^{\#}$	4.1±0.2	5.3±0.3$^{\#}$
LVPWs (mm)	2.2±0.3	2.4±0.2*	1.9±0.2	2.4±0.3$^{\#}$	2.4±0.2	2.4±0.3	2.2±0.2	2.7±0.2$^{\#}$	2.3±0.2	2.8±0.3$^{\#}$	2.2±0.3	2.9±0.2$^{\#}$	2.4±0.3	2.6±0.3
LVM (g)	0.5±0.1	0.7±0.1$^{\#}$	0.5±0.1	0.8±0.1$^{\#}$	0.5±0.1	0.8±0.1$^{\#}$	0.5±0.1	0.8±0.1$^{\#}$	0.6±0.0	0.8±0.1$^{\#}$	0.6±0.1	0.8±0.1$^{\#}$	0.7±0.1	0.9±0.1$^{\#}$
LA (mm)	3.2±0.3	3.4±0.4*	3.1±0.3	3.2±0.4	3.2±0.2	3.6±0.3$^{\#}$	3.3±0.4	3.9±0.5*	3.3±5	3.9±0.4$^{\#}$	3.6±0.5	3.9±0.2	3.6±0.2	4.0±0.5
Ao (mm)	3.0±0.2	3.2±0.3	3.1±0.2	3.2±0.4	3.1±0.2	3.4±0.3$^{\#}$	3.3±0.3	3.3±0.3	3.4±0.4	3.6±0.3	3.8±0.3	3.9±0.2	3.9±0.1	4.3±0.5*
LA/Ao	1.0±0.1	1.1±0.1	1.0±0.1	1.0±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
LV (mm)	9.0±0.4	10.6±0.6$^{\#}$	9.9±0.3	10.8±0.6$^{\#}$	9.7±0.4	10.7±0.4$^{\#}$	9.7±0.3	10.5±0.8$^{\#}$	9.9±0.6	10.5±0.6$^{\#}$	9.3±0.4	10.1±0.5$^{\#}$	10.1±0.6	11.3±0.4*

All parameters show in median ± standard deviation
IVSd = interventricular septum at end diastole; LVIDd = left ventricular internal diameter at end diastole; LVPWd = left ventricular posterior wall at end diastole; IVSs = interventricular septum at end systole; LVIDs = left ventricular internal diameter at end systole; LVPWs = left ventricular posterior wall at end systole; LVM = left ventricular mass index; LA = internal diameter of left atrium at short axis transaortic view; Ao = internal diameter of aorta at short axis transaortic view; LA/Ao = LA by Ao ratio; LV = left ventricular internal diameter from apex to closed mitral valve.
* $P < 0.01$ and $^{\#}$ $P < 0.005$ compared to Females at the same time-point

Table 3 Cardiac function parameters.

Parameters	Age 4 weeks		Age 6 weeks		Age 8 weeks		Age 10 weeks		Age 12 weeks		Age 16 weeks		Age 20 weeks	
	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)
EDV (mL)	0.3±0.0	0.4±0.1$^{\#}$	0.3±0.1	0.5±0.1$^{\#}$	0.3±0.1	0.5±0.1$^{\#}$	0.3±0.1	0.5±0.1$^{\#}$	0.4±0.1	0.6±0.1$^{\#}$	0.4±0.1	0.6±0.7$^{\#}$	0.4±0.1	0.6±0.1$^{\#}$
ESV (mL)	0.0±0.1	0.1±0.1	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.0$^{\#}$	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
EF (%)	83.5±3.4	80.0±4.7	70.6±8.0	81.3±5.5*	82.7±2.9	78.4±4.7$^{\#}$	79.9±5.0	81.1±5.0	79.9±4.3	80.1±3.4	81.5±5.5	79.7±4.3	81.5±5.3	75.1±3.5$^{\#}$
FS (%)	45.1±3.7	41.5±5.4	33.5±6.9	42.9±4.9*	44.2±3.0	39.7±4.1$^{\#}$	41.4±11.1	42.7±5.7	41.5±4.4	41.6±3.3	43.1±5.4	41.2±4.3	43.1±4.7	37.1±3.0$^{\#}$
IVRT (ms)	21.0±2.0	20.5±3.0	17.0±2.0	17.0±3.0	18.0±3.3	20.0±1.9	14.0±3.8	17.0±2.6	20.0±2.5	21.0±2.5	17.0±1.3	17.0±2.7	16.0±0.5	24.0±3.0$^{\#}$
CO (mL/m)	60.0±17.6	100.0±14.1$^{\#}$	80.0±15.3	80.0±26.6	70.0±15.8	100.0±26.2$^{\#}$	60.0±23.1	80.0±13.5*	90.0±14.0	130.0±24.9$^{\#}$	70.0±24.2	80.0±18.5	85.0±29.1	90.0±10.7
SV (mL)	0.3±0.1	0.6±0.1#	0.4±0.1	0.5±0.2	0.4±0.1	0.6±0.2$^{\#}$	0.4±0.1	0.5±0.1$^{\#}$	0.5±0.1	0.6±0.1$^{\#}$	0.4±0.1	0.5±0.1#	0.5±0.2	0.6±0.1
PEP (ms)	29.0±2.6	24.0±2.6$^{\#}$	27.0±2.0	27.0±2.6	25.0±3.0	27.0±2.0	25.0±2.9	25.0±2.1	28.0±2.2	27.0±2.1	27.0±3.7	25.0±2.4	24.0±3.6	27.0±2.2
ET (ms)	92.0±7.1	88.0±11.5	74.0±4.2	72.5±5.2	76.0±4.8	81.0±5.6*	72.0±3.8	76.0±7.4	77.0±6.0	70.0±4.5$^{\#}$	76.0±6.8	84.0±4.5	79.5±4.0	84.0±4.5$^{\#}$

