# The morphological investigations on the heart and some vessels of bovine foetus between the 15<sup>th</sup> and 25<sup>th</sup> weeks of gestation

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**Background:** The aim of this study was to define the morphological and morphometric development of the foetus heart obtained from the domestic cattle in the gestation period of 15–25 weeks.

Materials and methods: For this purpose, a total of 30 hearts belonging to cattle foetuses (15 males, 15 females) were used. The ages of foetuses were calculated according to the forehead-to-tail length and examined in three different groups. After dissection; biometric, macroanatomic, morphometric and histological findings were obtained from the foetal hearts according to the groups. In addition, mean values of the morphometric findings were determined.

**Results:** As a result of the study, it was found that with the advancing age the convexity of margo ventricularis dexter increased and margo ventricularis sinister transformed from a convex-concave shape to a flat shape. The heart-to-body weight ratio was determined as 0.08% for Group II female foetuses and 0.09% for all other groups. The heart heights for Groups I, II, and III females were identified as 26.21, 41.00, and 46.27 mm, respectively, and for the males 26.45, 34.89, and 47.15 mm, respectively. In the statistical analysis, it was determined that all the morphometric values measured from the heart correlated significantly with the forehead-to-tail length.

**Conclusions:** The data obtained as a result of the study is thought to help understand the morphological and morphometrical development of the heart, pioneer the attempts to create a foetal cattle heart model, and thus help in the diagnosis of the foetal heart pathologies. (Folia Morphol 2019; 78, 3: 524–534)

Key words: bovine foetus, heart, macroanatomy, morphology, morphometry

# **INTRODUCTION**

The embryonic/foetal deaths in cattle cause serious economic losses for the producers and consumers [36]. Foetal deaths are caused by the reasons such as foetal and placental anomalies, environmental impact, inadequate maternal support, and inappropriate maternal-foetal interaction [14, 41]. Given the high costs in livestock sector, the diagnosis of embryonic/foetal aliveness is very important [32]. Besides, the pregnancy and embryonic development processes of the domes-

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tic animals take an important place for the scientific research and economy as well [36]. Therefore, the cattle foetuses are often used in scientific researches [1, 8, 9, 12, 32].

The heart is one of the early developing organs in amniotes [42]. The embryo heart that transforms from a simple tube to a four-chambered structure providing pulmonary and systemic circulation is formed in two different processes as the growth and degeneration. The development of the heart occurs in three phases, namely, the formation of the heart tube, embodiment of the cardiac circulation, and the septation of the heart and the exit trunks [2, 29]. Morphological development of the heart in some animal species was previously described in prenatal and postnatal periods [4, 30, 34]. However, the morphological studies [26, 38, 39] of the heart in the prenatal period in domestic cattle have been limited only to determining the heart weight. Senos et al. [35] emphasized the need to know the normal structure of the heart in order to identify the heart diseases such as myocardial fibrosis, chronic myocarditis and ventricular dilation in mammals. In this study, in the light of the literature knowledge, we aimed to define the morphological and morphometric development and structure of the foetus heart obtained from the domestic cattle in the pregnancy period of 15–25 weeks.

# **MATERIALS AND METHODS**

#### Sample collection

In the study, a total of 30 Holstein breed's foetus hearts obtained from the domestic cattle (15 males, 15 females) in the pregnancy period of 15–25 weeks were used. The foetuses were provided from the healthy animals sent to the compulsory slaughter in the slaughterhouses located in Burdur and nearby cities. The permission needed for the study was obtained from Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (No: 183, Date: 30.03.2016).

# Determination of the groups

As in previous studies [3, 11, 12] of foetus, in this study the ages of the foetuses were calculated according to the following formula [25]: Y = 2.5(X + 21), where: Y — foetal age (day); X — the distance between the anterior end of os frontale and tuberositas ischiadicum (foetal crown-rump length [CRL]; taken with the flexible tape between the two eyes across the back to the base of the tail [cm]). The foetuses used in the study were examined in three groups in total with the equal number of samples for each group according to their ages calculated:

- Group I: CRL 21–30 cm;
- Group II: CRL 31–40 cm;
- Group III: CRL 41–50 cm.

#### **Biometric and morphometric examination**

After calculating the CRL value, foetal body weights were measured. The hearts were removed from the chest cavity and macroanatomical findings were obtained. After this, the hearts were washed with saline by a cannula inserted into aorta. In this way, the blood in the hearts was drained. Then, the vessels (aorta, tr. pulmonalis, vv. pulmonalis, v. cava caudalis, v. cava cranialis, v. azygos) were removed from their origin. This was followed by the measurement of heart weight (HW), pericardium weight (PW), and pulmonary weight (LW). Also the heart-tobody weight ratio (HW/BW) was calculated. A total of 20 morphometric measurements of the heart described below were performed with the help of digital calliper (0.00 BTS, UK).

