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Echocardiographic evaluation of mares at different gestational ages

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Abstract
Aim: The aim of this study was to monitor cardiac adaptations in mares from the fifth to the ninth month of gestation.
Materials and Methods: Echocardiographic parameters of 10 Quarter Horse mares were established, followed during different gestational months, all kept in a semi- extensive regime, with ages ranging from 3 to 17 years. Data were collected in five stages, all with resting animals. The mares were evaluated at 5 months of gestation, at 6 months, at 7 months, at 8 months and at the last 9 months of gestation, seeking to establish a comparative parameter of possible adaptations.
Results: The results passed through statistical analysis by the Dunett's Multiple Test and analysis of variance one way ANOVA, considering P<0, 05 as statistical significant. 12
Parameters (85, 72%) showed statistical significance (*P*< 0, 05) and 2 parameters (14, 28%) didn't showed statistical significance (P >0, 05).
Conclusion: It was possible to observe that in the evaluated mares, the echocardiography was efficient in determining the cardiac functional indices acquired at different gestational ages.

Keywords: Pregnancy, quarter mile, echocardiogram, horse

1. Introduction

Cardiovascular changes represent an important adaptation during the gestational period and are widely studied in humans, but not yet fully understood in some species, such as horses. It is known that throughout the gestational period the circulatory system undergoes changes, redirecting blood flow and oxygen availability in order to better supply the reproductive tract, which results in an adaptive physiological deviation of a determined duration, whose main purpose is preserve the systemic demands necessary for the proper growth and development of the fetus [1].

Among the changes that occur during the gestational period can be mentioned the increase in plasma volume with consequent decrease in red cell concentration, since the increase in blood plasma is greater than the increase in blood cells [1, 2]. Decreased peripheral vascular resistance and increased uterine blood flow are also noted, causing increased blood volume and myocardial hypertrophy [3, 4].

These changes during the gestational period lead to an increase of about 20% in cardiac output from the eighth week of gestation, and subsequently, the decrease in peripheral vascular resistance is verified. Immediately after delivery, there is a sudden increase in cardiac output due to clearance of the inferior vena cava, followed by a rapid decline in cardiac output about one hour after delivery [1].

The aim of this study was to follow, through echocardiographic evaluation, cardiac changes during gestation and puerperal period in Quarter Horse mares.

2. Material and Methods

2.1Animals

Ten Quarter Horse (QM) mares, aged 3 to 17 years, kept in semi-extensive regime were used. To minimize external effects, the animals were previously adapted to the handling related to the exam, thus ensuring the minimum stress.

2.2 Clinical Evaluation

Prior to echocardiographic evaluation, all animals underwent a complete clinical examination: mucosal evaluations, pulmonary cardiac auscultation, skin turgor evaluation, capillary filling time and weighing of the animals employed by electronic scales (Toledo MGR 3000 Junior).

2.3 Echocardiographic evaluation

All animals were evaluated at five different times. The first echocardiographic evaluation occurred at 5 months of gestation (M5), the second at 6 months (M6), the third at 7 months (M7), the fourth at eighth month (M8) and the last at 9 months of gestation (M9).

For echocardiographic evaluation, ultrasound gel was applied and the evaluation was started by approaching the right parasternal window using ultrasound with a convex broadband transducer with frequencies from 2 to 4 MHz (Sonosite Elite Plus). The longitudinal section images were obtained through the right parestemal window with visualization of the left atrium and ventricle and the left ventricular outflow tract with the aorta. Therefore, they were used to position the one-dimensional bundle (M-mode) at the height of the chordae tendinea, perpendicular to the interventricular septum and the left ventricular free wall. The M-mode image of the left ventricle obtained in this way was used to measure the thickness of the interventricular septum and the left ventricular free wall, as well as the internal dimensions of the left ventricle, in systole and diastole, following the recommendations of the American Society. Of echocardiography (Lang *et al*., 2015). The longitudinal section with the ventricular outflow tract was also used to measure the diameter and radius (r) of the aortic root. Thus, it was possible to measure the aortic sectional area (area = π.r2). The shortening fraction (EF %) was calculated according to the following formula: SF% = [(LVIDd - LVIDs) / LVIDd] x 100. Where: LVIDd = left ventricular internal diameter in diastole; LVIDs = left ventricular internal diameter in systole.

