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Effect of lactation on functional and morphological echocardiographic variables in adult dairy cows

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Abstract *Objectives:* To study the effect of pregnancy and lactation on echocardiographic parameters in Holstein dairy cows.
Animals: Nine multiparous high milk producing (HMP) dairy cows (producing more than 40 kg of milk per day in peak production) and 9 low milk producing (LMP) cows (producing less than 30 kg or milk per day in peak production).
Methods: Echocardiography was performed twice; one month before calving and two months after calving.
Results: The heart rate of HMP cows in the early lactation period was significantly higher than in the dry period. In LMP cows, there was a significant increase in left ventricular dimension in the early lactation period as compared to the dry period, and the interventricular septum in systole (IVSs) in the dry period was significantly thicker than early lactation period. In HMP cows, there was an increase in the right ventricular diameter in systole in the early lactation period as compared to the dry period. Left ventricular and aortic dimensions in the dry period of HMP were significantly higher than those of LMP cows. When the data were corrected for body weight, comparison of values of the dry period of HMP and LMP cows showed that left ventricular volume in systole in HMP was significantly higher and that IVSs, left

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ventricular fractional shortening and ejection fraction were significantly lower than in LMP cows.
Conclusions: This study demonstrated that lactation influences the intracardiac dimensions. The amount of milk production can influence echocardiographic parameters in dairy cows.

Abbreviations

Ao	aortic diameter in diastole
Ao-cs	aortic diameter, cross section
AoS	aortic sinus diameter in diastole
BCS	body condition score
CI	cardiac index
CO	cardiac output
ECG	electrocardiogram
EF	ejection fraction
HMP	high milk producing
HR	heart rate
IVSs	interventricular septum during systole
LFS	left ventricular fractional shortening
LMP	low mild producing
LVDd	left ventricular diameter in diastole
LVDs	left ventricular diameter in systole
LVVd	left ventricular volume in diastole
LVVs	left ventricular volume in systole
MV	mitral valve; SE, standard error
SV	stroke volume

Introduction

Lactation challenges the homeostatic mechanisms of ruminants, especially those controlling the cardiovascular system. Blood flow to the mammary gland is directly related to milk production.[1,2] In dairy goats, although cardiac dimensions are not influenced by milking and pregnancy status, cardiac output is increased during pregnancy and lactation when compared to the dry period.[3] Echocardiography is a non-invasive method for assessment of the heart and hemodynamic status in veterinary species. Echocardiography has been used in small animals and horses for the diagnosis and follow-up of many cardiac and non-cardiac disorders.[4–10] However, echocardiographic studies on cattle are limited to healthy calves,[11–13] healthy adults,[14–19] and cows with various cardiac diseases and congenital anomalies.[20–26] To date, no study has been performed concerning the impact of late pregnancy (the dry period) and early lactation on the echocardiographic parameters of Holstein cows. It is of particular interest for the clinician to know the impact of late pregnancy and peak milk production on the cardiovascular function of cows. This would potentially help in the interpretation of echocardiograms of cows suspected to have cardiac dysfunction. The purpose of this study was therefore to assess the effect of lactation and the dry period on the cardiac dimensions and function in adult dairy cows. We hypothesized that the echocardiographic variables of Holstein cows would be influenced by their production status (late pregnancy or peak milk production) and their milk yield.

Animals, materials and methods

Study animals

Eighteen clinically healthy adult Holstein cows from a 400 milking cow dairy farm were used during this study for echocardiographic measurements. The feed consisted of corn silage, dried legumes and grasses, and a concentrate mixture as a total mixed ration. The quantity of feed was adjusted on the basis of dairy cows' nutritional requirements as established by the National Research Council and was given three times daily in adequate amounts for the reproductive period, the lactation phase, and the amount of milk production. Cows had free access to water and salt blocks. The cows were above four years of age (greater than second parity); 9 cows were high milk producing (HMP) cows and 9 cows were low milk producing (LMP) cows. Milk production was obtained from information regarding the last 2 milk production cycles. In this study, cows with daily milk production $>$ 40 L during peak production (second month of lactation) were considered HMP cows (expected 305-day lactation $>$ 9,000 L[27]) and cows with daily milk production $<$ 30 L were considered LMP cows (expected 305-day lactation $<$ 7,000 L). Cows with peak production between 30 and 40 L were not included in the study to increase the chances to observe differences between groups. All cows received a full clinical examination and were deemed apparently healthy. This study was approved by the School of Veterinary Medicine, Shiraz University Ethics and Welfare Committee.

