



# Measurement of M-mode and B-mode echocardiographic Reference values in Domestic rabbits '*Oryctolagus cuniculus*'

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## ABSTRACT

**Objective:** To assess B-mode and M-mode echocardiographic reference values in Domestic rabbits (*Oryctolagus cuniculus*). **Animals:** 100 healthy domestic rabbits. **Procedures:** M-mode and B-mode measurements of the LVFWd, AO, LA, LA/AO, LVFWs, LVIDd, LVIDs, IVSd, IVSs, and HR were determined. **Results:** Mean±SD heart rate during echocardiographic tests was 204.33±3.929 beats/min. Mean±SD measurements in diastole and systole for the interventricular septum thickness, left ventricular internal diameter, and left ventricular free wall thickness were 2.5496±0.125 mm and 3.4232±0.134 mm; 15.5428±0.579 mm and 10.9706±0.439 mm; and 2.355±0.0977 mm and 3.6119±0.155 mm, respectively. Mean ± SD left atrial-to-aortic diameter ratio was 1.2265 ±0.0202, and Mean ± SD for fractional shortening and ejection fraction were 29.6047±0.941% and 60.0756±1.328%, respectively. All echocardiographic parameters were studied separately in males and females and in different weights. **Conclusions and Clinical Relevance:** Echocardiographic reference values for non-anesthetized domestic rabbits are presented providing reference values for future studies. Most of the results were comparable to those reported in non-anesthetized rabbits and may offer a good alternative when this data is necessary.

**Key Words:** Echocardiography, Rabbit, Reference values, *Oryctolagus cuniculus*.

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## INTRODUCTION

Domestic rabbit (*Oryctolagus cuniculus*) is becoming gradually popular as a companion animal. The cardiac disease has been reported in pet rabbits [1, 2]. Echocardiography is a valuable method for diagnosing cardiovascular illness in small animals [3]. In addition to dogs and cats, rabbits are popular companion animals in the world with millions kept as pets. Nearly 60% of these rabbits are registered with a veterinary practice. The number of reported or anecdotal reports on congenital or acquired cardiac diseases is growing, which leads to a

growing demand to perform echocardiography in pet rabbits as clinical cases. Reports of normal echocardiographic findings for comparison with potentially unhealthy rabbits are very limited, and most of them involve the description of particular echocardiographic parameters in small cohorts of young rabbits in a research setting. It has been utilized for cardiac imaging and assessment in experimental and clinical settings and has become an essential tool in the specialty of veterinary cardiology since it facilitates noninvasive measurement of cardiac structures. The results in healthy animals is crucial for the explanation of findings in clinical subjects. Values

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obtained from clinically normal animals by application of 2-dimensional (B-mode) and M-mode echocardiography have been described in a variety of animals, including dogs [4, 5], cats [6], hamsters [7], and birds [8], but, reference values for rabbits have not been published in Iran and many other countries. The goal of this investigation was to define reference values for echocardiographic M-mode and B-mode in healthy domestic rabbits in Iran.

