

M-MODE ECHOCARDIOGRAPHIC MEASUREMENTS IN PREGNANT AND NON-PREGNANT HOLSTEIN-FRIESIAN X SAHIWAL CROSSBRED DAIRY CATTLE

Jezie A. Acorda¹, Jesalyn L. Constante¹, Antonio A. Rayos²
and Arville Mar Gregorio A. Pajas¹

ABSTRACT

The study was conducted to determine the M-mode echocardiographic measurements in pregnant and non-pregnant dairy cattle and identify possible relationships between the echocardiographic measurements and pregnancy status. Ninety-one (91) 2-13 year-old Holstein-Friesian x Sahiwal crossbred dairy cattle, weighing 275-450 kg were utilized in the study. The animals were grouped by status of pregnancy into pregnant (63 animals) and non-pregnant (28 animals). Pregnant animals were further grouped into easy breeders (with 1-2 inseminations/ pregnancy) and repeat breeders (with more than 2 inseminations/ pregnancy). The heart was examined on the right thorax using an ultrasound machine equipped with a 3.5 MHz convex scanner. The cardiac structures were identified using B-mode ultrasonography and measurements were made using M-mode echocardiography. No significant differences were observed between pregnant and non-pregnant animals, except for higher interventricular septum at systole in pregnant compared to non-pregnant animals. No differences were observed between easy breeders and repeat breeders. The results suggest that status of pregnancy does not affect the echocardiographic measurements. The echocardiographic values obtained in the study can be useful in diagnosing cardiovascular disorders of pregnant and non-pregnant crossbred dairy cattle.

Key words: cattle, echocardiography, heart, M-mode, pregnancy, ultrasound

INTRODUCTION

Efficient milk production necessitates the selection of a dairy animal with a good history of lactation, proper nutrition and good management procedures, including prevention and treatment of diseases. In the Philippines, establishment of a Holstein-Friesian x Sahiwal crossbred dairy cattle has been done to obtain an animal with relatively high production of milk and high resistance to diseases. However, the milk production of this dairy animal continues to be inadequate due to the inability to select an animal with ideal reproduction performance and the occurrence of diseases and disorders, including diseases of the heart. Diseases of the cardiovascular system in dairy cattle are difficult to recognize by symptoms alone because these are mostly non-specific (Buczinski *et al.*, 2010a). In addition, ancillary tests, such as hematology and serum biochemistry lack the sensitivity or specificity to detect heart diseases (Buczinski, 2009).

Many cardiovascular disorders in dairy cattle have been previously diagnosed using

ultrasonography. These include valvular endocarditis (Yamaga and Too, 1987), fibrinous pericarditis (Boon, 2011), bacterial endocarditis, congenital heart disease, cardiomyopathy (Buczinski *et al.*, 2010a and 2010b), ventricular septal defect (Bonagura and Pipers, 1983; Buczinski *et al.*, 2006), tetralogy of Fallot, atrial septal defect, mitral valve regurgitation (Bonagura and Pipers, 1983) and traumatic pericarditis (Bakos and Vörös, 2011).

Determination of the normal features and normal ultrasonographic measurements of the heart are important for comparison with values obtained in diseased heart and comparison of cattle with different characteristics, such as status of pregnancy and lactation. Differences in echocardiographic measurements have been found between double-musled calves and in calves with standard conformation (Amory *et al.*, 1992) and between Jersey and Holstein-Friesian cows (Hallowell *et al.*, 2007). Although some ultrasonographic features of the heart had been determined in dairy cattle, there are no reported studies relating these features with the pregnancy status of the animal. The study aims to provide basic M-mode ultrasonographic measurements of the heart in pregnant (easy and repeat breeders) and non-pregnant dairy cattle. The echocardiographic measurements that were obtained, hopefully, will be useful in the diagnosis of cardiovascular diseases and disorders, and increasing reproduction performance of Holstein-Friesian x Sahiwal crossbred dairy cattle.

MATERIALS AND METHODS

Ninety-one (91) 2-13 year-old Holstein-Friesian x Sahiwal crossbred dairy cattle, weighing 275-450 kg from two dairy cattle farms in the province of Laguna, Philippines were utilized to determine the echocardiographic measurements. The animals were grouped by status of pregnancy into pregnant (63 animals) and non-pregnant (28 animals). Pregnant animals were further grouped into easy breeders (with 1-2 inseminations/pregnancy, 34 animals) and repeat breeders (with more than 2 inseminations/ pregnancy, 24 animals).