Apical section evaluation was performed with the left ventricular outflow tract and the aorta through the left caudal parestemal window. Subsequently, the pulsed Doppler sampling volume was positioned at the root of the aorta to obtain the velocity-time spectral graph of the aortic flow, which served to determine the velocity-time integral (TVI). Left ventricular systolic volume (mm3) was obtained by multiplying the aortic sectional area (mm2) by the IVT (mm). Finally, cardiac output (L / min) was measured by multiplying the systolic volume by the heart rate (HR) obtained by electrocardiography.

The data obtained were presented as mean and standard deviation. In order to compare the parameters obtained at different gestational ages, the Kolmogorov-Smirnov normality test was initially applied. Then the parameters for each age were evaluated by applying one-way analysis of variance ANOVA and Dunnett's multiple comparison test, considering $P<0.05$ as statistically significant.

3. Results

The results for each of the evaluated parameters were based on the application of statistical treatment for animals at different gestational ages for each of the five groups (M5, M6, M7, M8, M9) analyzing their morphological characteristics separately (Table 1). The M5 group was compared with the M6, M7, M8 and M9 groups to evaluate the parameter changes during pregnancy

Table 1: Representation of cardiac functional indices of mares with different gestational ages

Parameters	**M5**	**M6**	**M7**	**M8**	**M9**
IVSTs	3,74±0, 29abc	4,39 ±0, 25ab	6, 21±0, 4 5ac	4, 05±0, 32	3,9 8±0, 52
IVSTd	2,65±0,28[a]	2,87±0,27	3,40±0,53[a]	2,59±0,37	2,61±0, 24
LVWTs	3,65±0,37abc	3,42±0,67	3,51±0,25	3,80±0,39	3,92±0, 2
		ab	ac		9
LVWTd	2,44±0, 29[a]	2,44±0, 75	1,68±0, 27[a]	2,39±0, 64	2,40±0, 30
LVIDs	4,56±1, 26[a]	6,32±0, 95[a]	5,55±0, 34	5,54±1, 18	5,00±0, 89
LVIDd	8,52±0,79abc	10,05±0, 95ab	9,39±0, 27	9,67±1, 10ac	9,03±1, 21
AD	5,75±0, 33	5,47±0, 50	6,21±0, 45	5,45±0, 71	5,29±0, 41
SF%	45,54±10, 59[a]	36,25±4, 62[a]	43,61±2, 23	43,58±6, 08	44,44±4, 42
jFE%	81,29±9, 90	74,80±6, 30	77,99±5, 70	80,99±6, 02	82,95±5, 46
SV	1476,00±452,40abcd	2308,00±529, 20ab	2082,00±175, 10[ac]	2182,46±591, 20ad	1572,00±773, 20
fSV	383,2±281,4[a]	881,70±36 2, 80[a]	499,60±72, 26	564,66±35 6, 57	389,5±143, 40
DV	1915±491,6[a]	3124,00±7	2588,00±1	2747,12±9	1943, 00±
		88, 60[a]	33, 90	25, 48	983, 70
CD	65,71±15,39 abc	91,77±20, 31	110,30±19, 57ab	116,80±42, 18ac	90,92±38, 05
HR	44,40±6,11	46,20±6, 70	45,10±3, 14	53,60±14, 37	46,50±6, 13

Interventricular septal thickness in systole (IVSTs); interventricular septal thickness in diastole (IVSD); left ventricular free wall thickness in systole (LVPS); left ventricular free wall thickness in diastole (LVWTd); left ventricular internal diameter in systole (LVIDs), left ventricular internal diameter in diastole (LVIDd); Aorta Diameter (AD); shortening fraction (EF);shortening fraction (jFE%) systolic volume (SV);final systolic volume (fSV); diastolic volume (RVF); cardiac output (CD); ; heart rate (HR). In all groups, 10 animals were analyzed. Five months of gestation (M5); Six months of gestation (M6); Seven months of gestation (M7); Eight months of pregnancy (M8); Nine months of gestation (M9). Data are presented as (mean ± standard deviation). Equal lowercase letters on the same line express statistical difference in Dunnett's multiple comparisons test ($p<0.05$).