## MATERIALS AND METHODS

100 rabbits between 6 to 12 months of age without sedation or anesthesia were used in this study. Rabbits were free of signs of cardiovascular or respiratory tract ailment and were determined to be clinically normal according to a physical examination and radiology. For the right parasternal views, rabbits were placed in right lateral recumbency over a gap on the tabletop through which the ultrasound probe was brought from below and located on a shaved area on the cranial aspect of the lower portion of the right thoracic wall. The hair was cut in the subcostal portion of the abdominal wall for the subcostal apical 4-chamber view, which was obtained with rabbits positioned in dorsal recumbency [9]. Echocardiographic measurements were carried out from standard views. Transthoracic 2-dimensional (B-mode) and M-mode echocardiography were performed with a system that included B-Mode and M-Mode capabilities with a 9-MHz transducer. Calipers were utilized to measure structures to the nearest millimeter by means of a leading-edge-to-leading-edge technique based on accepted echocardiographic standards for rabbits. From the right parasternal short-axis view, 2-dimensional (B-mode) guided M-mode tracings were made just below the mitral valve at the level of the papillary muscles for examinations of the IVS, LVID, and LVFW in diastole and systole and left atrial diameters were evaluated at the level of the aortic valve. These measurements were performed from the leading edge of the first endocardial surface to the leading edge of the second endocardial surface. Assessments of LVID and IVS in diastole and systole, the thickness of the LVFW in diastole and systole, and the left atrial appendage and aortic diameters were extensively utilized for assessment of cardiac morphology. The echocardiographic values were determined for FS, ejection fraction (EF). An ultrasound system (ZONCARE Q9) with a phased array sector transducer of 7.5 MHz and a micro convex transducer of 9 MHz were utilized for all of the echocardiographic examinations. Based on published standards [10], rabbits were located in right lateral recumbency and manually restrained without sedation. A complete B-mode and Motion-mode (M-Mode) exam was carried out with standard right parasternal long and short axis views [9, 11]. Standard cardiac wall and chamber thicknesses were then determined. Any suspected anomalies were subsequently scanned in numerous planes

to guarantee that the heart was normal. Subjective comparisons of the left ventricle (LV), intraventricular septum (IVS) and right ventricle (RV) wall thicknesses to lumen size, as well as the right heart to left heart structures were made. Left atrial (LA) diameter and aortic root (Ao) were measured on M-mode views [12]. The overall appearance of the heart was also assessed accurately on the short axis images: round symmetrical LV circumference [13]. The left ventricular internal diameter in diastole was examined in B-mode and M-mode. M-mode measurements included left ventricular internal diameter at end-diastole (LVIDd) and end-systole (LVIDs), Ao at end-diastole, LA at end-systole, interventricular septal thickness at end-diastole (IVSd) and end-systole (IVSs), and left ventricular posterior wall thickness at end-diastole (LVFWd) and systole (LVFWs). The percent fractional shortening (%FS) was determined by the standard formula:  $LVIDd - LVIDs$  divided by LVIDd. The LA:Ao ratio was measured from M-mode measurements.

## Statistical analyses

The data were stored in MS Excel and analyzed with the statistical software. Means and proportions of the different features (age, weight, and echocardiographic measurements) were determined. The normality of interval measured data within male and female groups was evaluated utilizing a Kolmogorov Smirnov test. Means and proportions were contrasted between male and female rabbits utilizing the appropriate two-sample tests (unpaired T-test, Mann Whitney). To explain the relationship between the different numeric (interval and score) parameters for age and body weight, a Spearman's rank correlation test was utilized. P-values < 0.05 were considered statistically significant.

## RESULTS

50 rabbits (50 %) were female and 50 rabbits (50 %) were male. The mean age of all rabbits in the research was 9.08 months (ranged from 6 to 12 months). The mean age of female rabbits was not significantly less than that of the male. The mean weight of the female population was 2517.20 gr. The mean weight of males was significantly higher than that of females. The mean heart rate of the 50 female rabbits was 207.66 beats/min, which was significantly higher than the mean heart rate of the males (201 beats/min). All evaluations of echocardiographic dimensions are depicted in Table 1. The mean values of LA, LA:Ao and LVIDs of the male population were significantly higher than those of the female population. LA, Ao, LVIDd, and LVFWd differed significantly between male and female rabbits (Table 2). Echocardiographic parameters such as the IVSs, IVSd, LVIDs, LVIDd, LVFWs, and LVFWd increased parallel to BW. LVFW was higher ( $p < 0.05$ ) in males than in females.

It was revealed that fractional shortening (FS) and ejection fraction (EF) values reduced based on BW. It was also demonstrated that the left atrial dimension (LAD) and aortic root dimension (AOD) enhanced in association with the enhancement in BW; of these parameters, LAD and AOD were higher ( $p < 0.05$ ) in males than in females, and only the CA amplitude was statistically higher ( $p < 0.05$ ) in females than in males. All echocardiographic parameters for eight weights were investigated separately in this study (Table 2).