The dairy cow was restrained while in standing position in a squeeze chute and the right foot was pulled forward and tied. The cow was shaved on its right 2nd to 5th intercostal spaces, from the costochondral junction to the sternum. The animal was examined on the right thorax between the 3rd to 4th intercostal spaces using an ultrasound machine (WED-3100V, Shenzhen Well.D Medical Electronics Co., Ltd., Shenzhen, China) equipped with a 3.5 MHz convex scanner. Liberal amount of ultrasound gel was used to maximize skin-transducer contact. Ultrasound images of the heart were recorded using a video graphic printer (Sony UP-897 MD, Sony Corporation, Tokyo, Japan).

B-mode ultrasonography through a right short axis view was used to identify the structures of the heart (Figures 1 to 3) and M-mode echocardiography was utilized to measure the thickness of the walls and diameter of the different chambers of the heart. M-mode echocardiograms through the right ventricle, interventricular septum and left ventricle and through the right atrium, aorta and left atrium were obtained (Figures 1 to 3). From these ultrasonograms, measurements were made during diastole and systole of the right ventricular wall (RVWd and RVWs), right ventricular internal diameter (RVIDd and RVIDs), left ventricular wall (LVWd and LVWs), left ventricular internal diameter (LVIDd and LVIDs), interventricular septum (IVSd and IVSs), aorta (Ao), right atrium (RA) and left atrium (LA). Based on the measurements, fractional shortening (%FS), left ventricular diastolic volume (LVVd), left ventricular systolic volume (LVVs), stroke volume (SV) and ejection fraction (EF) were calculated according to the formula of Boon (2011).

Differences in the echocardiographic measurements between pregnant and non-pregnant animals and between easy and repeat breeders were analyzed using Student's t-test. The protocol used in the study has been approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, UP Los Baños.

¹Department of Veterinary Clinical Sciences, College of Veterinary Medicine, ²Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, Laguna, Philippines (email: jaacol32@gmail.com).

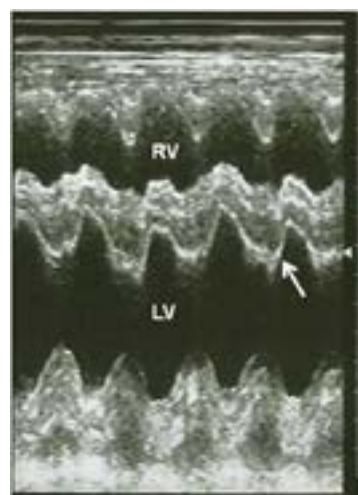


Figure 1. Right short axis M-mode echocardiogram of the right ventricle (RV), left ventricle (LV) and interventricular septum (arrow) in a pregnant easy breeder dairy cattle.

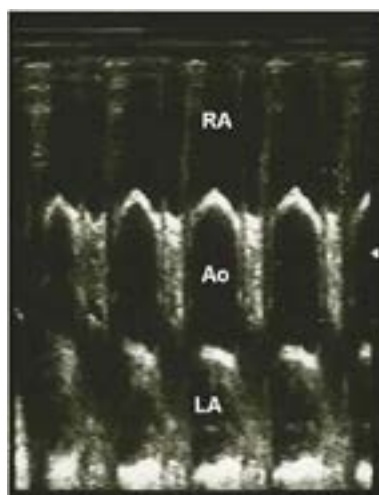


Figure 2. Right short axis M-mode echocardiogram of the right atrium (RA), aorta (Ao) and left atrium (LA) of a non-pregnant dairy cattle.

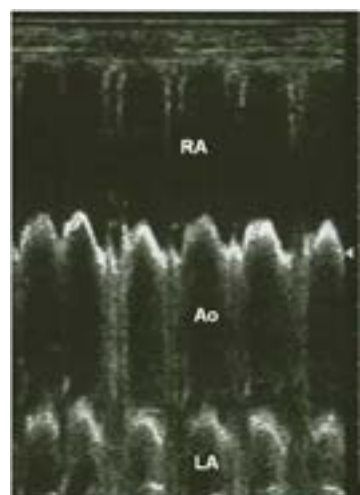


Figure 3. Right short axis M-mode echocardiogram of the right atrium (RA), aorta (Ao) and left atrium (LA) of a repeat breeder pregnant dairy cattle. The far wall of the LA could not be visualized in this sonogram.

