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Electrocardiographic Parameters in Lambs after Birth until 120 Days of Age and Maternal and Neonatal Cardiac Biomarkers

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Abstract

Objective: The objectives of the study were to evaluate the behaviour of electrocardiographic parameters in lambs during development and the concentrations of maternal and neonatal cardiac biomarkers NT-proBNP and troponin I. These results significantly contribute to medical research, since the sheep heart is similar to the human heart.

Methods: Ten Dorper lambs were evaluated after birth until 120 days of age. A computerized electrocardiographic was performed from the record of six leads. The determination of NT-proBNP was performed by the ELISA method, the troponin I was performed by the ELFA technique with a Vidas® Troponin I Ultra kit.

Results: The heart rate decreased at 21 days of age, and the PR interval was elevated after that period. The P-wave had a longer duration at 90 and 120 days of life. There was no difference in the duration of the QRS complex across the age groups. The duration of the QT interval increased from 30 days of age, the duration of the T-wave increased from 21 days, and its amplitude was increased from 24 hours after delivery up to 7 days of age. There were no differences in the concentrations of the maternal and lamb biomarkers in any of the evaluated periods.

Conclusion: From 21 days, there was a reduction in HR, which illustrates the participation of the Autonomic Nervous System (ANS) and the balance between vagal and sympathetic activities in this age group. As development progresses, there are changes in cardiac electrical conduction. The cardiac biomarkers NT-proBNP and troponin I do not change during development.

Keywords: Electrocardiogram; Biomarker; Development; Autonomic nervous system; Neonatal

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Introduction

As in humans, in animals, the characteristics of electrocardiographic tracing must be evaluated according to the patient's age. A newborn's ECG reflects the haemodynamic repercussions on the right ventricle in intrauterine life, right ventricular overload pattern and the anatomical-physiological changes resulting from the transition from foetal circulation to neonatal circulation, a transition that can take up to two years to complete in children [1]. The changes are due to physiological development, increases in body size, the position of the heart in relation to the body and variations in the structural conformation of the cardiac chambers and pulmonary vessels [2,3].

The sheep species has been widely used in several experimental protocols in veterinary and human medicine [4-6]. Models of ischaemic heart disease and heart failure have been developed in sheep and have been increasingly explored in research; however, extrapolating their results to the clinical scenario requires knowledge of the physiological cardiac function in neonates of this species [7-9].

The humoral and haemodynamic forces influence cardiac development and the proliferation and maturation of cardiomyocytes, although the mechanisms that regulate the normal growth process of the heart are not well understood. The regulation of this development process is essential, given that the endowment of cardiomyocytes is essentially determined at the time of birth [10].

In 1988, a new peptide from the family of natriuretic peptides was identified in the pig brain, which, for this reason, was renamed brain natriuretic peptide or B-type Patriuretic Peptide (BNP), and in 1990, a third member of the family was then identified and named Natriuretic Peptide type-C (CNP) [11]. Since then, the family of natriuretic peptides has been widely studied, as they perform a vital function in cardiovascular regulation, acting as regulators of blood pressure and homeostasis of body fluids through their diuretic, natriuretic and vasodilator effects [12].

In patients with congestive heart failure, plasma BNP levels rise considerably, and BNP is the main natriuretic peptide found in blood plasma [13]. Natriuretic peptides have been shown to be important regulators of the Renin-Angiotensin-Aldosterone System (RAAS) and Sympathetic Nervous System (SNS) as hormones that counterbalance the increase in pressure and blood volume. Some studies have shown that BNP concentrations in animal and human foetuses are higher than in adults and neonates [14].

Cardiac natriuretic peptides are secreted during development in response to physiological stimuli, similar to those that cause them to be secreted in adults. Some studies in sheep foetuses have shown that Atrial Natriuretic Peptide (ANP) secretion is stimulated in response to swelling, hyperosmolarity, hypoxia and vasoconstrictors such as angiotensin II and phenylephrine, vasopressin and endothelin [15].

The objectives of the present study were to evaluate the electrocardiographic parameters in Dorper lambs during neonatal development as well as the concentration of cardiac biomarkers (NT-proBNP and troponin I) in this period. Since development causes a series of hormonal and haemodynamic changes, where there is a rearrangement of cardiomyocytes during development, there may also be changes in cardiac electrical stimuli. This study also aimed to assess the concentration of maternal cardiac biomarkers to compare the neonatal and maternal concentrations and their behaviour during the gestational and neonatal periods. Thus, since sheep are currently being used as experimental models in translational research, illustrating the cardiovascular changes that occur during early sheep development can contribute to improvements and advancements of cardiovascular research in human children.

Materials and Methods

Study location

This study was conducted according to the animal well-being guidelines and approved by the Ethics Commission on Animal Use (CEUA, Comissão de Ética no Uso de Animais) of the School of Veterinary Medicine and Animal Science at Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu Campus, under protocol CEUA-0174/2016. The owners of the animals consented to the experimental plan and to the procedures performed. The study was conducted at the city of Botucatu, State of São Paulo, Brazil, at the Rubião Júnior District, latitude S-22.902107 and longitude W-48.516534, from December 2017 to April 2018. The present study was conducted without any type of anesthetic protocol.