Study protocol

Cows were weighed and a body condition score (BCS) was calculated using a 5 point scale[28] prior to echocardiographic examination. Age, parity, and the month of pregnancy were obtained from the animal's individual records. The cows were restrained in boxes and allowed to acclimatize to this environment for 10 min. All cows received a full clinical examination, and heart rate (HR), respiratory rate, and body temperature were recorded. Cows were excluded from the study if a pathological cardiac murmur was detected on auscultation.[29] A base-apex electrocardiogram (ECG) lead[e] was acquired in each animal.

Echocardiography protocol

The echocardiographic examination was performed in each cow twice; at eight months of pregnancy and eight weeks after parturition (peak milk production). All echocardiographic examinations were performed in the standing non-sedated animal. The thoracic limb was moved cranially on the same side that echocardiographic examinations were performed to facilitate better contact between the probe and intercostal space. Transthoracic echocardiography was performed using a 2.5 MHz phased-array probe connected to a portable ultrasound unit[f]. The maximal depth was set at 27 cm. A simultaneous single lead ECG was recorded during the studies. For quantitative data analysis, end diastole was defined as the time point corresponding to the beginning of the QRS complex on the ECG and end-systole to the end of the T-wave. For all M-mode variables, the leading edge method was used for measurements. For 2-dimensional variables, the inner-edge method was used. At least three cardiac cycles were measured and the mean value for each parameter was obtained. All images were stored on a DVD disk and analyzed off-line.

Three, two dimensional parasternal views were obtained from the right side as described previously.[16,18,19] Additionally, three M-mode images were obtained from the right parasternal caudal short-axis view at the chordal level, mitral valve (MV) and aortic valve. In the right parasternal long-axis view of the left ventricle, images were obtained of the right ventricle, tricuspid valve, left ventricle, and MV. The right parasternal caudal long-axis view of the left ventricular outflow tract allowed measurement of the diastolic aortic diameter (Ao) and diastolic aortic sinus diameter (AoS). The right parasternal cranial long-axis view of the right ventricular outflow tract allowed measurement of the diastolic pulmonary artery diameter. The left parasternal caudal long-axis view allowed assessment of the end diastolic left atrial diameter and the mitral valve diameter. Echocardiographic recording was performed using intracardiac landmarks to orient the transducer position in order to obtain standardized images and correct measurements according to the previously published methods.[16–19] Right parasternal M-Mode echocardiography was used to determine measurements in diastole and systole of the interventricular septal thickness, left ventricular diameter (LVDd and LVDs), and left ventricular free wall thickness. Right parasternal short-axis view at the chordal level was used to determine right ventricular diameter in diastole and systole. An M-mode echocardiogram of the MV was obtained from the right parasternal caudal short-axis view for measurement of the distance between the maximal opening of the septal leaflet of the MV in early diastole (the E point) and the maximal excursion of the septum (EPSS), and the slope of the line from E to point of partial closure of the septal leaflet of the MV in mid diastole (the F point). The cross sectional diameter of the aorta (Ao-cs) was measured in diastole using an M-mode echocardiogram from the right parasternal caudal short-axis view of the aortic valve.

The calculated measurements included the fractional shortening the left ventricle (LFS) using the formula: $FS = 100 \times ((LVDd - LVDs)/LVDd)$, the ratio of the pulmonary artery diameter to the aortic diameter, and the left ventricular ejection fraction (EF) that was calculated as previously described.[11,18] The left ventricular volume in systole and diastole (LVVs and LVVd) were calculated in the basis of Teichholz formulas as follows: $LVVs = 7\,(LVDs^3)/(2.4 + LVDs)$ and $LVVd = 7\,(LVDd^3)/(2.4 + LVDd)$, respectively. The stroke volume (SV), cardiac output (CO) and cardiac index (CI) of each cow were calculated as $SV = (LVVd - LVVs)$, $CO = SV \times HR$ and $CI = CO/\text{body weight}$.