RESULTS AND DISCUSSION

The dairy cattle were examined in a squeeze chute using just physical restraint without the use of any chemicals, with the right front leg restrained to allow examination of the right thorax. This is similar to the procedure recommended by Boon (2011). However, some animals refused to have their right leg tied, making examination through ultrasonography impossible, as noted by Boon (2011). Thus, these animals were excluded from the study.

In 20 out of 63 pregnant animals and 5 out of 28 non-pregnant animals, the left ventricular far wall was not observed (Table 1). In addition, in 36 out of 63 animals and 13 out of 28 animals, the left atrial far wall was not observed. For pregnant animals, in 14 out of 34 easy breeders and 5 out of 24 repeat breeders, the left ventricular far wall was not visualized (Table 2). In addition, in 19 out of 34 easy breeders and 13 out of 24 repeat breeders, the left atrial far wall was not visualized. Figure 3 shows an M-mode echocardiogram where the far wall of the left atrium is difficult to visualize. The failure to visualize the left atrial far wall and left ventricular far wall in some animals could be attributed to the depth of these structures which could not be penetrated by the frequency of the scanner used. For complete visualization of cardiac structures including the far wall of the heart in adult cattle, lower frequencies were recommended including 2.5-3.5 MHz (Buczinski, 2009), 3.0 MHz (Braun and Schweizer, 2001; Braun *et al.*, 2001) and 2.5 MHz (Boon, 2011). However, in non-pregnant normal buffaloes, El-Khodery *et al.* (2010) observed that a 3.5-MHz convex transducer proved to be sufficient for examination of all cardiac structures. In addition to the frequency of scanner used, the narrow intercostal space and the cranial position of the heart in the chest could also limit ultrasound visualization of cardiac structures (Buczinski, 2009).

Table 1. M-Mode echocardiographic measurements (Mean \pm SD) in pregnant and non-pregnant Holstein-Friesian x Sahiwal crossbred dairy cattle.

Parameter	Pregnant (N= 63)		Non-Pregnant (N= 28)	
	n	Mean \pm SD	n	Mean \pm SD
RVIDd	n= 63	2.63 \pm 0.58	n= 28	2.59 \pm 0.73
RVIDs	n= 63	1.38 \pm 0.40	n= 28	1.41 \pm 0.61
RVWd	n= 63	1.19 \pm 0.20	n= 28	1.12 \pm 0.15
RVWs	n= 63	1.82 \pm 0.30	n= 28	1.70 \pm 0.30
LVIDd	n= 56	6.12 \pm 0.65	n= 27	6.10 \pm 0.78
LVIDs	n= 60	2.83 \pm 0.57	n= 27	2.86 \pm 0.52
LVWd	n= 43	1.69 \pm 0.18	n= 23	1.69 \pm 0.16
LVWS	n= 54	2.43 \pm 0.27	n= 26	2.45 \pm 0.20
IVSd	n= 63	1.74 \pm 0.19	n= 28	1.73 \pm 0.23
IVSs	n= 63	2.61 \pm 0.26 ^a	n= 28	2.44 \pm 0.29 ^b
RA	n= 62	5.23 \pm 0.67	n= 28	5.13 \pm 0.72
Ao	n= 63	5.21 \pm 0.66	n= 28	5.12 \pm 0.69
LA	n= 27	4.59 \pm 0.60	n= 15	4.60 \pm 0.70
LA/Ao	n= 27	0.97 \pm 0.02	n= 15	0.96 \pm 0.02
% FS	n= 56	54.46 \pm 7.21	n= 27	53.05 \pm 7.09
IVSd/LVWd	n= 43	1.01 \pm 0.09	n= 23	1.00 \pm 0.08
LVVd	n= 56	191.15 \pm 46.14	n= 27	191.09 \pm 51.93
LVVs	n= 60	32.47 \pm 15.74	n= 27	32.66 \pm 14.03
SV	n= 56	159.57 \pm 38.79	n= 27	158.43 \pm 43.98
EF	n= 56	83.85 \pm 6.61	n= 27	82.71 \pm 6.26

