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Original Article

Measurement of Echocardiographic Ejection Fraction Reference Values and Evaluation between Body Weight and Ejection Fraction in Domestic Rabbits (*Oryctolagus cuniculus*)

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ARTICLE INFO	ABSTRACT
<p><i>Article History:</i></p> <p>Received 27 July 2021 Revised 3 January 2022 Accepted 22 January 2022 Online 22 January 2022</p> <hr/> <p><i>Keywords:</i></p> <p>Echocardiography Ejection fraction Rabbit Heart</p>	<p>Domestic rabbits (<i>Oryctolagus cuniculus</i>) are an excellent model for cardiovascular research because the size of these animals is more suitable for study and experimentation than smaller animals. One of the most important diagnostic imaging methods is echocardiography, which is used today to evaluate the anatomical and functional cardiovascular system and is one of the most accurate and sensitive non-invasive methods for examining heart disease. Ventricular function indices can be assessed with cardiac imaging techniques. One of these important cardiac parameters is the ejection fraction (EF), which has a valuable place along with other involved parameters. EF is a measure of the percentage of blood that comes out of the heart with each contraction. For this study, 100 adult and young standard domestic rabbits, six months to one year old and in both sexes (50 female and 50 male rabbits) without anesthesia and sedation were used. In this study, the mean EF in domestic rabbits studied in males was 58.753 ± 6.889 and females 61.397 ± 6.530, which are comparable to the items mentioned in the valid books and the average size of EF measured in this study, there is no significant difference between this research and other research. There was no significant difference in the percentage of EF between most weight groups, but there was a significant difference ($p < 0.05$) in weight groups (2161–2320 g and 2481–2640 g). Echocardiographic EF reference values for domestic rabbits (<i>Oryctolagus cuniculus</i>) non-anesthetized with are presented providing reference values for future studies.</p>

Introduction

The domestic rabbit (*Oryctolagus cuniculus*) is becoming increasingly popular as a companion animal. Cardiac disease has been described in pet rabbits.^{1,2} Echocardiography is a useful technique for diagnosing

cardiovascular disease in small animals.³ Next to dogs and cats, rabbits are popular companion animals in the World with millions kept as pets. Almost 60% of these rabbits are registered with a veterinary practice. The number of reported or anecdotal reports describing congenital or acquired cardiac diseases is increasing,

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which results in a growing demand to perform echocardiography in pet rabbits as clinical cases. Reports of normal echocardiographic findings for comparison with potentially diseased rabbits are very scarce, and most involve the description of selected echocardiographic parameters in small cohorts of young rabbits in a research setting. It has been used for cardiac imaging and evaluation in experimental and clinical settings and has become an indispensable tool in the specialty of veterinary cardiology because it enables noninvasive measurement of cardiac structures. Knowledge of findings in healthy animals is essential for interpretation of results in clinical patients. Values obtained from clinically normal animals by use of 2-dimensional (B-mode) and Motion-mode (M-mode) echocardiography have been reported in a variety of animals, including dogs,^{4,5} cats,⁶ ferrets, chinchillas, guinea pigs, hamsters,⁷ and birds,⁸ but, reference values for domestic rabbits have not been published in Iran. Ventricular function indices can be assessed with cardiac imaging techniques. One of these important cardiac parameters can be referred to the ejection fraction (EF), which has a valuable position along with other parameters involved. EF is a measure of the percentage of blood that comes out of the heart with each contraction. At each heartbeat, the heart contracts and expands. When the heart contracts, blood is expelled from the two chambers (ventricles) of the heart. As the heart expands, the ventricles fill with blood again. But it must be said that no matter how strong the contraction, the ventricles will never be completely empty. The term "ejection fraction" refers to the percentage of blood that comes out of a blood-filled ventricle with each heartbeat. The left ventricle is the main site of pulse of the heart, so usually only the left ventricular EF is measured. The purpose of this study was to determine reference values for echocardiographic and ejection fraction in healthy domestic rabbits (*Oryctolagus cuniculus*) in Iran.