The morphometric parameters determined on the hearts were as follows: CCD - craniocaudal diameter (at the level of basis cordis), DSD - dexter-sinister diameter (at the level of basis cordis), PLSC — peripheral length of sulcus coronaries, LSIS length of sulcus interventricularis subsinuosus, LSIP — length of sulcus interventricularis paraconalis, LMVD — length of margo ventricularis dexter, LMVS — length of margo ventricularis sinister, TVD - thickness of ventriculus dexter (at the centre level of the margo ventricularis dexter), TVS — thickness of ventriculus sinister (at the centre level of the margo ventricularis sinister), DA — diameter of aorta (at the origin level of arcus aorta), DTP — diameter of truncus pulmonalis (at the origin level of tr. pulmonalis), DDA — diameter of ductus arteriosus (at the centre level of ductus arteriosus), IHDA — internal height of ductus arteriosus (at the centre level of ductus arteriosus), IWDA — internal width of ductus arteriosus (at the centre level of ductus arteriosus), LDA — length of ductus arteriosus, LAD — length of auricula dextra (at the longest edge), HAD ---height of auricula dextra (at the highest level), LAS length of auricula sinistra (at the longest edge), HAS — height of auricula sinistra (at the highest level), DBCAC — distance from basis cordis to apex cordis (height of cordis).



Figure 1. The view of the bovine heart from facies auricularis and facies atrialis; A. Group I; B. Group I; C. Group II; AC — apex cordis; AD — atrium dextrum; AO — aorta; AS — atrium sinistrum; DA — ductus arteriosus; FT — fat tissue; IAC — incisura apices cordis; MVD — margo ventricularis dexter; MVS — margo ventricularis sinister; SIS — sulcus interventricularis subsinuosus; TB — truncus brachiocephalicus; TP — truncus pulmonalis; VD — ventriculus dexter; VS — ventriculus sinister.

## Histological examination

After being fixed in 10% formaldehyde solution for 24 h, tissue samples were put through the routine histological processes and blocked in paraffin. Modified Mallory's triple staining (triple) was applied to the 5  $\mu$ m sections taken from these blocks to show the general structure of the tissue. Slides were examined under the light microscope (Olympus BX51, Tokyo, JAPAN).

## Statistical analysis

The morphometric and biometric findings of the heart were statistically analysed, considering the gender difference, with the Independent Samples t-test in the Statistical Package for the Social Sciences (IBM, SPSS 20.0) package programme. The correlation value between the morphometric parameters and CRL was determined by Pearson's correlation test in the same package program.

# RESULTS

# Macroanatomical results

It was observed that in the 15–25-week-old cattle foetuses, the heart was located between the two lung lobes on the left side of the mediastinum medium ventrale in the third and fifth intercostal space. In Groups II and III, in the advanced ages with the increased convexity of the heart, the rear end of the margo ventricularis dexter was found to extend towards the front edge of the sixth costa. It was observed that apex cordis was connected to the diaphragm with ligamentum phrenicopericardiacum. In the Group I, the heart was found to be a flat cone. It was determined that the convexity of margo ventricularis sinister increased with the advancing age and in all groups proximal 2/3 was formed by ventriculus dexter and distal 1/3 by ventriculus sinister. It was determined that, in Groups I and II, margo caudalis was convexo-concave in shape from basal to apex, and in Group III, it was vertically (flat) shaped (Fig. 1). It was found that the apex of the heart was sharp in Groups I and II, rounded in the Group III and it was formed by ventriculus sinister in all groups. On the outer surface of the heart, four grooves were observed, namely, sulcus interventricularis paraconalis et subsinuosus, sulcus intermedius, and sulcus coronarius. It was determined that, in the hearts of Group III, a small amount of fat tissue began to form in the proximal of sulcus coronarius, sulcus interventricularis paraconalis, and at the origin of truncus (tr.) pulmonalis.

#### **Biometrical results**

Table 1 shows the mean and standard deviation values of the foetuses' age, body weight, CRL, pulmonary weight, heart weight, pericardium weight, and heart-to-body weight ratio and heart-to-lung weight ratio. Accordingly, the average ages of the male foetuses in Groups I, II and III were determined as 117.00 ± 6.22, 135.00 ± 5.90 and 162.91 ± 9.38 days, respectively, and those of the female foetuses as  $115.83 \pm 6.78$ ,  $144.37 \pm 3.14$  and  $161.50 \pm 6.92$ days, respectively. When the heart weight was examined, it was observed that the heart weights of the male foetuses in Groups I, II, and III were 6.70, 17.04, and 33.16 g, respectively, and those of the female foetuses as 7.08, 25.50 and 36.5 g, respectively. Thus, it was determined that the heart weights of the male foetuses in Group III were 1.94 times higher than in