All parameters show in median ± standard deviation
EDV = end diastolic volume; ESV = end systolic volume; EF = ejection fraction; FS = fractional shortening; IVRT = isovolumic relaxation time;
CO = cardiac output; SV = stroke volume; PEP = pre-ejection period; ET = ejection time;
* $P < 0.01$ and $^{\#}$ $P < 0.005$ compared to Females at the same time-point

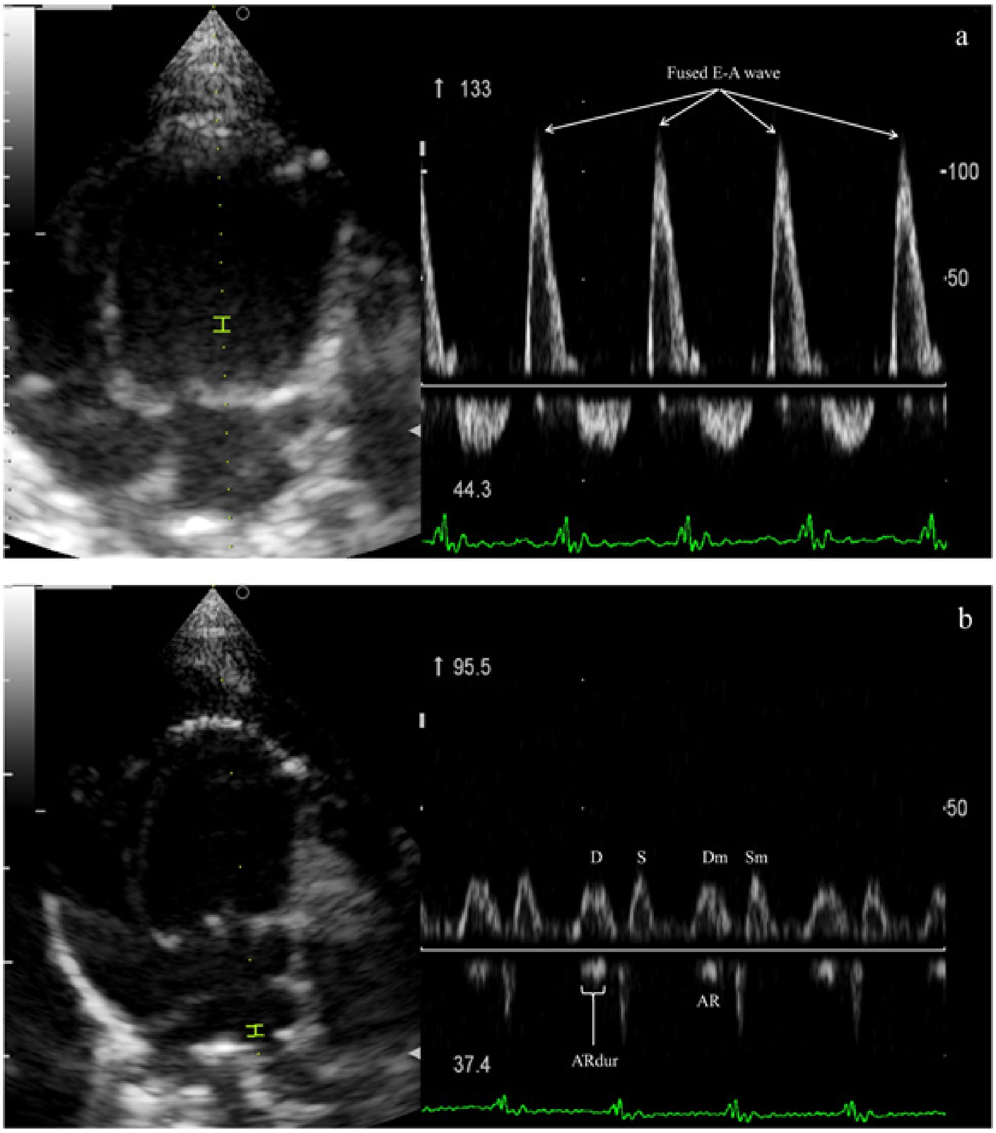

Figure 4 (a) Parasternal long-axis apical 4-chamber view and sample volume is placed over the mitral valve measuring the mitral inflow Doppler. In this study, because of high heart rate in rats, early and late diastole were fused (E-A wave); (b) Pulmonary vein Doppler is also measure in apical 4-chamber view; S = systolic wave; Sm = maximum velocity of the systolic wave, D = diastolic wave; Dm = maximum velocity of the diastolic wave; AR = retrograde end-diastolic flow; ARdur = duration of retrograde end-diastolic flow.

Table 4 Pulsed - wave Doppler parameters.

Parameters	Age 4 weeks		Age 6 weeks		Age 8 weeks		Age 10 weeks		Age 12 weeks		Age 16 weeks		Age 20 weeks	
	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)
Fused E-A (cm/s)	99.1±11.2	119.3±12.8$^{\#}$	105.7±9.3	108.8±9.3	106.4±7.4	110.4±7.5	103.8±14.9	123.3±11.8	100.0±16.7	97.2±14.9	106.4±13.2	109.2±6.2	98.8±19.4	111.1±6.2