Materials and Methods

100 rabbits between 6 to 12 months of age (8.9 ± 0.56 months in females and 9.26 ± 0.557 months in males) without sedation or anesthesia were used in this study. Rabbits were free of signs of cardiovascular or respiratory tract disease and were determined to be clinically normal on the basis of a physical examination and radiology. For the right parasternal views, rabbits were placed in right lateral recumbency over a gap in the table top through which the ultrasound probe was

brought from below and placed on a shaved area on the cranial aspect of the lower portion of the right thoracic wall. The hair was clipped 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 obtained from standard views. Transthoracic 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 used to measure structures to the nearest millimeter by means of a leading-edge-to-leading-edge technique according to accepted echocardiographic standards for rabbits. From the right parasternal short-axis view, B-mode and M-mode tracings were made just below the mitral valve at the level of the papillary muscles for measurements of the intraventricular septal thickness, left ventricular internal diameter, and left ventricular posterior wall thickness at end diastole and systole (IVSd, LVIDd, LVFWd, IVSs, LVIDs, and LVFWs, respectively) and left atrial diameters were evaluated at the level of the aortic valve. These measurements were made from the leading edge of the first endocardial surface to the leading edge of the second endocardial surface. Left ventricular EF was calculated by use of the cube method according to this formula:

$$\text{Ejection fraction} = [(LVIDd3 - LVIDs3)/LVIDd3] \times 100$$

Measurements of LVID in diastole and systole, IVS in diastole and systole, thickness of the LVFW in diastole and systole, and the left atrial appendage and aortic diameters (AO) are widely used for evaluation of cardiac morphology. The echocardiographic values calculated for EF. An ultrasound system (ZONCARE Q9) with a phased array sector transducer of 7.5 MHz and micro convex transducer of 9 MHz were used for all of the echocardiographic examinations. According to published standards,¹⁰ rabbits were positioned in right lateral recumbency and manually restrained without sedation. A complete B-mode and M-Mode exam was performed with standard right parasternal long and short axis views (Figures 1 and 2).¹¹ Standard cardiac wall and chamber thicknesses were then measured. Any suspected abnormalities were subsequently scanned in multiple planes to ensure that the heart was normal. Subjective comparisons of the left ventricle (LV), IVS, and right ventricle (RV) wall thicknesses to lumen size, as well as right heart to left heart structures were made. Left atrial (LA) diameter and AO were measured on M-mode views.¹² The general appearance of the heart was also evaluated objectively on the short

axis images: round symmetrical LV circumference.¹³ LVIDd was measured in B-mode and M-mode. M-mode measurements included: LA at end systole, AO at end diastole, LVIDd and LVIDs, IVSd and IVSs, and LVFWd and LVFWs. The percent fractional shortening (% FS) was calculated by the standard formula: $LVIDd - LVIDs$ divided by LVIDd. The LA:AO ratio was calculated from M-mode measurements.

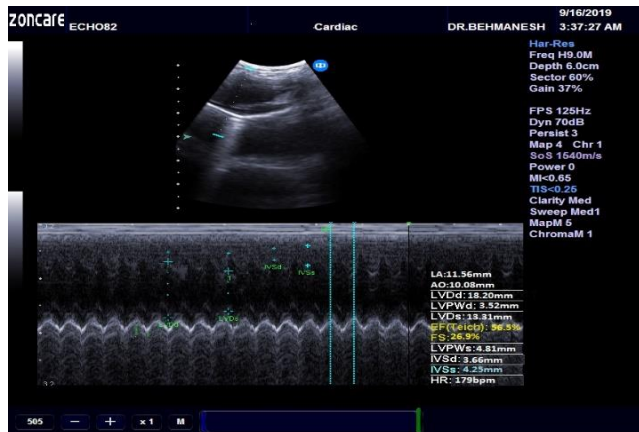


Figure 1. Echocardiographic long-axis section of the left ventricle at the four chamber level at both end-diastolic and end-systolic phases of the cardiac cycle.