## DISCUSSION

Domestic rabbit is a good model for cardiovascular research owing to its size, which makes surgical manipulation of the heart more practicable than in smaller animals; they are less expensive to procure and maintain than dogs, and the composition of rabbit myosin and the kinetics of calcium in the rabbit are comparable to those in human myocardium [14].

Pet rabbits can develop cardiovascular disease. Radiography, electrocardiography, and echocardiography are useful noninvasive diagnostic procedures that can be used in the evaluation of cardiac disease to provide a highly specific assessment of cardiac size, dysrhythmias, and internal structure and function (dynamics), respectively [3]. Measurements of the thickness of the LVFW in diastole and systole, IVS in systole and diastole, LVID in systole and diastole, and the left atrial appendage and aortic diameters are extensively utilized for assessment of cardiac morphology. The echocardiographic values determined for

FS, ejection fraction, were utilized to assess systolic, and diastolic function.

The mean  $\pm$  SD heart rate of the rabbits in our research was  $204.33 \pm 3.929$  beats/min, a range that was lower than the range of mean heart rates (180 to 250 beats/min) previously reported for conscious rabbits [15]. Echocardiographic M-mode measurements described for dogs differ proportionally with body size (weight), which varies by breed [16]. The M-mode measurements reported for cats [17, 18], ponies, horses [19], ferrets [20], and chinchillas [21], however, do not vary with body size, probably because different breeds of these species are comparable in size. Although the bodyweight of rabbits in the present investigation varied from 2000 to 3250 gr, significant associations between body weight and echocardiographic measurements were noticed. Because there were significant changes in echocardiographic values within this range of body weights, the authors speculate that cardiac measurements do not enhance with improved weight after rabbits reach maturity. Values for the echocardiographic variables in this investigation can represent reference values for application in the echocardiographic examination of all domestic rabbits.

## CONCLUSIONS:

Echocardiographic reference values for non-anesthetized domestic rabbits are presented providing reference values for future studies. Most of the results were comparable to those reported in non-anesthetized rabbits, and may offer a good alternative when this data is necessary.

## Appendices

**Table 1: Comparison of echocardiographic parameters, age, weight and heart rate between healthy adult male and female rabbits examined.**

	Sex	N	Mean	Std. Deviation	Std. Error Mean	P-value
Body weight (gr)	Male	50	2663.20	273.604	38.693	
	Female	50	2517.20	315.802	44.661	0.381
Heart rate	Male	50	201.00	(.352)	2.878	
	Female	50	207.66	19.579	2.769	0.007
LA mm)	Male	50	10.7754	1.654376	.233964	0.021
	Female	50	10.0252	1.869933	.264448	
AO (mm)	Male	50	8.7752	1.04592	.14791	0.006
	Female	50	8.0970	1.25949	.17812	
LA/AO	Male	50	1.2200	.09943	.01406	
	Female	50	1.2330	.10835	.01532	0.475
LVIDd (mm)	Male	50	15.9890	2.81264	.39777	0.103
	Female	50	15.0966	3.08259	.43594	
LVIDs (mm)	Male	50	11.4090	2.08494	.29485	0.06
	Female	50	10.5322	2.34959	.33228	

LVFWd (mm)	Male	50	2.4956	.52592	.07438	0.004
	Female	50	2.2144	.43642	.06172	
LVFWs(mm)	Male	50	3.8328	.77389	.10944	0.0008
	Female	50	3.3910	.76538	.10824	
IVSd (mm)	Male	50	2.7078	.60289	.08526	0.007
	Female	50	2.3914	.64146	.09072	
IVSs (mm)	Male	50	3.6224	.63876	.09033	0.003
	Female	50	3.2240	.68665	.09711	
FS (percent)	Male	50	28.5774	4.76851	.67437	0.041
	Female	50	30.6320	4.70638	.66558	
EF (percent)	Male	50	58.7536	6.88923	.97428	0.065
	Female	50	61.3976	6.53087	.92360	