E-A DecT (ms)	45.0±16.0	46.0±9.0	55.0±9.0	73.0±15.0	37.0±4.6	48.0±7.8$^{\#}$	45.0±7.3	55.0±9.8*	40.0±5.8	45.0±9.1*	47.5±7.3	55.0±8.0*	50.5±7.3	55.0±12.1
RVOT (cm/s)	103.2±12.5	114.±14.0$^{\#}$	85.8±17.3	103.8±22.9*	94.2±14.8	102.1±10.0*	96.9±13.3	111.6±22.7*	93.4±19.6	106.7±12.9$^{\#}$	86.6±10.8	96.4±12.5	86.0±9.5	95.5±9.7*
LVOT (cm/s)	83.0±11.8	115.0±9.2$^{\#}$	96.3±8.3	114.9±13.4$^{\#}$	87.5±11.8	118.3±9.2$^{\#}$	97.1±5.4	120.4±13.6$^{\#}$	95.5±10.3	123.0±18.8$^{\#}$	86.8±7.5	114.3±11.0$^{\#}$	99.0±10.3	104.0±8.3
PV Sm (cm/s)	14.7±2.5	22.6±3.8$^{\#}$	18.7±2.9	18.9±2.4	13.5±2.7	18.7±3.7$^{\#}$	18.3±1.9	23.2±2.1$^{\#}$	23.5±2.1	20.8±1.6$^{\#}$	19.6±3.3	17.4±4.6*	19.6±2.9	18.0±1.2
PV Dm (cm/s)	25.9±3.3	28.0±4.1	23.9±5.0	22.3±5.8	23.2±6.8	27.0±5.6	33.9±8.5	35.3±7.4	27.0±7.9	26.6±4.2	30.0±9.8	21.0±1.7$^{\#}$	28.3±3.4	21.1±3.3$^{\#}$
PV AR (cm/s)	13.8±2.3	13.3±1.0	13.0±1.8	11.1±2.1	13.7±0.8	12.6±2.0	13.8±1.6	14.8±1.0	14.2±1.3	14.2±1.4	13.5±1.1	11.8±1.5$^{\#}$	13.8±1.9	14.5±2.3
PV ARdur (ms)	23.0±2.9	41.0±7.0$^{\#}$	39.0±5.0	47.0±12.8	36.0±2.4	40.0±10.7	29.0±2.5	32.0±2.5	32.0±3.7	33.0±3.4	29.0±3.4	28.0±5.9	31.0±5.4	33.0±6.0