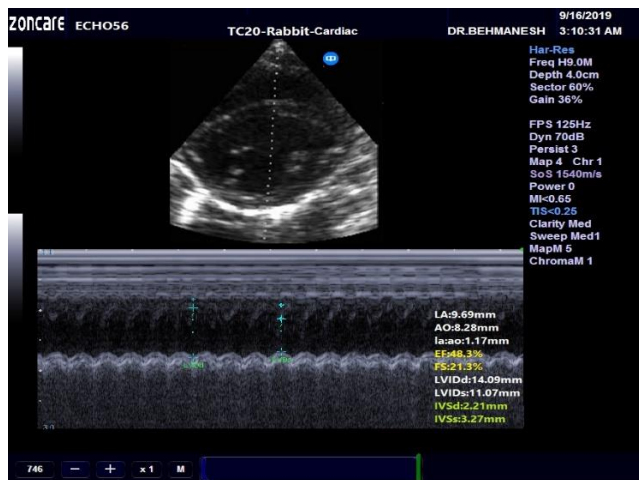


Figure 2. Echocardiographic short-axis section of the left ventricle at the papillary muscle level at both end-diastolic and end-systolic phases of the cardiac cycle.

Statistical Analysis

The data were stored in MS Excel and analyzed with the statistical software. Means and proportions of the various characteristics (age, weight, echocardiographic measurements) were calculated. Normality of interval measured data within male and female groups was assessed using a Komolgorov-Smirnov test. Means and proportions were compared between male and female

rabbits using the appropriate two-sample tests (unpaired t-test, Mann-Whitney). To describe the relation between the various numeric (interval and score) parameters for age and body weight, a Spearman's rank correlation test was used. *p* values less than 0.05 were considered statistically significant.

Results

50 % (50) of rabbits were female and 50 % (50) of them were male. The mean age of all rabbits in the study was 9.08 months (8.9 ± 0.56 in females and 9.26 ± 0.557 in males). 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 g. The mean weight of males was significantly higher than that of females. Mean heart rate of the 50 female rabbits was 207.66 ± 19.579 beats/min; significantly higher than the mean heart rate of the male (201 ± 20.352 beats/min). All comparisons of echocardiographic dimensions are summarized 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. There was no significant difference in LA, AO, LVIDd, and LVFWd between male and female rabbits in this study ($p < 0.05$) (Table 2).

In this study, there was no significant difference in the percentage of EF of most weight groups (eight weight groups) except for the 2481-2640 and 2161-2320 weight groups, while the mean weight of male rabbits in this study was significantly higher in the age range of 6 to 12 months. Therefore, according to the results, the authors believe that the factor of weight, sex and age (6 to 12 months) in the case of health and lack of cardiovascular problems in domestic rabbits has no significant effect on the rate of jump fraction.

Echocardiographic parameters such as IVSs, IVSd, LVIDs, LVIDd, LVFWs, and LVFWd increased parallel to body weight (BW), and LVFW was higher ($p < 0.05$) in males than in females. It was observed that EF values decreased according to BW. It was also determined that the left atrial dimension (LAD) and aortic root dimension (AOD) increased in connection with the increase in BW and that of these parameters, LAD and AOD were higher ($p < 0.05$) in males than in females, and that of these parameters only the CA amplitude was statistically higher ($p < 0.05$) in females than in males. All echocardiographic parameters for eight weights were analyzed separately in this study. (Table 2).

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

	Sex	N	Mean	Std. Deviation (SD)	Std. Error Mean (SEM)	p-value
Body weight (g)	Male	50	2663.20	273.604	38.693	0.381
	Female	50	2517.20	315.802	44.661	
Heart rate	Male	50	201.00	20.352	2.878	0.007
	Female	50	207.66	19.579	2.769	
LA (mm)	Male	50	1.07754E1	1.654376	0.233964	0.021
	Female	50	1.00252E1	1.869933	0.264448	
AO (mm)	Male	50	8.7752	1.04592	0.14791	0.006
	Female	50	8.0970	1.25949	0.17812	
LA/AO	Male	50	1.2200	0.09943	0.01406	0.475
	Female	50	1.2330	0.10835	0.01532	
LVIDd (mm)	Male	50	15.9890	2.81264	0.39777	0.103
	Female	50	15.0966	3.08259	0.43594	
LVIDs (mm)	Male	50	11.4090	2.08494	0.29485	0.06
	Female	50	10.5322	2.34959	0.33228	
LVFWd (mm)	Male	50	2.4956	0.52592	0.07438	0.004
	Female	50	2.2144	0.43642	0.06172	
LVFWs(mm)	Male	50	3.8328	0.77389	0.10944	0.0008
	Female	50	3.3910	0.76538	0.10824	
IVSd (mm)	Male	50	2.7078	0.60289	0.08526	0.007
	Female	50	2.3914	0.64146	0.09072	
IVSs (mm)	Male	50	3.6224	0.63876	0.09033	0.003
	Female	50	3.2240	0.68665	0.09711	
FS (%)	Male	50	28.5774	4.76851	0.67437	0.041
	Female	50	30.6320	4.70638	0.66558	
EF (%)	Male	50	58.7536	6.88923	0.97428	0.065
	Female	50	61.3976	6.53087	0.92360	