**Table 2: Echocardiographic parameters, age, weight and heart rate in healthy adult male and female rabbits examined.**

Body weight (gr)		Heart rate	LA(mm)	AO(mm)	LA/AO	LVIDd (mm)	LVIDs (mm)
2000 - 2160	Mean	222.60	8.24100	7.2700	1.1270	12.4230	8.6690
	N	10	10	10	10	10	10
	Std. Deviation	18.374	1.122452	.93824	.03234	1.78268	1.34918
	Std. Error of Mean	5.810	.354951	.29670	.01023	.56373	.42665
	Minimum	193	6.750	5.90	1.08	9.20	6.35
	Maximum	245	10.300	8.68	1.18	15.53	10.74
2161 - 2320	Mean	209.46	8.44000	7.1692	1.1669	12.8008	8.5600
	N	13	13	13	13	13	13
	Std. Deviation	14.655	1.647387	.96183	.08864	2.60627	1.58414
	Std. Error of Mean	4.065	.456903	.26676	.02458	.72285	.43936
	Minimum	183	6.630	5.98	1.07	9.20	6.35
	Maximum	231	11.640	8.78	1.33	17.97	11.07
2321 - 2480	Mean	215.85	9.70692	7.9462	1.2169	14.6923	10.4215
	N	13	13	13	13	13	13
	Std. Deviation	19.527	1.327481	.94853	.09911	2.87288	2.19636
	Std. Error of Mean	5.416	.368177	.26307	.02749	.79679	.60916
	Minimum	185	7.900	6.20	1.10	10.87	6.81
	Maximum	254	11.370	9.71	1.41	19.04	13.14
2481 - 2640	Mean	207.78	10.56444	8.4350	1.2500	15.3417	11.2278
	N	18	18	18	18	18	18
	Std. Deviation	19.952	1.328313	.87405	.11386	2.63979	1.92911
	Std. Error of Mean	4.703	.313086	.20602	.02684	.62220	.45470
	Minimum	177	8.900	5.93	1.09	10.87	6.81
	Maximum	245	13.210	10.11	1.50	19.18	13.76
2641- 2800	Mean	200.22	11.19481	9.0222	1.2385	16.7411	11.7515
	N	27	27	27	27	27	27
	Std. Deviation	12.801	1.296381	.95910	.06274	2.28652	1.69133

	Std. Error of Mean	2.464	.249489	.18458	.01207	.44004	.32550
	Minimum	181	8.890	6.92	1.13	11.71	8.81
	Maximum	230	13.460	10.22	1.36	19.86	14.92
2801 - 2960	Mean	186.38	12.14500	9.7475	1.2462	18.5212	13.6462
	N	8	8	8	8	8	8
	Std. Deviation	19.661	.940167	.37724	.08749	.83297	.96411
	Std. Error of Mean	6.951	.332399	.13337	.03093	.29450	.34087
	Minimum	167	10.930	8.85	1.14	16.69	11.78
	Maximum	228	14.210	10.07	1.44	19.21	14.92
2961 - 3120	Mean	190.57	11.96000	8.7771	1.3714	17.6343	12.0829
	N	7	7	7	7	7	7
	Std. Deviation	22.729	.359722	.93111	.15636	1.98705	1.80257
	Std. Error of Mean	8.591	.135962	.35192	.05910	.75103	.68131
	Minimum	169	11.280	7.84	1.13	13.89	8.83
	Maximum	238	12.480	9.91	1.52	19.08	13.43
3121 - 3280	Mean	176.75	12.10250	9.8900	1.2200	18.2175	12.6175
	N	4	4	4	4	4	4
	Std. Deviation	6.898	.399614	.08756	.05416	1.51454	1.61550
	Std. Error of Mean	3.449	.199807	.04378	.02708	.75727	.80775
	Minimum	168	11.520	9.83	1.14	15.96	10.21
	Maximum	184	12.430	10.02	1.26	19.09	13.55
Total	Mean	204.33	10.40030	8.4361	1.2265	15.5428	10.9706
	N	100	100	100	100	100	100
	Std. Deviation	20.148	1.796509	1.20114	.10366	2.96982	2.25346
	Std. Error of Mean	2.015	.179651	.12011	.01037	.29698	.22535
	Minimum	167	6.630	5.90	1.07	9.20	6.35
	Maximum	254	14.210	10.22	1.52	19.86	14.92