Gender	Groups	Hearts number	Age [day]	Crown-rump length [cm]	Pulmonary weight (LW) [g]	Heart weight (HW) [g]	Pericardium weight (PW) [g]	Body weight (BW) [g]	HW/BW	HW/LW
Female	I	10	115.83 ± 6.78 <sup>a,b</sup>	25.33 ± 2.71 <sup>a,b</sup>	$26.75 \pm 6.02^{a,b}$	7.08 ± 1.80 <sup>a,b</sup>	0.48 ± 0.27 <sup>b</sup>	680.91 ± 190.81ª,b	$\begin{array}{c} 0.009 \ \pm \\ 0.0005 \end{array}$	0.26 ± 0.51
	II	10	144.37 ± 3.14	36.75 ± 1.25 <sup>*,₀</sup>	89.75 ± 19.80*	25.50 ± 10.51	1.87 ± 1.03	2469.87 ± 466.89*	$\begin{array}{c} 0.008 \pm \\ 0.0009 \end{array}$	0.27 ± 0.60
	III	10	161.50 ± 6.92	43.60 ± 2.77	125.40 ± 50.19	36.5 ± 16.29	2.70 ± 1.78	3678.70 ± 1327.00	0.009 ± 0.001	0.28 ± 0.36
	Mean		138.66 ± 21.24	34.46 ± 8.49	76.43 ± 52.75	21.80 ± 16.57	1.59 ± 1.46	2157.23 ± 1533.48	$\begin{array}{c} 0.009 \ \pm \\ 0.0009 \end{array}$	0.27 ± 0.04
Male	I	10	117.00 ± 6.22 <sup>d,e</sup>	25.80 ± 2.48 <sup>d,e</sup>	$\begin{array}{c} 25.20 \pm \\ 6.62^{\rm d,e} \end{array}$	6.70 ± 2.30 <sup>d,e</sup>	1.00 ± 0.86°	660.20 ± 184.63°	0.009 ± 0.001	0.26 ± 0.47
	II	10	135.00 ± 5.90 <sup>f</sup>	$33.00 \pm 2.36^{f}$	56.50 ± 15.77	17.04 ± 3.86 <sup>f</sup>	1.78 ± 0.63 <sup>ŕ</sup>	1586.78 ± 468 ± 41 <sup>f</sup>	$\begin{array}{c} 0.009 \pm \\ 0.0003 \end{array}$	0.30 ± 0.42
	III	10	162.91 ± 9.38	44.16 ± 3.75	101.50 ± 42.02	33.16 ± 6.75	3.16 ± 1.04	3296.33 ± 753.85	$\begin{array}{c} 0.009 \ \pm \\ 0.0005 \end{array}$	0.34 ± 0.08
	Mean		134.58 ± 17.92	32.83 ± 7.16	55.06 ± 33.95	16.83 ± 10.40	2.00 ± 1.38	1619.83 ± 1056.73	0.009 ± 0.0007	0.29 ± 0.05

Table 1. Mean and standard deviation values of biometric parameters in foetus groups

\*Refers to p < 0.05 in female and male foetuses, al and II in female foetuses; al and III in female foetuses; all and III in female foetuses; and II in male foetuses; and III in male foetuse; and III in male foe

Table 2. Mean and standard deviation values of morphometric parameters in bovine foetus heart

Gender	Groups	CCD	DSD	LSIP	LSIS	LMVD	LMVS	TVD	TVS	DA	DTP	DDA	PLSC
Female	Ι	21.44 ± 2.44 <sup>a,b</sup>	16.07 ± 1.36 <sup>a,b</sup>	19.78 ± 3.80ª,b	17.76 ± 2.26 <sup>a,b</sup>	25.58 ± 3.03 <sup>a,b</sup>	23.83 ± 2.80 <sup>a,b</sup>	3.76 ± 1.21 <sup>a,b</sup>	5.97 ± 1.87 <sup>b</sup>	7.63 ± 1.02 <sup>b</sup>	6.21 ± 0.92 <sup>a,b</sup>	4.43 ± 0.85 <sup>a,b</sup>	52.21 ± 4.41 <sup>a,b</sup>
	II	33.32 ± 3.99	23.86 ± 2.57	30.50 ± 5.06	28.89 ± 3.91*	40.16 ± 3.70*	37.43 ± 2.28*	5.68 ± 0.88°	7.09 ± 1.40°	10.92 ± 2.41	9.45 ± 1.15*	6.70 ± 1.32	87.05 ± 13.59
	III	38.70 ± 5.37	30.92 ± 6.62	34.83 ± 3.77	30.40 ± 2.02	45.33 ± 4.57	39.89 ± 4.08	7.86 ± 1.21	9.31 ± 0.74	12.01 ± 1.99	10.44 ± 1.82	7.42 ± 0.71	106.74 ± 21.26
	Mean	30.36 ± 8.67	23.10 ± 7.60	27.66 ± 7.87	24.94 ± 6.59	36.05 ± 9.73	32.09 ± 7.85	5.64 ± 2.09	7.23 ± 2.08	9.97 ± 2.61	8.48 ± 2.32	6.03 ± 1.64	79.68 ± 27.85
Male	Ι	21.74 ± 2.00 <sup>d,e</sup>	16.53 ± 0.84 <sup>d,e</sup>	20.47 ± 0.85 <sup>d,e</sup>	16.59 ± 2.41 <sup>d,e</sup>	26.19 ± 2.76 <sup>d,e</sup>	24.27 ± 2.18 <sup>d,e</sup>	3.84 ± 1.09 <sup>d,e</sup>	6.61 ± 1.74°	7.09 ± 0.67 <sup>d,e</sup>	5.53 ± 0.36 <sup>d,e</sup>	4.10 ± 0.63 <sup>d,e</sup>	56.74 ± 4.09 <sup>d,e</sup>
	II	29.24 ± 3.31	22.68 ± 1.91	27.43 ± 3.09	22.25 ± 1.43	33.84 ± 2.07	30.63 ± 1.91	5.59 ± 1.32 <sup>f</sup>	6.84 ± 1.55 <sup>f</sup>	9.54 ± 1.07	7.71 ± 1.06	5.21 ± 0.68 <sup>f</sup>	77.00 ± 4.57
	III	39.19 ± 6.53	32.84 ± 7.34	36.11 ± 6.58	31.38 ± 5.12	47.45 ± 9.55	42.54 ± 10.03	7.89 ± 0.36	9.41 ± 1.12	11.16 ± 1.38	10.83 ± 1.66	7.46 ± 0.51	106.46 ± 22.77
	Mean	28.73 ± 7.27	22.66 ± 6.71	26.85 ± 6.60	22.19 ± 5.96	34.01 ± 8.81	30.89 ± 7.88	5.47 ± 1.82	7.06 ± 1.96	9.04 ± 1.82	7.61 ± 2.16	5.29 ± 1.36	76.14 ± 20.48