LA: left atrium, AO: aortic diameter, LVIDd: left ventricular internal diameter end diastole, LVIDs: left ventricular internal diameter end systole, LVFWd: left ventricular posterior wall thickness end diastole, LVFWs: left ventricular posterior wall thickness end systole, IVSd: intraventricular septal thickness end diastole, IVSs: intraventricular septal thickness end systole, FS: fractional shortening, EF: ejection fraction.

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

Body weight (g)	Heart rate (beats/min)	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
	SD	18.374	1.122452	0.93824	0.03234	1.78268	1.34918
	SEM	5.810	0.354951	0.29670	0.01023	0.56373	0.42665
	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
	SD	14.655	1.647387	0.96183	0.08864	2.60627	1.58414
	SEM	4.065	0.456903	0.26676	0.02458	0.72285	0.43936

	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
	SD	19.527	1.327481	0.94853	0.09911	2.87288	2.19636
	SEM	5.416	0.368177	0.26307	0.02749	0.79679	0.60916
	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
N		18	18	18	18	18	18
SD		19.952	1.328313	0.87405	0.11386	2.63979	1.92911
SEM		4.703	0.313086	0.20602	0.02684	0.62220	0.45470
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
	SD	12.801	1.296381	0.95910	0.06274	2.28652	1.69133
	SEM	2.464	0.249489	0.18458	0.01207	0.44004	0.32550
	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
	SD	19.661	0.940167	0.37724	0.08749	0.83297	0.96411
	SEM	6.951	0.332399	0.13337	0.03093	0.29450	0.34087
	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
	SD	22.729	0.359722	0.93111	0.15636	1.98705	1.80257
	SEM	8.591	0.135962	0.35192	0.05910	0.75103	0.68131
	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
	SD	6.898	0.399614	0.08756	0.05416	1.51454	1.61550
	SEM	3.449	0.199807	0.04378	0.02708	0.75727	0.80775
	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
	SD	20.148	1.796509	1.20114	0.10366	2.96982	2.25346
	SEM	2.015	0.179651	0.12011	0.01037	0.29698	0.22535
	Minimum	167	6.630	5.90	1.07	9.20	6.35
	Maximum	254	14.210	10.22	1.52	19.86	14.92

Table 2. Continue.

Body weight (gr)		LVFWd (mm)	LVFWS (mm)	IVSd (mm)	IVSs (mm)	FS (%)	EF (%)
2000 - 2160	Mean	1.8650	2.8730	1.9760	2.7830	30.2800	61.2600
	N	10	10	10	10	10	10
	SD	0.37925	0.57643	0.63554	0.72630	3.64655	5.10124
	SEM	0.11993	0.18228	0.20098	0.22968	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
	SD	0.17086	0.36041	0.40449	0.39348	4.63953	6.41468
	SEM	0.04739	0.09996	0.11218	0.10913	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
	SD	0.39958	0.64817	0.56256	0.56700	5.89527	8.38157
	SEM	0.11082	0.17977	0.15603	0.15726	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
	SD	0.37773	0.65284	0.53231	0.54331	4.98926	7.14840
	SEM	0.08903	0.15387	0.12547	0.12806	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
	SD	0.36014	0.47287	0.41980	0.49357	4.19863	5.94091
	SEM	0.06931	0.09100	0.08079	0.09499	0.80803	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
	SD	0.42795	0.85745	0.27339	0.28379	4.27215	5.00278
	SEM	0.15130	0.30316	0.09666	0.10033	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
	SD	0.40836	0.59905	0.11715	0.33076	3.34991	4.52685
	SEM	0.15435	0.22642	0.04428	0.12501	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
	SD	.45800	.30576	.29240	.12728	3.39939	4.60751
	SEM	0.22900	0.15288	0.14620	0.06364	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
	SD	0.50114	0.79729	0.63940	0.68949	4.82531	6.80934
	SEM	0.05011	0.07973	0.06394	0.06895	0.48253	0.68093
	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