Statistical methods

Data are presented as the mean ± standard error (SE). Measurements between the dry period and early lactation period in HMP and LMP cows were compared using a paired *t*-test and measurements between HMP and LMP cows in the dry period and early lactation period were compared using an independent *t*-test. Then data, except CO and CI,

[e] ECG, Cardiostate 701, Siemens, Germany.
[f] Megas, Esaote Biosound, Florence, Italy.

were indexed for body weight; each individual measurement was divided by the individual cow's body weight and data between HMP and LMP cows in the dry period and early lactation period were again compared using an independent *t*-test. All the statistical analyses were performed using SPSS V.11.5 for Windows[g] and the differences were considered significant at $p < 0.05$.

Results

Nine HMP cows and nine LMP cows were used in this study. The mean values and standard errors for the BCS, body weight, HR, and respiratory rate and measured and calculated echocardiographic variables, during the dry period and early lactation period in HMP and LMP cows are summarized in Table 1.

The mean $\pm$ SE of milk production on the day of examination during the early lactation period was 25 $+/-$ 3 L in LMP cows and 43 $+/-$ 1 L in HMP cows and the mean $\pm$ SE of 305 day milk production was 5660 $+/-$ 406 L and 9, 820 $+/-$ 303 L in LMP and HMP cows respectively. Comparison of values of dry period HMP and LMP cows showed that LVVd and LVVs in HMP were significantly higher and that IVSs, LFS and EF were significantly lower ($p < 0.05$) in HMP than in LMP cows. Comparison of corrected values of the early lactation period of HMP and LMP cows revealed significantly smaller values ($p < 0.05$) of Ao, AoS, LFS, right sided fractional shortening, and EF in HMP than LMP cows (Table 1). Despite higher absolute values of Ao and AoS in HMP cows in the early lactation period, Ao and AoS were significantly higher in LMP cows when corrected for body weight ($p < 0.05$).

Discussion

This is the first study focusing on echocardiographic changes in lactating vs. dry cows in relation to production status. We found that both lactation and level of production had an impact on echocardiographic parameters in Holstein dairy cows.

The results of our study are in agreement with previous studies in normal adult cows, except for CO and CI.[17,18] In this study, the CO and CI were calculated from internal dimensions of the left ventricle using the Teicholtz formula. A different technique was used for the calculation of these parameters with pulsed wave Doppler in a previous study.[18] This difference explains why the CO and CI were different from those described by Hallowell et al.[18] To the authors' knowledge, there are no published studies concerning the agreement between echocardiographic techniques to assess CO and CI in cattle. It is interesting to note that despite the fact that no morphological changes have been reported in goats between lactation, late pregnancy and the dry period,[3] the present study found some differences in cardiac morphology in cows. In women there are moderate changes during late pregnancy. Typically, the left atrium increases in size by 10%—15% and the left ventricle by 5—10%. Dilation of the right atrium and the right ventricle is often more obvious.[30,31] In LMP cows, there was a significant increase in LVVd and LVDd in early lactation period when compared with the dry period. In LMP cows, IVSs in the dry period is significantly higher than in early lactation which may indicate a stronger systolic contraction in the dry period, left or right ventricular hypertrophy secondary to increased blood flow associated with the late pregnancy, or occult cardiomyopathy. In women, late pregnancy is associated with a global increase of the heart cavities when compared to early pregnancy.[32] In HMP cows, echocardiographic parameters showed increased right ventricular diameter during systole during early lactation when compared to the dry period. Echocardiographic parameters of LVDd, LVDs, Ao, AoS, LVVd, LVVs and SV in the dry period of HMP were higher than those of LMP cows, which may be due to higher body and cardiac size. In the early lactation period, LVDd, Ao-cs, LVVd, SV and CO in HMP cows were higher than those in LMP cows. These differences could also be due to bigger body weight and cardiac size in HMP cows when compared to LMP cows. In a study carried out in cattle, larger Ao, AoS, Ao-cs and LVDd have been demonstrated in Holstein cows when compared to the smaller Jersey breed.[18] The difference here could also be due to increased mammary blood flow demand and higher cardiac work in HMP cows. Mammary blood flow is correlated with milk yield in ruminants.[33]

After data correction for body weight, all significant differences except larger Ao and AoS in LMP cows in the early lactation period may be an indicator of higher ventricular afterload in HMP than LMP cows. In a study reported by Olsson et al., a lower blood pressure was observed in goats after using an angiotensin converting enzyme or ACE inhibitor during pregnancy and milk production when compared to the dry period. This reflects higher activity of the renin-angiotensin

[g] SPSS for windows 11.5, Chicago, IL, USA.