RVIDd: right ventricular internal diameter at diastole, RVIDs: right ventricular internal diameter at systole, RVWd: right ventricular wall at diastole, RVWs: right ventricular wall at systole, LVIDd: left ventricular internal diameter at diastole, LVIDs: left ventricular internal diameter at systole, LVWd: left ventricular wall at diastole, LVWs: left ventricular wall at systole, IVSd: interventricular septum at diastole, IVSs: interventricular septum at systole, Ao: aorta, LA: left atrium, RA: right atrium, LA/Ao: left atrium to atrial ratio, FS: fractional shortening, IVSd/LVWd: interventricular septum at diastole to left ventricular wall at diastole ratio, LVVd: left ventricular diastolic volume, LVVs: left ventricular systolic volume, SV: stroke volume, EF: ejection fraction. Means with different superscripts among rows are different ($P < 0.05$).

Table 1 shows the echocardiographic measurements of pregnant and non-pregnant crossbred dairy cattle. In both groups, RVIDd and LVWd values obtained in the study were lower compared to those obtained by Pipers *et al.* (1978) but higher than those obtained in Jersey and Holstein-Friesian cows by Hallowell *et al.* (2007). LVIDd, LVIDs, Ao values obtained in the study were lower compared to those obtained by Pipers *et al.* (1978) and Hallowell *et al.* (2007) in Jersey and Holstein-Friesian cows. RVIDs and LVWs in the present study were higher but the IVSd and IVSs were lower than those obtained by Hallowell *et al.* (2007) in Jersey and Holstein-Friesian cows.

The LA diameter in both groups was lower than that obtained by Pipers *et al.* (1978). In all animals, %FS obtained in the study was higher than in the study of Pipers

Table 2. M-Mode echocardiographic parameters (Mean \pm SD) in pregnant Holstein-Friesian x Sahiwal crossbred dairy cattle (easy and repeat breeders).

Parameter	Easy Breeders (N= 34)		Repeat Breeders (N= 24)	
	n	Mean \pm SD	n	Mean \pm SD
RVIDd (cm)	n= 34	2.64 \pm 0.54	n= 24	2.65 \pm 0.66
RVIDs (cm)	n= 34	1.42 \pm 0.41	n= 24	1.34 \pm 0.41
RVWd (cm)	n= 34	1.22 \pm 0.21	n= 24	1.14 \pm 0.19
RVWs (cm)	n= 34	1.86 \pm 0.28	n= 24	1.73 \pm 0.28
LVIDd (cm)	n= 28	6.11 \pm 0.64	n= 23	6.05 \pm 0.65
LVIDs (cm)	n= 31	2.91 \pm 0.61	n= 24	2.75 \pm 0.53
LVWd (cm)	n= 20	1.73 \pm 0.19	n= 19	1.63 \pm 0.15
LVWS (cm)	n= 28	2.46 \pm 0.32	n= 21	2.40 \pm 0.21
IVSd (cm)	n= 34	1.77 \pm 0.15	n= 24	1.68 \pm 0.22
IVSs (cm)	n= 34	2.61 \pm 0.23	n= 24	2.58 \pm 0.29
RA (cm)	n= 34	5.30 \pm 0.69	n= 23	5.07 \pm 0.61
Ao (cm)	n= 34	5.28 \pm 0.67	n= 24	5.04 \pm 0.63
LA (cm)	n= 15	4.70 \pm 0.58	n= 11	4.45 \pm 0.64
LA/Ao	n= 15	0.97 \pm 0.03	n= 11	0.96 \pm 0.02
% FS	n= 28	53.43 \pm 8.51	n= 23	55.14 \pm 5.91
IVSd/LVWd	n= 20	1.03 \pm 0.08	n= 19	1.01 \pm 0.10
LVVd (cm ³)	n= 28	190.55 \pm 44.74	n= 23	186.01 \pm 46.27
LVVs (cm ³)	n= 31	34.67 \pm 17.38	n= 24	29.89 \pm 13.79
SV (cm ³)	n= 28	157.68 \pm 39.64	n= 23	157.67 \pm 36.81
EF	n= 28	82.75 \pm 7.90	n= 23	84.67 \pm 5.19