\*Refers to p < 0.05 in female and male foetuses: \*I and II in female foetuses; \*I and III in female foetuses; \*I and III in female foetuses; \*I and III in male foetuses; \*I and \*II in male foetuse; \*I and \*II a

the Group II, 4.94 times higher than in Group I; and the heart weights of the female foetuses in Group III were 1.43 times higher than in Group II, 5.15 times higher than in Group I. In the study, it was observed that the heart-to-lump weight ratios of the male foetuses in Groups I, II, and III were 0.26, 0.30, and 0.34, respectively, and those of the female foetuses were 0.26, 0.27 and 0.28, respectively.

#### Morphometrical results

The mean and standard deviation values of the morphometric parameters measured from the heart are shown in the Tables 2 and 3. Accordingly, the mean values of CCD, DSD, TVD, TVS, and DBCAC in the male foetus were determined as 28.73, 22.66, 5.47, 7.06, and 34.53 mm, respectively and those in the female foetus as 30.36, 23.10, 5.64, and 36.84 mm, respectively.

Gender	Groups	LDA	LAD	HAD	LAS	HAS	DBCAC	IHDA	IWDA
Female	I	$4.72\pm0.90^{\text{a,b}}$	$15.11\pm2.38^{\scriptscriptstyle a,b}$	$7.83 \pm 2.01^{\text{a,b}}$	$16.13 \pm 1.68^{\text{a,b}}$	$11.20\pm1.32^{\text{b}}$	$26.21\pm2.99^{\text{a,b}}$	$2.35\pm0.35^{\text{a,b}}$	$1.59\pm0.26^{\text{b}}$
	II	7.01 ± 1.27	$23.42\pm3.48$	$10.82 \pm 1.26^{\circ}$	$23.90\pm4.29$	$17.40\pm4.16$	$41.00\pm4.33$	4.14 ± 1.28	$2.54\pm0.37$
	III	8.30 ± 1.13	$29.30\pm5.02$	$14.64 \pm 1.93$	$29.00\pm3.97$	$21.48 \pm 4.33$	$46.27\pm4.95$	$4.93\pm0.76^{*}$	$3.14\pm0.61$
	Mean	$6.52 \pm 1.89$	$22.06\pm7.19$	$10.90\pm3.44$	$22.49\pm6.51$	$16.58\pm5.54$	$36.84 \pm 9.96$	$3.69 \pm 1.39$	$2.46\pm0.70$
Male	I	$5.52\pm0.45^{\rm d,e}$	$16.60 \pm 1.88^{\rm d,e}$	$10.06\pm1.05^{\rm d,e}$	$16.46\pm2.02^{\rm d,e}$	$12.68\pm2.01^{\circ}$	$26.45 \pm 1.81^{\text{d,e}}$	$2.61\pm0.21^{ m de}$	$1.85\pm0.30^{\circ}$
	II	7.51 ± 1.17	$20.28 \pm 1.83$	$10.92\pm1.54$	$21.38\pm2.12$	$15.07\pm1.87$	$34.89\pm3.41$	$3.01\pm0.38$	$2.38\pm0.48$
	III	$7.78 \pm 1.94$	$26.00\pm5.20$	$13.56\pm1.65$	$29.50\pm5.28$	$18.16\pm2.08$	$47.15\pm9.05$	$3.50\pm0.35$	$3.00\pm0.005$
	Mean	$6.90 \pm 1.49$	$20.20 \pm 4.26$	11.16 ± 2.00	21.36 ± 5.46	14.89 ± 2.70	$34.53 \pm 8.66$	$2.97 \pm 0.44$	$2.24 \pm 0.63$