Body weight (gr)		LVFWd (mm)	LVFWS (mm)	IVSd (mm)	IVSs (mm)	FS (percent)	EF (percent)
2000 - 2160	Mean	1.8650	2.8730	1.9760	2.7830	30.2800	61.2600
	N	10	10	10	10	10	10
	Std. Deviation	.37925	.57643	.63554	.72630	3.64655	5.10124
	Std. Error of Mean	.11993	.18228	.20098	.22968	1.15314	1.61315
	Minimum	1.61	2.40	1.47	2.16	25.60	54.60
	Maximum	2.90	4.01	2.92	3.91	36.30	69.40
2161 - 2320	Mean	1.7662	2.6362	1.7554	2.5362	32.9769	64.9154
	N	13	13	13	13	13	13
	Std. Deviation	.17086	.36041	.40449	.39348	4.63953	6.41468
	Std. Error of Mean	.04739	.09996	.11218	.10913	1.28677	1.77911
	Minimum	1.58	2.25	1.40	2.11	21.30	48.30
	Maximum	2.14	3.58	2.55	3.27	38.90	72.70
2321 - 2480	Mean	2.2600	3.4531	2.3738	3.3754	29.1615	59.5077
	N	13	13	13	13	13	13

	Std. Deviation	.39958	.64817	.56256	.56700	5.89527	8.38157
	Std. Error of Mean	.11082	.17977	.15603	.15726	1.63505	2.32463
	Minimum	1.70	2.25	1.66	2.21	19.80	45.70
	Maximum	2.97	4.22	3.40	4.20	37.40	70.80
2481 - 2640	Mean	2.3967	3.5778	2.5889	3.4667	26.7278	56.0100
	N	18	18	18	18	18	18
	Std. Deviation	.37773	.65284	.53231	.54331	4.98926	7.14840
	Std. Error of Mean	.08903	.15387	.12547	.12806	1.17598	1.68490
	Minimum	1.78	2.21	1.59	2.15	19.80	45.70
	Maximum	2.97	4.65	3.42	4.35	37.40	70.80
2641- 2800	Mean	2.4789	3.8911	2.7589	3.6148	29.9185	60.4326
	N	27	27	27	27	27	27
	Std. Deviation	.36014	.47287	.41980	.49357	4.19863	5.94091
	Std. Error of Mean	.06931	.09100	.08079	.09499	.80803	1.14333
	Minimum	1.79	2.55	1.98	2.01	20.20	46.20
	Maximum	2.94	4.55	3.29	4.15	35.90	68.90
2801 - 2960	Mean	2.8600	4.3737	3.1663	4.1575	26.9625	55.6750
	N	8	8	8	8	8	8
	Std. Deviation	.42795	.85745	.27339	.28379	4.27215	5.00278
	Std. Error of Mean	.15130	.30316	.09666	.10033	1.51043	1.76875
	Minimum	2.02	2.46	2.71	3.86	21.10	47.70
	Maximum	3.52	5.35	3.66	4.75	34.20	60.20
2961 - 3120	Mean	2.7743	4.3371	3.1029	3.9400	31.6529	63.3286
	N	7	7	7	7	7	7
	Std. Deviation	.40836	.59905	.11715	.33076	3.34991	4.52685
	Std. Error of Mean	.15435	.22642	.04428	.12501	1.26615	1.71099
	Minimum	2.15	3.10	2.87	3.21	28.00	58.20
	Maximum	3.54	5.02	3.25	4.21	36.40	69.60
3121 - 3280	Mean	3.0350	4.6225	3.3450	4.2000	30.9250	62.2250
	N	4	4	4	4	4	4
	Std. Deviation	.45800	.30576	.29240	.12728	3.39939	4.60751
	Std. Error of Mean	.22900	.15288	.14620	.06364	1.69969	2.30376
	Minimum	2.69	4.20	3.15	4.08	29.00	59.60
	Maximum	3.71	4.93	3.78	4.35	36.00	69.10
Total	Mean	2.3550	3.6119	2.5496	3.4232	29.6047	60.0756
	N	100	100	100	100	100	100
	Std. Deviation	.50114	.79729	.63940	.68949	4.82531	6.80934
	Std. Error of Mean	.05011	.07973	.06394	.06895	.48253	.68093
	Minimum	1.58	2.21	1.40	2.01	19.80	45.70
	Maximum	3.71	5.35	3.78	4.75	38.90	72.70