Table 1 Body condition scores, body weights, respiratory rates and echocardiographic measurements of high and low milk producing cows in dry and early lactation period.

Parameters	High milk producing cows				Low milk producing cows			
	Dry period		Early lactation period		Dry period		Early lactation period	
	N	Mean ± SE	*N*	Mean ± SE	*N*	Mean ± SE	*N*	Mean ± SE
BCS	9	3.5 ± 0.11[a]	9	2.53 ± 0.13[a]	9	3.47 ± 0.11[b]	9	2.61 ± 0.18[b]
BW (Kg)	9	696.67 ± 32.91[a,c]	9	653.11 ± 32.29[a,d]	9	577.78 ± 30.51[b,c]	9	512.78 ± 26.05[b,d]
RR (number/min)	9	28.33 ± 2.04[a]	9	37.44 ± 2.34[a]	9	30.22 ± 1.87[b]	9	39.11 ± 1.42[b]
HR (beat/min)	9	68.56 ± 2.43[a,c]	9	79.89 ± 1.65[a,d]	9	77.56 ± 2.30[c]	9	73.33 ± 1.67[d]
PA (cm)	7	5.48 ± 0.36	7	5.94 ± 0.24	8	5.08 ± 0.22	8	5.38 ± 0.24
Ao (cm)	8	6.59 ± 0.24[c]	8	5.92 ± 0.22[f]	9	5.39 ± 0.17[c]	9	5.49 ± 0.14[f]
PA:Ao	6	0.92 ± 0.04[e]	6	1.08 ± 0.05	8	0.97 ± 0.05[e]	8	0.99 ± 0.03
AoS (cm)	8	7.96 ± 0.22[c]	8	7.59 ± 0.33[f]	9	6.72 ± 0.20[c]	9	6.86 ± 0.18[f]
Ao − cs (cm)	9	6.12 ± 0.51	9	6.67 ± 0.43[d]	9	5.04 ± 0.20	9	5.37 ± 0.14[d]
LAD (cm)	3	9.65 ± 0.62	3	10.67 ± 0.36	5	10.04 ± 0.65	5	9.65 ± 0.47
MVD (cm)	4	8.24 ± 0.25	4	8.13 ± 0.07	6	7.54 ± 0.39	6	7.71 ± 0.31
EPSS (cm)	8	1.17 ± 0.15	8	1.00 ± 0.12	8	1.10 ± 0.20	8	0.89 ± 0.20
EF slope (cm/s)	8	17.37 ± 1.39	8	18.45 ± 1.70	8	20.20 ± 2.18	8	21.10 ± 2.27
RVDd (cm)	9	3.24 ± 0.39	9	3.78 ± 0.29	9	2.49 ± 0.21	9	3.26 ± 0.28
RVDs (cm)	9	1.70 ± 0.33[a]	9	2.14 ± 0.27[a]	9	1.23 ± 0.18	9	1.53 ± 0.22
LVDd (cm)	9	9.78 ± 0.27[c]	9	9.67 ± 0.22[d]	9	8.04 ± 0.31[b,c]	9	8.53 ± 0.35[b,d]
LVDs (cm)	9	5.14 ± 0.32[c]	9	4.98 ± 0.28	9	3.71 ± 0.20[c]	9	4.25 ± 0.21
IVSd (cm)	9	2.14 ± 0.13	9	2.13 ± 0.08	9	2.06 ± 0.12	9	1.93 ± 0.08
IVSs (cm)	8	3.58 ± 0.07[e]	8	3.48 ± 0.18	9	3.72 ± 0.16[b,e]	9	3.19 ± 0.15[b]
LVFWd (cm)	8	1.81 ± 0.17	8	1.74 ± 0.12	9	1.57 ± 0.08	9	1.45 ± 0.05
LVFWs (cm)	9	2.71 ± 0.23	9	2.42 ± 0.12	9	2.31 ± 0.18	9	2.18 ± 0.11
FS (%)	9	47.63 ± 2.52[e]	9	48.58 ± 2.47[f]	9	53.40 ± 2.90[e]	9	49.68 ± 2.66[f]
RFS (%)	9	50.63 ± 4.88	9	44.87 ± 3.94[f]	9	50.85 ± 5.95	9	54.04 ± 5.11[f]
EF (%)	9	76.14 ± 2.69[e]	9	77.16 ± 2.50[f]	9	82.04 ± 2.56[e]	9	78.55 ± 2.45[f]
LVVd (ml)	9	541.53 ± 31.77[c,e]	9	526.97 ± 26.04[d]	9	353.69 ± 28.43[b,c,e]	9	404.39 ± 34.01[b,d]
LVVs (ml)	9	131.83 ± 19.16[c,e]	9	121.18 ± 15.57	9	60.73 ± 8.26[c,e]	9	83.29 ± 9.09
SV (ml)	9	409.70 ± 24.04[c]	9	405.79 ± 22.87[d]	9	292.97 ± 27.24[c]	9	321.97 ± 31.59[d]
CO (L/min)	9	28.00 ± 1.77	9	32.36 ± 1.84[d]	9	22.77 ± 2.34	9	23.75 ± 2.48[d]
CI (ml/Kg/min)	9	40.43 ± 2.10[a]	9	50.51 ± 3.70[a]	9	38.99 ± 2.10	9	45.36 ± 3.42