Interventricular septal thickness in systole (IVSTs) showed a significant difference (p = 0.0009) in the analysis of variance and Dunnett's multiple comparison test between groups M5 and M6 and M5 and M7. M7 and then progressively decreased to M9. Diastole interventricular septal thickness (dSSI) showed a significant difference ($p<0.0001$) in the analysis of variance and Dunnett's multiple comparison test, from M5 to M7. M7 and decreased to M9.

Systolic Left Ventricular Free Wall Thickness (LVPS) showed a significant difference ($p<0.0001$) in the analysis of variance and also in Dunnett's multiple comparison test, between M5 and M6 and M5 and M7.

Left Ventricular Free Wall Thickness in diastole (LVWTd) showed a significant difference (p= 0.0042) in the analysis of variance and Dunnett's multiple comparison test between the values of M5 and M7. It is noteworthy that they remained in the M5 and M6 group at the same value. In M7 there was a reduction in LVDP, however, in the interval between M7 and M9, there was a progressive increase in values.

In the analysis of systolic left ventricular internal diameter (LVIDs) there was a significant difference (p = 0.0036) in the analysis of variance and Dunnett's multiple comparison test between M5 and M6, which occurred between these two groups. Groups a significant increase in diameter. However, LVIDs decreased in subsequent groups, with the largest reduction occurring between M6 and M7. Regarding the left ventricular internal diameter in diastole (LVIDd), there was a significant difference (p = 0.0071) in the analysis of variance and Dunnett's multiple comparison test between M5 and M6, and M5 and M8.

Aorta diameter (AD) showed a significant difference (p = 0.0015) in the analysis of variance, but did not show a significant difference in Dunnet's multiple comparison test. However, it is noteworthy that this parameter increased until M7 and decreased in M8 and M9.

Regarding the shortening fraction (EF%), a significant difference (p = 0.0146) was observed in the analysis of variance, and a significant difference was also observed in Dunnett's multiple comparison test between groups M5 and M6, vanedo, however, progressive growth of EF% up to M9. jFE% showed no significant difference (p = 0.0802) in the analysis of variance and no statistical difference in Dunnet's multiple comparison test. It should be noted, however, that it decreased until M7, growing again in M8 and M9.

It was possible to observe that the Systolic Volume (SV) showed significant difference (p = 0.0016) in the analysis of variance and Dunnet's multiple comparison test, between M5 and M6, M5 and M7 and, M5 and M8. a large increase in M6, a slight decrease in M7, a further increase in M8, and finally a sharp decrease in M9. The fSV showed significant difference (p= 0.0008) in the analysis of variance and Dunnett's multiple comparison test between M5 and M6, where it had a large increase, decreasing in M7, slightly increasing in M8 and decreasing in M9. The DV showed significant difference (p = 0.0018) in the analysis of variance and Dunnet's multiple comparison test between M5 and M6, where there was a large increase. In M7 there was a slight decrease, followed by a slight increase in M8 and finally a considerable but not significant decrease in M9.

Cardiac output (CD) showed a significant difference (p= 0.0030) in the analysis of variance and Dunnet's multiple comparison test, with significant difference between M5 and M7, and M5 and M8. There was no significant difference (p = 0.1057) in the analysis of variance and Dunnet's multiple heart rate (HR) test.