RVIDd: right ventricular internal diameter at diastole, RVIDs: right ventricular internal diameter at systole, RVWd: right ventricular wall at diastole, RVWs: right ventricular wall at systole, LVIDd: left ventricular internal diameter at diastole, LVIDs: left ventricular internal diameter at systole, LVWd: left ventricular wall at diastole, LVWs: left ventricular wall at systole, IVSd: interventricular septum at diastole, IVSs: interventricular septum at systole, Ao: aorta, LA: left atrium, RA: right atrium, LA/Ao: left atrium to aortic ratio, FS: fractional shortening, IVSd/LVWd: interventricular septum at diastole to left ventricular wall at diastole ratio, LVVd: left ventricular diastolic volume, LVVs: left ventricular systolic volume, SV: stroke volume, EF: ejection fraction.

Means with different superscripts among rows are different (P<0.05).

et al. (1978) and Hallowell *et al.* (2007) in Jersey and Holstein-Friesian cows. The EF value was lower than that obtained in Jersey but higher than in Holstein-Friesian cows (Hallowell *et al.*, 2007). The aortic diameter in both groups was larger than the left atrial diameter. However, the LA/Ao ratio was almost 1.0% (0.97%). In contrast, Pipers *et al.* (1978) observed significantly larger diameter of the aorta than the left atrium.

RVIDd and LVIDd values were higher than the corresponding RVIDs, LVIDs values in all animals. This could be attributed to the expansion of the chambers in diastole, compared to the contraction in systole. Braun and Schweizer (2001) and El-Khodery *et al.* (2010) observed larger diameter of the left and right ventricles during diastole than during systole in cattle and in non-pregnant buffaloes, respectively. The larger LVIDd compared

to LVIDs agrees with the finding of Hallowell *et al.* (2007). Boon (2011) also noted a great variability in measuring the RVID and its wall due to the varying right ventricular conformations between animals.

In both pregnant and non-pregnant groups, RVWd, LVWd and IVSd values were lower than the corresponding RVWs, LVWs, IVSs values. This could be due to the reduction of the thickness of the walls and septum as they expand during diastole. Braun and Schweizer (2001) in cattle, El-Khodery *et al.* (2010) in non-pregnant buffalo and Michima *et al.* (2007) in Holstein calves also observed that the interventricular septum and the left ventricular wall were thicker during systole than during diastole. In buffaloes, however, Acorda and Pilapil (2008) observed no significant differences in the thickness of the LVW and IVS in diastole and systole.

In all animals, the right ventricular dimensions (RVIDd, RVIDs, RVWd and RVWs) were lower than the corresponding left ventricular dimensions (LVIDd, LVIDs, LVWd and LVWs). The higher values could be attributed to the smaller size of the right ventricle compared to the left ventricle. Significantly smaller right ventricular diameters at diastole and systole than corresponding measurements in the left ventricle were also observed in cattle by Braun and Schweizer (2001) and in non-pregnant buffaloes by El-Khodery *et al.* (2010). Hallowell *et al.* (2007) also noted differences in left and right ventricular dimensions between Jersey and Holstein-Friesian cows.

No significant differences in the echocardiographic measurements were observed between pregnant and non-pregnant animals, except for higher IVSs in pregnant compared to non-pregnant animals. This implies that the status of pregnancy of the animal, whether pregnant or non-pregnant, has practically no effect on the measurements of the heart.

When easy breeders and repeat breeders were compared, no significant differences were observed in all the echocardiographic measurements (Table 2). This finding suggests that the number of inseminations for the animal to get pregnant had no effect on the different measurements of the heart of crossbred dairy cattle.

B-mode and M-mode can both be used to obtain echocardiographic measurements. However, M-mode was utilized in this study because of the difficulty in determining the maximum opening of the different chambers due to the movement of the heart. In addition, a high degree of correlation between B-mode and M-mode echocardiographic measurements has already been observed by Amory *et al.* (1991) and Zhang and Chen (1996).

The present study was able to determine the echocardiographic measurements in pregnant and non-pregnant Holstein-Friesian x Sahiwal crossbred dairy cattle, with no differences in the measurements between pregnant and non-pregnant animals. These can be used as references for diagnosing cardiovascular diseases and disorders and improvement of reproductive efficiency in Holstein-Friesian x Sahiwal crossbred dairy cattle.

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