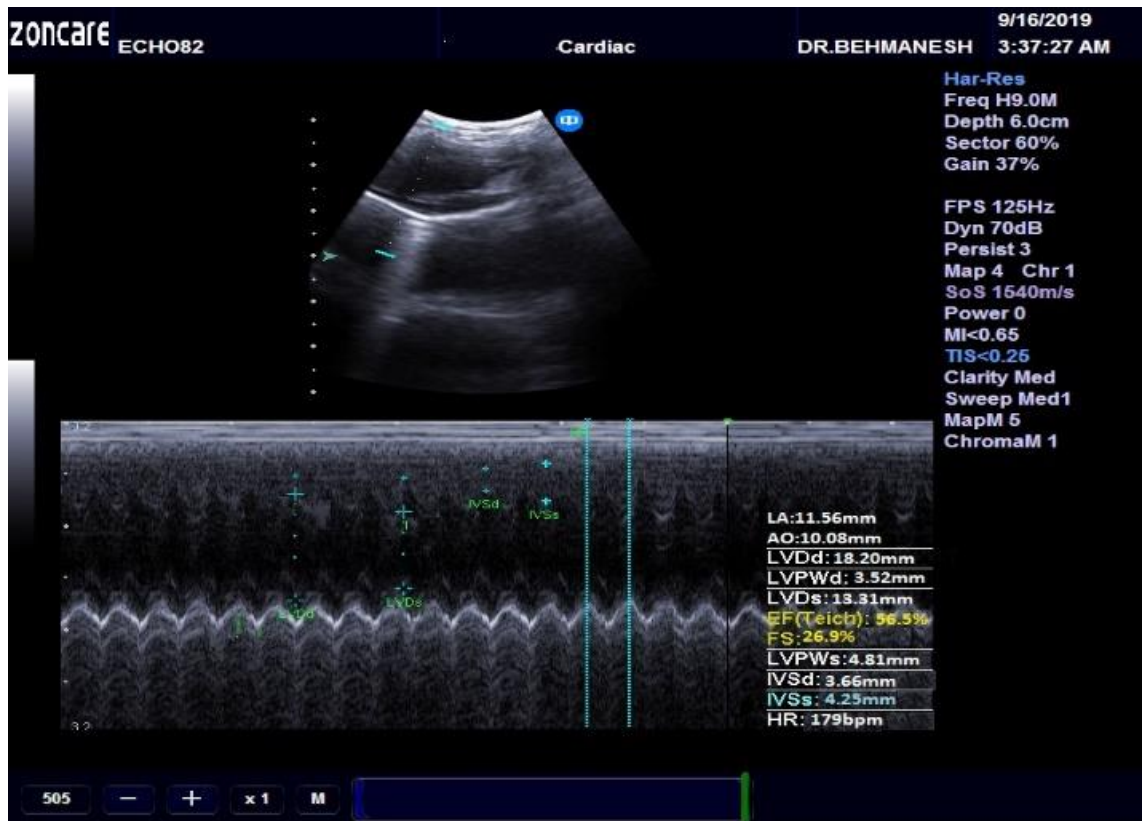


Figure 1. Echocardiographic long-axis section of the left ventricle at the four chamber level at both end-diastolic and end-systolic phases of the cardiac cycle.