All parameters show in median ±standard deviation
E-A = peak velocity of the fusion of early and late diastole; E-A DecT = deceleration time of E-A; RVOT = right ventricular outflow tract velocity at pulmonic valve; LVOT = left ventricular outflow tract velocity at aortic valve; PV Sm, Dm and AR = peak velocity of pulmonary venous flow at systole, early diastole and late diastole (atrial contraction), respectively; ARdur = AR duration.
* P < 0.01 and $^{\#}$ P < 0.005 compared to Females at the same time-point

Table 5 Tissue Doppler parameters.

Parameters	Age 4 weeks		Age 6 weeks		Age 8 weeks		Age 10 weeks		Age 12 weeks		Age 16 weeks		Age 20 weeks	
	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)	Female (n = 15)	Male (n = 15)
Sep *s'* (cm/s)	3.4±0.6	4.4±0.9	3.8±0.2	3.8±1.1	3.8±0.7	3.8±1.0	5.1±1.3	6.4±1.3	4.5±0.6	6.0±0.8$^{\#}$	4.9±0.8	4.7±0.6	5.3±0.6	6.0±1.2
Sep *e'-a'* (cm/s)	5.1±0.4	5.6±1.6$^{\#}$	5.4±0.9	5.4±2.2	7.5±1.0	6.0±1.0$^{\#}$	8.5±2.0	9.0±2.1	7.4±1.4	8.0±1.4	6.5±2.3	5.3±0.6	7.6±0.5	8.0±1.9
Lat *s'* (cm/s)	4.4±1.7	5.3±0.4*	5.6±1.0	5.1±0.7	5.1±0.6	4.9±0.3	5.1±1.4	6.7±1.0$^{\#}$	5.4±0.9	6.1±1.4	5.6±0.8	6.0±0.7	5.6±0.4	6.3±0.9$^{\#}$
Lat *e'-a'* (cm/s)	6.1±1.2	7.7±1.3	7.8±1.1	7.5±1.5	8.4±0.8	7.7±2.1	9.0±1.0	9.3±1.6	8.5±0.6	8.6±1.6	8.1±2.0	7.7±2.0	8.8±1.3	9.3±1.2

All parameters show in median ± standard deviation
Sep *s'* and *e'-a'* = tissue Doppler of systolic, and fusion of early and late diastolic wave velocities at septal annulus; Lat s' and e'-a' = tissue Doppler of systolic, and fusion of early, and late diastolic wave velocities at lateral annulus.
* P < 0.01 and $^{\#}$ P < 0.005 compared to Females at the same time-point

Table 6 Comparison between male and female rats.