LA: left atrium, AO: aortic diameter, LVIDd: left ventricular internal diameter end diastole, LVIDs: left ventricular internal diameter end systole, LVFWd: left ventricular posterior wall thickness end diastole, LVFWs: left ventricular posterior wall thickness end systole, IVSd: intraventricular septal thickness end diastole, IVSs: intraventricular septal thickness end systole, FS: fractional shortening, EF: ejection fraction. SD: standard deviation, SEM: standard error of mean.

Discussion

Domestic rabbits (*Oryctolagus cuniculus*) are a good model for cardiovascular research because their size makes surgical manipulation of the heart more feasible 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 similar to those in human myocardium.¹⁴

Pet rabbits can develop cardiovascular disease, and radiography, electrocardiography, and echocardiography are useful noninvasive diagnostic procedures that can be used in evaluation of cardiac disease to provide highly specific assessment of cardiac size, dysrhythmias, and internal structure and function (dynamics), respectively.³ Measurements of LVID in diastole and systole, IVS in diastole and systole, thickness of the LVFW in diastole and systole, and the left atrial appendage and aortic diameters are widely used for evaluation of cardiac morphology. The echocardiographic values calculated for FS and EF are used to assess systolic and diastolic function.

The mean \pm SD heart rate (HR) of the rabbits in our study was 204.33 ± 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 The HR during examination was lower than in two echocardiographic studies, one performed with awake research New Zealand rabbits (238 beats/min),¹⁶ and one with sedated/anaesthetised research rabbits (258 beats/min),¹⁷ and higher than in other studies with research rabbits under sedation or anesthesia (155 to 198 beats/min).^{18,19}

Echocardiographic M-mode measurements reported for dogs vary proportionally with body size (weight), which varies by breed.²⁰ The M-mode measurements reported for cats,²¹ ponies and horses,²² ferrets,²³ and chinchillas,²⁴ however, do not vary with body size, presumably because different breeds of these species are similar in size. Although the body weight of rabbits in the present study varied from 2000 to 3250 g, significant associations between body weight and echocardiographic measurements were detected. Because there were significant changes in echocardiographic values within this range of body weights, the authors speculate that cardiac measurements do not increase with increased weight after rabbits reach maturity. In the present study, there was no significant difference in the percentage of jump fraction of most weight groups (eight weight groups) except for the 2481-2640 and 2161-2320 weight groups, while the mean weight of male rabbits in this study was significantly higher in the age range of 6 to 12 months. It has been made of females. Therefore, according to the results, the authors believe that, the factor of weight, sex and age in the domestic rabbits (healthy and with any cardiovascular problems) has no significant effect on the rate of EF. echocardiographic values in this study can represent reference values for use in echocardiographic examination of all domestic rabbits. $61.29 \pm 4.66\%$, mean \pm SD for EF reported in 2006 by Ana Patrícia N. Fontes-Sousa in 52 healthy male rabbits.²⁵ Comparing the results of this research does not show a significant difference with our research.

In conclusion, The mean values of the ejection fraction obtained in this study, which is an ideal indicator of ventricular contractility and the amount of blood removed after the diastolic volume stage of the heart, and other parameters measured for the first time in domestic rabbits can be used as a reference for echocardiographic tests of domestic rabbits in future clinical echocardiographic research and examinations. Most of the results were comparable to those found in non-anaesthetised rabbits, and may offer a good alternative when this data is necessary.

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Conflict of Interest

None.

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