N = number of measurements used in analysis.

Ao : aorta; Ao-cs : cross sectional diameter of the aorta; AoS : aortic sinus diameter; BCS : body condition score; CI : cardiac index; CO : cardiac output; EF : ejection fraction; EPPS : E-point to septal separation; HMP : high milk production; HR : heart rate; IVSs : interventricular septum; LAD : left atrial diameter; LVD : left ventricular internal diameter; LVFW : left ventricular free wall; LVOT : left ventricular outflow tract; LVV : left ventricular volume; LMP : low milk production; MV : mitral valve; MVD : mitral valve diameter; PA : pulmonary artery; RR : respiratory rate; RVD : right ventricular internal diameter; RVOT : right ventricular outflow tract; SV : stroke volume; d: diastole; s: systole.

[a] Shows significant differences between dry and early lactation periods in high milk producing cows.

[b] Shows significant differences between dry and early lactation periods in low milk producing cows.

[c] Shows significant differences between high and low milk producing cows in dry period.

[d] Shows significant differences between high and low milk producing cows in early lactation period.

[e] Shows significant differences between high and low milk producing cows in dry period when corrected for body weight.

[f] Shows significant differences between high and low milk producing cows in early lactation period when corrected for body weight; $p < 0.05$.

system during caprine pregnancy and the milk production period.[33]

One of the important reasons for performing this study is to know from a practical point, if echocardiographic examination should be interpreted differently when performed in dry cows or early lactating cows and depending on their production level. This study shows that even if several changes can be observed among different groups, these changes were small and the importance of these variations should be further studied.

This study demonstrates that echocardiography is a non-invasive practical technique for the study of heart dimensions and function during lactation and the dry period in adult dairy cattle. Cows in the present study have higher BCS than cows used in a previous study.[18] High BCS and fat could pose difficulties in echocardiographic imaging and visualization, and this may explain why the image quality was in general lower than previously described.

This study must be considered to have limitations. We performed echocardiographic examinations on a small sample. Further studies should include multiple echocardiographic examinations in every cow in order to document the important changes of the cardiovascular system in late pregnancy and early lactation, especially in high producing dairy cows. Conclusions cannot be made on the repeatability of these findings. It still remains unknown if, as in athletic horses,[34] the echocardiographic examination can be a discriminatory tool in predicting the milk potential of dairy cows.

Conclusion

This study shows that the level of production during lactation has some effects on echocardiographic parameters in dairy cows. The LVDd, LVVd and SV were increased in HMP cows during the early lactation period when compared to LMP cows, indicating a more efficient cardiac function in these animals. Larger studies are required to ascertain whether echocardiography can be used to predict HMP cows as well.

Conflict of interest

No conflicts of interest declared.

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