Parameters	Female Age from 4- to 20 weeks (n = 15)				Male Age from 4- to 20 weeks (n = 15)				P-value
	Min	Max	Median	SD	Min	Max	Median	SD	
BW (g)	133.1	342.0	245.7	62.3	137.7	520.0	364.0	111.7	< 0.0001#
HR (bpm)	327.0	555.0	421.0	55.0	321.0	552.0	420.0	48.0	0.3287
SBP (mm Hg)	75.0	158.0	107.0	14.0	78.0	156.0	111.0	12.0	0.0134*
MBP (mm Hg)	40.0	105.0	75.0	13.0	47.0	116.0	77.0	12.0	0.1186
DBP (mm Hg)	21.0	89.0	60.0	14.0	20.0	104.0	60.0	15.0	0.5880
IVSd (mm)	1.0	1.8	1.5	0.2	1.1	2.1	1.6	0.2	< 0.0001#
LVIDd (mm)	5.3	7.8	6.9	0.5	6.0	9.0	8.1	0.6	< 0.0001#
LVPWd (mm)	1.2	2.3	1.6	0.2	1.4	2.6	1.8	1.2	< 0.0001#
IVSs (mm)	1.6	3.0	2.1	0.3	1.8	3.2	2.3	0.3	< 0.0001#
LVIDs (mm)	3.1	5.4	4.0	0.5	3.2	6.0	4.7	0.6	< 0.0001#
LVPWs (mm)	1.4	3.1	2.3	0.3	1.9	3.6	2.6	0.3	< 0.0001#
LVM (mg)	393.9	814.3	555.5	88.25	474.6	1114.0	835.2	124.3	< 0.0001#
EDV (mL)	0.2	0.5	0.3	0.1	0.2	0.7	0.5	0.1	< 0.0001#
ESV (mL)	0.0	0.1	0.1	0.0	0.0	0.2	0.1	0.0	< 0.0001#
LA (mm)	2.6	4.4	3.3	0.4	2.4	4.9	3.7	0.5	< 0.0001#
Ao (mm)	2.6	4.4	3.2	0.4	2.5	4.8	3.5	0.5	0.0003#
LA/Ao	0.7	1.3	1.0	0.1	0.7	1.4	1.0	0.1	0.0523
EF (%)	60.6	89.6	80.7	5.8	67.7	90.3	79.6	5.0	0.1675
FS (%)	26.7	53.2	42.6	5.5	31.4	54.1	41.1	5.0	0.1193
E-A (cm/s)	70.2	132.2	103.1	13.7	90.1	136.4	110.3	11.3	< 0.0001#
E-A DecT (ms)	32.0	86.0	45.0	9.9	35.0	81.0	51.0	11.6	< 0.0001#
IVRT (ms)	11.0	25.0	19.0	3.2	12.0	27.0	19.5	2.9	0.1404
Sep s' (cm/s)	3.0	7.1	4.4	1.0	2.8	7.8	5.0	1.3	0.0031#
E/Sep e'-a'	9.5	24.4	15.2	3.4	9.7	33.0	17.1	5.0	0.0179*
Lat s' (cm/s)	2.9	8.9	5.3	1.1	3.8	8.8	5.6	1.1	0.0022#
Lat e'-a' (cm/s)	4.7	10.5	8.1	1.4	4.2	11.2	8.0	1.8	0.8632
LV (mm)	0.8	1.1	1.0	0.1	0.9	1.2	1.1	0.1	< 0.0001#
RVOT (cm/s)	58.8	141.1	94.5	15.3	58.1	142.3	103.8	17.2	< 0.0001#
LVOT (cm/s)	71.3	117.8	92.9	10.6	88.5	154.5	115.4	13.6	< 0.0001#
CO (mL/m)	40.0	120.0	70.0	20.9	50.0	150.0	90.0	23.5	< 0.0001#
SV (mL)	0.3	0.8	0.4	0.1	0.3	0.8	0.6	0.1	< 0.0001#
PEP (ms)	19.0	32.0	27.0	3.0	20.0	32.0	27.0	2.4	0.1928
ET (ms)	65.0	105.0	76.5	8.2	63.0	102.0	80.0	8.2	0.3497
PV Sm (cm/s)	11.2	27.3	18.7	3.8	9.1	30.6	19.7	3.5	0.0216*
PV Dm (cm/s)	15.7	48.8	27.0	7.3	15.7	41.8	24.5	6.0	0.0057#
PV AR (cm/s)	9.4	17.5	13.7	1.7	8.1	17.2	13.3	2.0	0.0992
PV ARdur (ms)	20.0	47.0	31.0	5.9	23.0	59.0	33.0	8.0	< 0.0001#

BW = body weight; HR = heart rate; SBP = systolic blood pressure; MBP = mean blood pressure; DBP = diastolic blood pressure; IVSd = interventricular septum at end diastole; LVIDd = left ventricular internal diameter at end diastole; LVPWd = left ventricular posterior wall at end diastole; IVSs = interventricular septum at end systole; LVIDs = left ventricular internal diameter at end systole; LVPWs = left ventricular posterior wall at end systole; EDV = end diastolic volume; ESV = end systolic volume; LA = internal diameter of left atrium at short axis transaortic view; Ao = internal diameter of aorta at short axis transaortic view; EF = ejection fraction; FS = fractional shortening; E-A = peak velocity of the fusion of early and late diastole; E-A DecT = deceleration time of E-A; IVRT = isovolumic relaxation time; Sep *s'* and *e'-a'* = septal mitral annulus, tissue Doppler of systolic, and fusion of early and late diastolic wave velocities; Lat *s'* and *e'-a'* = lateral mitral annulus, tissue Doppler of systolic, and fusion of early and late diastolic wave velocities; LV = left ventricular diameter, measured from mitral valve to apex in apical four-chamber view; RVOT = right ventricular outflow tract velocity measured at pulmonic valve; LVOT = left ventricular outflow tract velocity measured at aortic valve; CO = cardiac output; SV = stroke volume; PEP = pre-ejection period; ET = ejection time; PV Sm, Dm and AR = peak velocity of pulmonary venous flow at systole early diastole and late diastole (atrial contraction), respectively; ARdur = AR duration