4. Discussion

Analyzing the values of the Interventricular Septum (SIV), Bonomo *et al*. (2011) [5], when evaluating the echocardiographic parameters of QM athlete mares, found values for SIV in systole (IVSTs) with a value of 4.00cm, very close to the values found in the present study for M8 and M9. Bonomo *et al*. (2014) [6], also evaluating the echocardiographic parameters of horses of the QM race horses, found values of 4.20 cm for IVSTs, closest to M6. Latorre *et al*. (2016) [7], for Mangalarga-Marchador mares found IVSTs that ranged from 3.73cm to 3.75cm, values lower than those found for the QM mares of the present study. Regarding diastole IVS values (IVSD) Bonomo *et al*. (2011) [5] quantified IVSTd for QM athletes mares with a value of 2.82 cm, while Bonomo *et al*. (2014) [5] quantified IVSTd with a value of 2.89 cm. Such values are close to every month except M7. Latorre *et al*. (2016) [7] found IVSTd that ranged from 2.40cm to 2.43cm for Mangalarga-Marchador mares, values lower than those found for the QM mares of the present study.

It is possible to conclude that after prolonged exercise, the mares have decreased thickness of the Interventricular Septum, which corroborates the one proposed by Bertone *et al*. (1987) [8], who evaluated horses after low-intensity, aerobic and prolonged exercise and found a significant reduction in interventricular septum thickness, facts that corroborate the decrease that occurred in both parameters in M8 and M9. Bonomo *et al*. (2014) [5] conclude that IVS has a greater contribution during systole, indicating that possibly cardiac contraction increased in M7. Soma-Pillay *et al*. (2016) [1] state that myocardial contractility increases during pregnancy, so it is possible to state that M7 corresponds to a month of intense myocardial activity during mares' gestation.

Regarding the left ventricular wall thickness values found, Bonomo *et al*. (2011) [5] found, for systolic PLVE (LVWTs) 4.61cm for QM athlete mares, values that are above the findings of the present study in all months, while Bonomo *et al*. (2014)[6] for QM athletes, quantified LVWTs that corresponded to 4.55cm. Latorre *et al*. (2016) [7] found, for Mangalarga-Marchador mares, LVWTs that ranged from 3.48cm to 3.62cm, closer to the findings of the present study.

With regard to diastolic PLVE (LVWTd), Bonomo *et al*. (2011) [4] quantified, for QM athletes mares, LVWTd of 3.33 cm, while Bonomo *et al*. (2014) [5] found 3.12cm for QM horses, which were above the findings of the present study for mares Latorre *et al*. (2016) [7] found values for Mangalarga Marchador mares, which ranged from 2.19cm to 2.22cm, values below those found in the present work.

In athlete animals with respect to the change in myocardial wall thickness after exercise, as in the present study, the findings varied among authors, but this change was reversible (Bertone *et al*.1987; Douglas *et al*., 1987; Gehlen *et al*., 2005; Michima, 2007) [8- 11]

In this study, we found that pregnant mares presented a smaller ventricular wall thickness than athlete animals, with values closer to the wall thickness of non-athlete Mangalarga Marchador mares. Bonomo *et al*. (2014) [6] in a study comparing QM and English Thoroughbred (PSI) high animals evaluated that QM animals had higher LVP, concluding by a tendency of QM breed to concentric and eccentric hypertrophy, being characteristic of predominantly anaerobic, strength work. Soma-Pillay (2016) [1] states that early in pregnancy the cardiac output is increased by 20%. Possibly the increase in blood volume in the heart chambers due to pregnancy causes a decrease in ventricular thickness, even in the beginning, since in the fifth month is already well below the findings for QM animals, even athletes.

Comparing with the parameters presented for the athlete horse this finding coincided with Dávila-Roman *et al*. (1997) [12], who observed signs of effort fatigue in animals, when there was an increase in global left ventricular function in the period immediately after exercise, reflecting in the reduction of systolic and diastolic volume. Similarly, Kalliokoski *et al*. (2004) [13] considered that slight changes in left ventricular function parameters, including systolic volume reduction, were characteristic signs of cardiac fatigue in human marathon runners.