Table 3. Mean and standard deviation values of morphometric parameters in bovine foetus heart

\*Refers to p < 0.05 in female and male foetuses: <sup>a</sup>l and II in female foetuses; <sup>b</sup>l and III in female foetuses; <sup>c</sup>II and III in female foetuses; <sup>a</sup>l and II in male foetuses; <sup>a</sup>l and II in male foetuses; <sup>a</sup>l and III in male foetuses; <sup>a</sup>l and III in male foetuses; <sup>b</sup>l and III in male foetuses; <sup>a</sup>l and II in male foetuses; <sup>b</sup>l and III in male foetuses; <sup>a</sup>l and II in male foetuses; <sup>b</sup>l and III in male foetuses; <sup>a</sup>l and II in male foetuses; <sup>b</sup>l and III  in male foetuses; <sup>b</sup>l and II in male foetuse; <sup>b</sup>l and II in male foet

Table 4. Correlation values of morphometric parameters according to crown-rump length

Gender	Groups	LW	HW	PW	BW	HW/BW	CCD	DSD	PLSC	LSIP	LSIS	LMVD	LMVS	TVD
Female	I	0.725	0.935**	0.647	0.968**	-0.262	0.749	-0.249	0.799	0.518	0.884*	0.978**	0.954**	0.107
	II	0.030	0.403	-0.32	0.141	0.208	0.323	0.978*	0.562	0.924	0.416	0.381	0.316	-0.425
	III	0.930*	0.937*	0.815	0.948*	0.589	0.755	0.973**	0.900*	0.498	0.920*	0.761	0.834	0.223
Male	I	0.936*	0.879*	0.870	0.954*	0.477	0.949*	0.759	0.566	0.633	0.816	0.740	0.915*	0.000
	II	0.827*	0.949**	0.804*	0.805	0.373	0.945**	0.498	0.817*	0.743	0.728	0.804*	0.701	0.796*
	III	0.898	1.000*	0.277	0.986	-0.885	0.992	0.996	0.983	0.993	0.989	0.983	0.996	0.891
Mean		897**	0.904**	0.791**	0.939**	-0,87	0.957**	0.924**	0.939**	0.923**	0.945**	0.962**	0.954**	0.859**

\*p < 0.05; \*\*p < 0.01; abbreviations — see text

Table 5. Correlation values of morphometric parameters according to crown-rump length

Gender	Groups	LDA	LAD	HAD	LAS	HAS	DBCAC	IHDA	IWDA	DA	DTP	DDA	TVS
Female	I	0.553	0.291	0.264	0.813*	0.80	0.907*	0.507	0.550	-0.112	0.620	0.909*	-0.512
	II	0.649	0.898	0.611	0.290	0.999**	0.507	-0.718	0.053	0.883	0.855	0.217	0.254
	III	0.174	0.970**	0.481	0.731	0.846	0.829	-0.360	0.545	-0.032	0.665	0.564	-0.454
Male	I	0.956*	0.850	-0.376	0.896*	0.717	0.825	0.424	0.728	-0.421	-0.162	0.084	-0.150
	II	-0.163	0.436	0.674	0.970**	0.622	0.918	0.371	0.608	0.106	0.009	0.027	0.683
	III	0.944	0.691	0.951	0.861	0.203	0.981	0.944	-0.038	0.995	0.465	0.339	0.861
Mean		897**	0.753**	0.905**	0.808**	0.941**	0.842**	0.964**	0.719**	0.854**	0.778**	0.880**	0.870**

\*p < 0.05, \*\*p < 0.01; abbreviations — see text

## Statistical results

The statistically significant data obtained as a result of the comparisons between the groups belonging to the biometric and morphometric parameters are shown in the Tables 1–3 and the correlation data in the Tables 4 and 5, respectively. Accordingly, a statistically significant difference was not observed in all of the biometric and morphometric data of the male and female foetuses in Group I (p > 0.05). A statistically significant difference was observed in all of the parameters except the IHDA datum (p < 0.05). In the male and female foetuses in Group II, a statistically significant difference was observed in the parameters of CRL, LW, BW, LSIS, LMVD, and LMVS (p < 0.05). In the comparison in which the gender was accepted as the constant factor, it was found that all of the data, except the proportional parameters in Groups I and III, had a statistically significance (p < 0.05). According to the correlation data obtained in the study, it was determined that the morphometric measurements had a positive correlation to the CRL. The highest correlation value in the morphometric parameters was found to belong to DBCAC



Figure 2. Female cattle foetus, aorta (A), truncus pulmonalis (B), ductus arteriosus (C); L — lumen; I — intima; M — media; A — adventitia (triple).



Figure 3. Female cattle foetus, vena cava cranialis (A, B); L — lumen; I — intima; M — media; A — adventitia (triple).

(r = 0.964). In the other groups except Group II, a statistically significant (p < 0.05) correlation value was identified between the data of heart weight and CRL.