* $P < 0.01$ and $^{\#}$ $P < 0.005$ compared to Females at the same time-point

DISCUSSION

Geometric parameters such as LV, LVID, IVS, LVPW, LVM, EDV and ESV in human and rat are generally similar, with normal values in males higher than in females (Otto, 2019). In this study, all parameters significantly differed throughout the experimental period (Table 6). In human echocardiography, LA and Ao increase gradually when subjects are growing, and LA diameter is the key major structural remodeling process occurring in response to pressure and volume overload. LA/Ao ratio remains stable in healthy individuals; however, LA size can be changed by variants such as age, sex and BW (Parajuli & Ahmed, 2021). Watson et al. (2004) showed that EF and SF in male Sprague-Dawley rats were similar to human, using a mixture of xylazine and ketamine as an anesthetic agent. When isoflurane was used for anesthesia, EF and FS were higher than the human reference. Kryzhanovskii et al. (2016) found higher EF and FS in male rats than our study when chloral hydrate was used for anesthesia. Weytjens et al. (2006) measured similar results to our experiment when pentobarbital was used. Our results showed that EF and FS were not different between male and female rats but values can differ depending on breed, sex and anesthesia protocol.

Mitral inflow patterns in anesthetized rats with isoflurane cannot be used to separate early and late diastolic flows because of high HR and fusion of E and A waves. Murakami et al. (2014) showed that HR in isoflurane anesthesia was higher and more stable than other anesthesia, suggesting that inhalation anesthesia was suitable to evaluate cardiovascular factors. Healthy rats commonly showed fusion of the two diastolic waves unless the anesthesia protocol depressed the HR to lower than 300 bpm. By contrast, Sanchez et al. (2017) recorded separation of E and A waves at HR over 300 bpm, even when using injection anesthesia. Therefore, both HR and anesthesia protocols play key roles in changing mitral inflow patterns (Stypmann et al., 2009). Furthermore, because mitral inflow in male rats was higher than in female rats, the higher velocity took longer to decelerate. Subsequently, deceleration time of E-A waves (E-A DecT) in males was higher than in female rats. Evaluation of diastolic functions or cardiac muscle movements during anesthesia, choice of breed and sex and anesthetic are crucial for designing experimental methods.

In this study, TDI was measured at both septal and lateral annuli, even though previous studies suggested that TDI measurement at the lateral mitral annulus gave unreliable visibility (Nguyen et al., 2013). Normally, the velocity of TDI s' is lower than e' in both human and animal studies but it changes when the disease develops (Ari et al., 2017; Nguyen et al., 2013; Rakha and Aboelenin, 2019). Zhou et al. (2018) showed that the s' and e' velocities became equal in hypertensive individuals and males gave higher velocities than females, similar to our study. E/e' values were not displayed in our experiment because fusion of e' and a' may cause unreliable data; however, to the best of our knowledge, they still provide a useful index of diastolic function when HR is high.

Pulmonary venous flows are normally used to evaluate left atrial pressure, and their parameters also correlate with mean pulmonary wedge pressure (Ito et al., 2000). In this study, the parasternal long-axis apical 4-chamber view was used, and the sample volume was placed close to the opening of the pulmonary vein. AR wave at the same time of Dm wave, as previously described that early and late diastole were fused in such a high HR condition (Figure 5). Pulmonary flow velocities averaged approximately one-third compared to human study (Iwashima et al., 2008). This characteristic is a good model of tachyarrhythmia in human medicine.

This study had some limitations. Sprague-Dawley rats are common rodents and widely used for testing. However, different rat species may show different values. The rats were only measured from 4 to 20 weeks of age as the period from premature to mature stage. Food, calorie intake and daily activities may cause changes in body score, with different heart sizes developing under varied conditions. All procedures should be performed using other anesthetic drugs to provide more information regarding cardiac functions under different conditions.