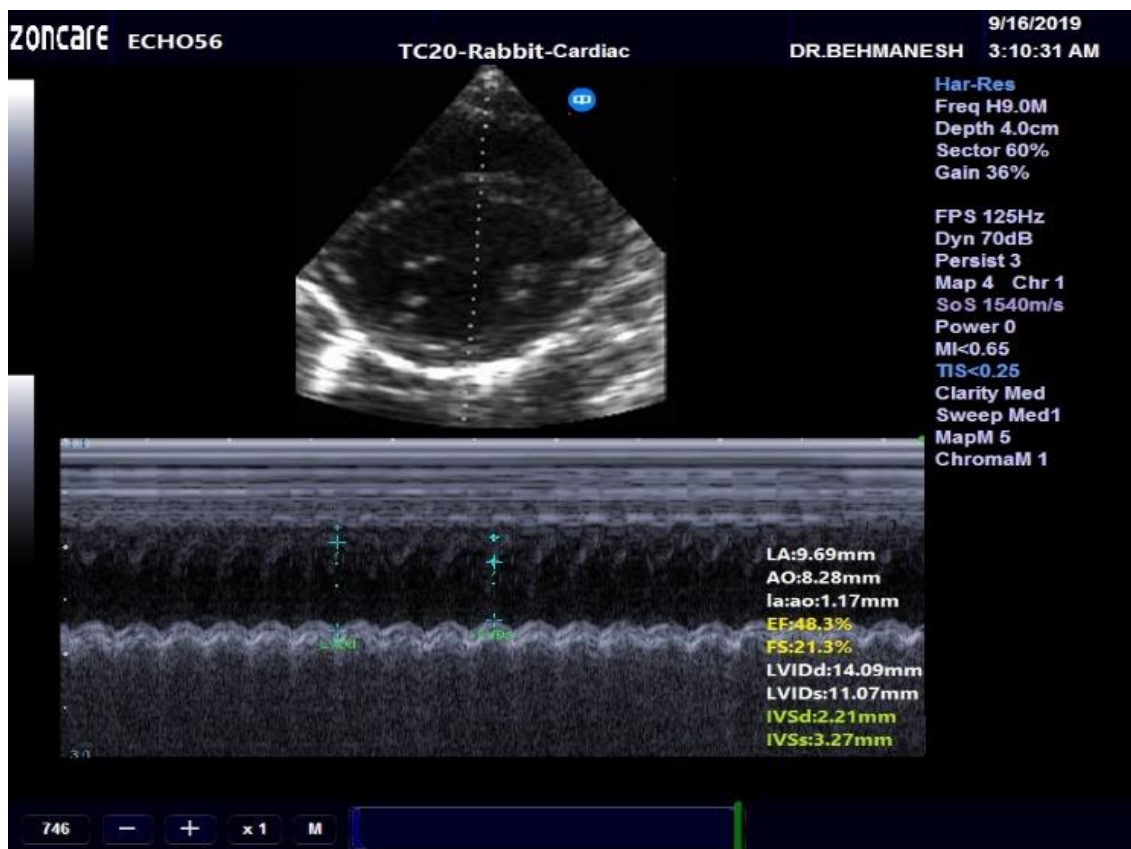


Figure 2. Echocardiographic short-axis section of the left ventricle at the papillary muscle level at both end-diastolic and end-systolic phases of the cardiac cycle.

## Abbreviations

Ao : aortic diameter  
bpm :Beats per minute  
FS :Fractional shortening  
HR :Heart rate  
IVSd :Interventricular septal wall thickness in diastole  
IVSs :Interventricular septal wall thickness in systole  
LA :Left atrium  
LVIDd :Left ventricular internal diameter in diastole  
LVIDs :Left ventricular internal diameter in systole  
LVFWd :Left ventricular free wall in diastole  
LVFWs :Left ventricular free wall in systole.

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