#### **Histological results**

Aorta, truncus pulmonalis, ductus arteriosus. In the cattle foetus of Groups I, II, and III; it was observed that the wall structure of aorta, truncus pulmonalis, and ductus arteriosus consisted of intima, media, and adventitia layers. It was established that the intima consisted of endothelial cells and subendothelium layer of the connective tissue. The media layer adjacent to the intima layer was observed to consist of smooth muscle cells. Nerve fibre bundles were found in the adventitia layer of the loose connective tissue (Fig. 2).

Vena cava cranialis. In the cattle foetus of Groups I, II, and III; it was seen that the intima layer of the vena cava cranialis consisted of single-layered flattened endothelial cells and subendothelium layer. It was observed that the media layer that is composed of smooth muscle cells was thinner than the adventitia layer of the loose connective tissue (Fig. 3).

Ventriculus sinister, ventriculus dexter, atrium sinistrum, atrium dextrum. In the cattle foetus of Groups I, II, and III; it was seen that the wall structure of ventriculus sinister, ventriculus dexter, atrium sinistrum, and atrium dextrum consisted of endocardium, myocardium and epicardium. The endocardium was observed to consist of the single-layered flattened endothelial cells and subendothelium layer. It was observed that, in the endocardium layer, the Purkinje fibres in the male foetuses were denser than the females. Myocardial layer consisted of cardiac muscle fibres. It was found that the subendocardial layer continued with the myocardial layer. It was also observed that the cardiac muscle cells displayed transverse banding and the intercalated discs existed in the connective regions and connected with the collateral ligaments (Figs. 4, 5).

# DISCUSSION

In this study, the morphological, morphometric, and histologic findings of the foetus hearts obtained from the domestic cattle in the gestation period of 15–25 weeks were examined according to the gender. The limitations of this study consisted of obtaining the foetus from healthy cattle, grouping the foetuses according to gender in order to evaluate them statistically, and the lack of detailed and sufficient literature to compare the findings. In fact, when these limitations are taken into consideration, the importance of the findings obtained in the study increases still more.

# Macroanatomical

Crick et al. [7] stated that the human heart was trapezoids in shape and the pig heart had the shape of a classic heart playing card. Anuradha [3] de-



Figure 4. Female cattle foetus, ventriculus sinister (A), ventriculus dexter (B), atrium sinistrum (C), atrium dextrum (D); E — endocardium; L — lumen; M — myocardium; arrow — Purkinje fibres (triple).



Figure 5. Male cattle foetus, ventriculus sinister (A), ventriculus dexter (B), atrium sinistrum (C), atrium dextrum (D); E — endocardium; L — lumen; M — myocardium, arrow — Purkinje fibres (triple).

scribed the foetus hearts of the buffalo as a cone with a broad basis cordis and narrow apex cordis. Although Getty [10] stated that the size and shape of the heart changed according to the degree of systole or diastole, in our study it was observed that the heart shape in the cattle foetuses resembled the literature data [3]. In

, 3					
Breed/sex*	Age*	HW/BW*	Foetus	HW/BW	
Cow	8	0.65%	Group I (male)	0.9%	
Cow	7	0.59%	Group II (male)	0.9%	
Cow	6	0.48%	Group III (male)	0.9%	
Bull	5	0.42%	Group I (female)	0.9%	
Cow	4	0.50%	Group II (female)	0.8%	
Cow	3	0.49%	Group III (female)	0.9%	
Calf	2/12	0.45%			
Calf	1/12	0.87%			

 Table 6. The ratio of heart weight (HW) to body weight (BW)

 by age in cattle

\*Nickel et al. [24]

addition to this, it was determined that, in the cattle foetuses in Groups I and II, margo ventricularis dexter et sinister had more curved boundaries than Group III.

## Biometrical

Lee et al. [19] stated that in all species except dogs, the heart-to-body weight ratio was higher in the newborns than in the adults. Kotch [18], Chakravarthy and Sastry [6] reported that this ratio was 0.5% in goats. Sathyamoorthy [34] reported that in the piglets the ratio was 0.87% at the age of 1 day, 0.50% at the age of 3 months, and 0.26% at the age of 4 years.

Gupta et al. [11] identified the heart-to-body weight ratio in the goats in the early, middle and late prenatal period as 1.53%, 1.04% and 0.80%, respectively. Ocal and Cakir [26] stated that the heart-to-body weight ratio in the adult and foetal cattle was 0.42% and 0.80%, respectively. For the purpose of an overall evaluation in the cattle, the heart-to-body weight ratio by age was taken from Nickel et al. [24] and shown in Table 6 together with findings of our study. According to this, as compatible with the literature data [19, 34], it is seen that the heart-to-body weight ratio of the cattle foetus was higher than that of the adult cattle.