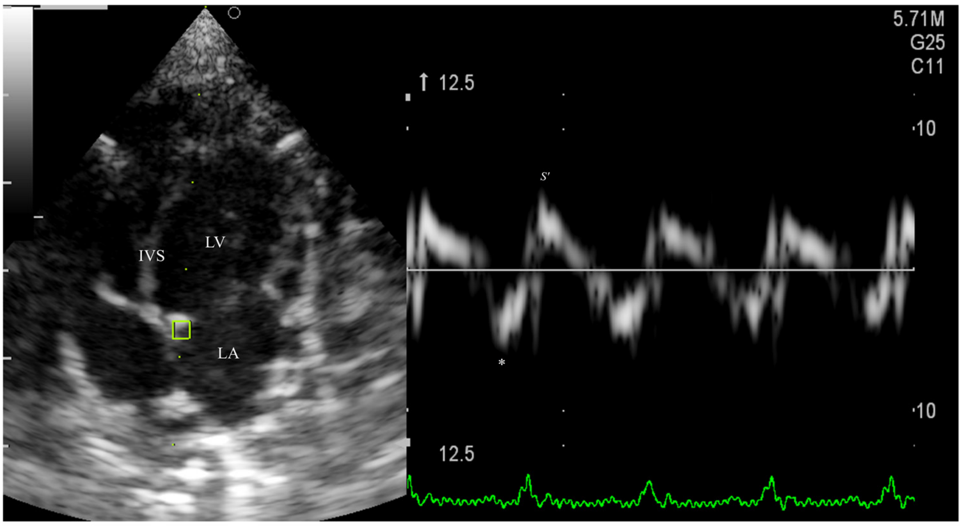

Figure 5 Showing parasternal long-axis apical 4-chamber view for measuring the spectral tissue Doppler imaging (TDI) at mitral annulus. LV = left ventricle; IVS = intraventricular septum; LA = left atrium; s' = systolic wave; * = fusion of early diastolic (e') and atrial contraction (a') velocities.

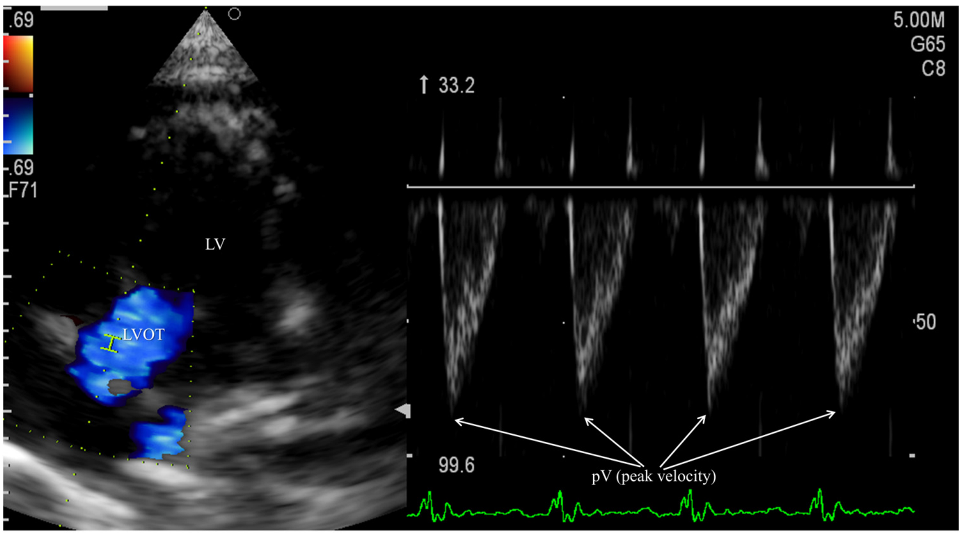

Figure 6 Showing parasternal long-axis apical 5-chamber view. The sample volume is placed at the aortic valve for measuring aortic Doppler velocity. LV = left ventricle; LVOT = left ventricular outflow tract.

CONCLUSIONS

Using rats as a model for cardiovascular experiments is challenging because changing several factors can result in different outcomes. The study method requires appropriate parameter design. Different age, sex, breed and anesthesia protocol can lead to diverse results. In this study, we used isoflurane that caused reduced side effects to cardiovascular functions. Rats are commonly used as models for cardiovascular research but this study revealed distinctions among sex, breed and age. Information concerning echocardiographic changes between male and female rats can be used as guidelines for cardiovascular study by researchers to select and design proper research protocol.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Pitipat Kitpipatkun performed the measurement of the echocardiographic parameters.
Catthareeya Sukwan analyzed the data.

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