Regarding the values found for the left ventricular internal diameter (DIVE) in systole (LVIDs) Bonomo *et al*. (2011) [5] found, for QM athletes, LVIDs that corresponded to 6.28cm and while Bonomo *et al*. (2014) [6] quantified the average value of 6.18cm for LVIDs when evaluating QM mares. Latorre *et al*. (2016) [7] found, for Mangalarga Marchador mares, LVIDs that ranged from 6.16 to 6.37cm.

Regarding DIVE in Diastole (LVIDd), Bonomo *et al*. (2011) [5] found 9.98cm for QM athlete mares, while Bonomo *et al*. (2014) [6] found 10.18cm for mares QM athletes. Latorre *et al*. (2016) [7] found values for LVIDd for Mares of the Mangalarga Marchador breed ranging from 10.22 to 10.56cm.

What can be concluded is that during the sixth month of gestation of the mares, the blood volume inside the heart chambers increases, which leads to a dilatation of the mares leading to an increase in the inner diameter of the ventricles. Soma-Pillay *et al*. (2016) [1] state that the heart during pregnancy is physiologically dilated. It is possible that this phenomenon occurs due to the need of blood supply to the fetus as it begins to grow rapidly.

Regarding the Aorta Diameter (AD) the values found remained below the findings of Bonomo *et al*. (2011) [5], who found values of 6.73 cm for quarter-mile athlete mares and the findings of Latorre *et al*. (2016) [7] where AD ranged from 6.48 to 6.59cm for Mangalarga Marchador mares. Gianicco *et al*. (2015) [13] states that the aortic diameter of non-athlete animals is expected to be smaller than that of athlete animals.

The shortening fraction finding differs from that proposed by Patteson (1996) [15] in that the EF% in resting adult horses ranged from 28-45% in this present study all animal groups had indices within normal range and the findings of Bonomo *et al*. (2011) [5], in which the values of EF% were 64.10%.

Corroborating what was described by Soma-Pillay *et al*. (2016) [1] Regarding cardiac changes during gestation, the increase in systolic volume (SV) as well as cardiac output (CO) progressively from group M5 to M7 was evaluated, with subsequent decrease in animals from M9. The CD values found in this study are well above the values proposed by Bonomo *et al*. (2011) [5], where the average values found were 11.61 for physiological Quarter Horse Mares. The increase in cardiac output is related to what was proposed by Marr *et al*. (1999) [16], when he stated that cardiac output was dependent on preload and its elevation was observed in situations associated with greater venous return and lower peripheral resistance, as observed during exercise, which possibly applies to mares in a state due to the need for blood supply due to the need for the fetus.

The resting horse's heart rate (HR) ranged from 28-45 beats per minute (bpm) and could rapidly increase to levels above 100 bpm due to arousal, fear, pain or pre-exercise processes such as warm-up (Boffi, 2006) [17]. In this context it can be seen that only group M5 was within the normal range of 22 to 45bpm (Table 1), as established by Fregin (1982) [18], for resting adult horses, further corroborating that presented by Fernandes (1994) [19]. Which found an average of 39.3bpm in Arabian enduro horses, 49bpm in Mangalargas and 51bpm in crossbred animals. Otherwise, Diniz (2006) [20] mentioned an average of 51bpm in jumping horses. The other groups of animals were just above the reference values, especially these data can be understood as being adaptations promoted by gestational age, ie fetal growth and changes in blood flow and supply. This coincided with the vascular and hemodynamic adjustments resulting from pregnancy (Clapp, 1985) [21].

Regarding the puerperal period, it is accepted that the adaptive changes developed during pregnancy progressively tend to disappear, thus restoring pre-gestational functional and anatomical conditions (Robson, 1987) [22]. The same was reported by Soma-Pillay *et al*. (2016) [1], when stating that for pregnant women who experience a gestational period free of complications, changes regress after delivery with minimal residual effects.

5. Conclusion

It was possible to observe that in the evaluated mares, the echocardiography was efficient in determining the cardiac functional indices acquired at different gestational ages. The changes noted were due to the adaptation of the animal's body due to pregnancy. Few studies have been found and related to pregnant mares, so more research is needed in this area in order to establish parameters for each group is better to subsidize these findings.

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