## Morphometrical

Panhwar et al. [28] found that, in the young male and female bison, the length of margo ventricularis dexter was 13.9 cm and 13.3 cm, respectively, and the length of margo ventricularis sinister was 12.1 cm and 11.8 cm, respectively. Gürbüz [13] reported that, in the Tuj and Hemşin sheep, the length of margo ventricularis dexter was 7.6 cm and 8.5 cm, respectively, and the length of margo ventricularis sinister was 7.0 cm and 7.4 cm, respectively. Nickel et al. [24] reported that, in the cattle, the length of the margo ventricularis dexter was greater than that of margo ventricularis sinister. Also in the study conducted, consistent with the findings of the literature [13, 24, 28], it was also found that, in all groups, the length of the margo ventricularis dexter was greater than that of margo ventricularis sinister (Table 2).

In the literature [21, 22], the average length of the sulcus coronarius in the buffalos and goats was reported as 38.75 cm and 13.83 cm, respectively. Panhwar et al. [28] reported that the length value of this groove was 26 cm and 27.6 cm in the young female and male buffalos, respectively, and 33.5 cm and 31.1 cm in the adult females and males, respectively. Mohan and Prakash [23] and Braun et al. [5] reported the length of the coronary groove in the cattle as 38.75 cm and 37 cm, respectively. Sathyamoorthy [34] reported the length values of this groove as 6.5, 25, and 31.68 cm in the pigs at the age of 1 day, 6 months, and 4 years, respectively. In our study, the average lengths of the sulcus coronarius in female and male cattle were detected as 79.68 mm and 76.14 mm, respectively (PLSC value, Table 2).

Gupta et al. [12] reported the average heart height of the goat foetuses in the early, middle, and late stages as 6.35, 14.87, and 28.34 mm, respectively. In our study, the heart height value of the male and female cattle foetuses in Groups I, II, and III were found to be as 26.45, 34.89, 47.15 and 26.21, 41.00, 46.27 mm, respectively. The cardiac morphometric values of the goat foetus and the findings of our study are given in Table 7. According to these results, considering that the pregnancy periods of the goats and cattle are 150 days and 284 days, respectively, it can be concluded that the increase in the height of the heart with age is greater in the cattle foetuses.

Malik et al. [22] reported that the average thickness of the right and left ventricular wall in the buffalo heart was 1.5 cm and 2.18 cm, respectively; whereas Malik et al. [21] reported that the average thickness of the right and left ventricular wall in the goats was 0.75 cm and 1.40 cm, respectively. Sathyamoorthy [34] reported that the thickness of the right and left ventricular wall in the pigs at the age of 1 day and 6 months were 0.25, 0.50 cm and 0.72, 1.52 cm, respectively. In our study, the wall thickness values of the right and left ventricle in female and male cattle foetuses were found as 5.64, 5.47 mm, and 7.23, 7.06 mm, respectively. The morphometric values and correlation data of the foetus heart with the CRL values between 20 and 40 cm in the literature [3] and in our study are shown comparatively in

Parameters	Goat*				Bovine (male	e)	Bovine (female)			
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III	
DBCAC	6.35	14.87	28.34	26.45	34.89	47.15	26.21	41.00	46.27	
LMVD	5.14	15.22	28.78	26.19	33.84	47.45	25.58	40.16	45.33	
LMVS	4.41	10.98	20.88	24.27	30.63	42.54	23.83	37.43	39.89	
LAD	2.19	4.36	8.80	16.60	20.28	26.00	15.11	23.42	29.30	
LAS	3.21	6.56	11.40	16.46	21.38	29.50	16.13	23.90	29.00	

 Table 7. The morphometric values of the heart in goat foetus and bovine foetus (early, middle and late pregnancy periods of Groups I, II vs. III in goat foetus, respectively. Values are in mm)

\*Gupta et al. [12]; abbreviations — see text

Table 8. The biometric and morphometric values of buffalo [3] and bovine foetus hearts with 20-40 cm crown-rump length

Parameters	Bu	ffalo	Bovine foetus					
			Fe	male	Male			
	Values	Correlation	Values	Correlation	Values	Correlation		
Age [day]	135.66	-	138.66	1.000**	134.58	_		
Heart weight [g]	11.17	0.915*	14.45	0.847**	12.75	0.972**		
LMVD [mm]	36.67	0.880*	31.41	0.967**	30.65	0.938**		
LMVS [mm]	23.83	0.934**	28.19	0.973**	27.98	0.948**		
LMVD/LMVS	1.53	0.427	1.10	0.711*	1.09	0.318		
TVS [mm]	7.50	0.967**	6.20	0.309	6.48	0.380		
TVD [mm]	5.51	0.558	4.53	0.653*	4.86	0.725**		
PLSC [mm]	75.33	0.688	66.15	0.918**	68.56	0.929**		

\*p < 0.05; \*\*p< 0.05; abbreviations — see text

Table 8. According to this, it was determined that the LMVD/LMVS ratio in the heart of buffalo foetus was higher than in the heart of the cattle foetus.

#### Statistical

Gupta et al. [12] emphasized that in the findings related to the heart morphometry of the goat foetuses, the heart weight in the early and mid-prenatal period was statistically significantly different from the late prenatal period. In the same study, it was stated that the difference between the groups in the parameter of the heart-to-body weight ratio was statistically significant. In our study, it was determined that, contrary to literature data, the difference in the heart weight between the groups in the male cattle foetuses and in the female cattle foetuses (except the comparison of Groups II and III in the females) was statistically significant. In addition, our study was also distinct from the available literature reports in that there was no statistically significant difference between the groups in the heart-to-body weight ratio [12].

In our study, the heart-to-lung weight ratio in Groups I, II, and III was determined as 0.26, 0.30, and 0.34 in the male foetuses, respectively, and as 0.26, 0.27, and 0.28 in the female foetuses, respectively (Table 1). According to this, as the age of the foetus increases, the examination showed no visible difference in the parameter of the heart-to-body weight ratio depending on the age of the foetus, but the increase in the parameter of the heart-to-lung weight ratio was observed. However, this difference was not statistically significant (p > 0.05).

Sutton et al. [37] observed that the right and left ventricular wall thicknesses in the hearts of the human foetuses did not display a statistically significant difference, but it had a direct proportion with the body weight and CRL value in the pregnancy period of 8–40 weeks. In addition, in the study conducted as the gender factor was fixed in the analysis of intra-group samples, the wall thicknesses of the right and left ventricle did not display a statistically significant difference, similar to the literature [37]. Resch and Papp [31] stated that there is a linear correlation between the size of the heart and the weight, age, and body weight of the heart. In their study in which the morphometric values of heart weight, external dimensions, and ventricular wall thicknesses were identified in the horse foetuses at the age of 130–190 days, Machida et al. [20] reported that all cardiac measurements were highly correlated with the foetal age. Similarly, also in the 15–25-week-old cattle foetuses used in the study, it was determined that all cardiac measurements were statistically significantly correlated with the foetal age (p < 0.05 or p < 0.01).

Vincze et al. [40] measured the aortic diameter as 2.07  $\pm$  0.14 cm by transabdominal ultrasonography and they found that there was a statistically significantly negative correlation (r = -0.41, p = 0.012) between the foetal heart weight and the foetal aortic diameter in 44 cattle foetuses in the late period of pregnancy. In the study conducted, it was determined that there was a statistically significantly positive correlation (r = 0.801, p < 0.01) between the foetal heart weight and the foetal heart weight and the foetal heart weight and the foetal heart weight and the foetal heart weight and the foetal heart weight and the foetal heart weight and the foetal heart weight and the foetal aortic diameter in cattle foetuses.

# Histological

In the literature [27, 33], it was emphasized that in the wall structure of the aorta, truncus pulmonalis, ductus arteriosus and vena cava cranialis, the intima was composed of the single layer endothelial cells and the subendothelial layer of the connective tissue; furthermore, there was an adventitia layer that was composed of the media layer of smooth muscle cells, collagen and elastic fibres, and the fibroblasts. In our study, it was also observed that the wall structure of the aorta, truncus pulmonalis, ductus arteriosus, and vena cava cranialis in the female and male cattle foetuses in Groups I, II, and III was composed of the intima, media and adventitia layers. It was also observed that the media layer of smooth muscle cells of the vena cava cranialis was thinner than the adventitia layer of loose connective tissue.

The heart wall, an organ of the cardiovascular system and pumping blood with rhythmic contractions, consists of three main layers from the inside out, namely, endocardium, myocardium, and pericardium [15–17]. In our study, in the female and male cattle foetuses in Groups I, II, and III, the wall structure of the ventricular sinister, ventriculus dexter, atrium sinistrum, and atrium dextrum was observed to be composed of the endocardium, myocardium, and epicardium layers. As is in our study, it was found that the endothelial layer, which is the inner surface of the atrium and ventriculus and also participates in the formation of the heart valves, was composed of the single-layered flattened endothelial cells and the subendothelial layer, and the Purkinje fibres sporadically existed in this layer [15]. It was emphasized that the myocardium layer consists of the heart muscle fibres and the subendocardial layer continues with the myocardial layer [33]. Typical feature of the heart muscle is the existence of the intercalated discs that cross the chains of heart cells with irregular intervals, and are dark dyed and seen as transverse lines. The cells are butted to these structures which are a specialised connection complex [15, 17]. Also in our study, it was observed that, in the female and male cattle foetuses in Groups I, II, and III, the heart cells displayed transverse banding, the intercalated discs existed in the connective regions and connected with the collateral ligaments.

# CONCLUSIONS

It is thought that especially the morphometric data obtained as a result of the study will help describe the heart numerically in the foetus ages examined, compare this values with the values to be obtained in the future by different methods (morphometric, radiological, etc.), understand the development of the heart, and thus, diagnose the foetal heart pathologies. Furthermore, in this study, it is thought that the findings on the morphological structure of the foetus hearts and vessels obtained from the domestic cattle in the gestation period of 15–25 weeks will contribute both to the foetal heart studies on the cattle foetus and other animals and to the objectives of forming a developmental model of cattle foetus heart.

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