Neonatal electrocardiogram

Ten Dorper lambs of both sexes were evaluated after birth until 120 days of age. The electrocardiogram was performed using the computerized method TEB® (Brazilian Electronic Technology, São Paulo-SP, Brazil) from the record of six leads from the coupling of electrodes to the animal limb, namely, DI, DII, DIII, aVR, aVL, and aVF, recorded at 25 mm/second and sensitivity N, for 90 seconds in the frontal plane. The animals were placed in the right lateral position and contained manually.

For frontal plane (PF) derivation, "alligator" type electrodes were attached, two to the forelimbs in the region of the humero-radio-ulnar joint and two to the lower limbs in the region of the femoro-tibio-patellar joint, and the insertion regions of the electrodes were pre-moistened with alcohol. Subsequently, the traces were analysed in the DII derivation, compiling the following electrocardiographic parameters: the duration of the P- and T-waves, the QRS complex and the PR and QT intervals in milliseconds (ms), the amplitude of the P-, R-, T-waves in millivoltage (mV), the T-wave polarity, rhythm, cardiac axis and HR.

Maternal and neonatal cardiac biomarkers

Serum samples from the lambs were acquired by jugular venipuncture and stored at -30°C until analysed within 15 days after collection. Maternal samples were also acquired by jugular venipuncture to assess the biomarkers during pregnancy. The determination of NT-proBNP was performed by ELISA (Enzyme-Linked Immuno Sorbent Assay) with the Sheep N-Terminal Pro-Brain Natriuretic Peptide ®Kit (NT-ProBNP) from MyBioSource (San Diego, California, USA) under the supervision of Profa. Dra. Eunice Oba.

The determination of the Troponin I biomarker was also carried out by the enzymatic immunoassay method using the ELFA technique (Enzyme-Linked Fluorescent Assay) with a Vidas ® Troponin I Ultra (TNIU) kit from Biomérieux (São Paulo-SP, Brazil). The troponin I concentration is given in micrograms per litre of blood (μg/L). The NT-proBNP biomarker concentration is given in picograms per millilitre of blood (pg/ml), and for the determination of the biomarker, the samples were diluted at a concentration of 1:3.

Statistical analysis

The results are illustrated with the mean, standard deviation, minimum and maximum values obtained. For the analysis of the parameters, the normality test employed was Kolmogorov-Smirnov; for the comparison between all of the proposed moments, the test used was Friedmann's, and later, the same test was used for comparisons between specific chosen moments (24 hours after birth, 7, 14 and 21 days of age). All analyses were conducted at a level of 5% significance.

Results

Table 1 illustrates the electrocardiographic parameters in lambs

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from 24 hours of birth to 120 days of age, and **Figures 1 and 2** illustrate the electrocardiographic tracings in the referred periods. As shown in the table, the HR declined after 21 days of age, with a significant difference between the age periods, and the lowest HR occurred at 120 days of age. The RR interval differed significantly across the periods, with the lowest records up to 14 days of age, and from that period, the duration of the intervals increased, following the HR inversely.

The duration of the P-wave also differed significantly, with 90 and 120 days having a longer duration; the PR interval also differed significantly, with longer durations obtained after 21 days of age. There was no difference in the duration of the QRS complex across the age groups, but a longer duration was observed after 21 days of age.

There was a significant difference regarding the duration of the QT interval, and after 30 days of age, the duration increased. There was also a significant difference for QTc, with its duration gradually increasing with advancing age. The duration of the T-wave also differed significantly across the age periods, and from 21 days onward, the duration increased.

There was no difference in the amplitude of the P-wave or in the amplitude of the R-wave, but greater amplitudes were observed at 21 days of age. The amplitude of the T-wave differed between the moments, with the lowest amplitudes at 14 days and 21 days of age and the highest amplitudes at 24 hours after delivery and 7 days of age.

In the assessment of specific periods (24 hours after birth up to 21 days of age), HR differed across all of these periods, except between 24 hours and 14 days, and the same occurred for the RR interval. The duration of the P-wave and the QT interval differed across all periods analysed, and the same occurred for the duration of the PR interval, except between 24 hours after birth and 7 days, where there was no difference. The duration of the T-wave differed across all periods analysed except between 7 and 14 days.

Table 2 illustrates the concentrations of the cardiac biomarkers NT-proBNP and troponin I in Dorper ewes during pregnancy. During this period, there was no significant difference during the months of pregnancy in the concentration of the biomarkers, but we observed that the concentrations of both were high in the third month of pregnancy.

Table 3 illustrates the concentrations of the cardiac biomarkers NT-proBNP and troponin I from ewes and lambs 2 hours, 8 hours, and 12 hours after delivery, and at 7, 14, 21, 30, 60, 90 and 120 days after delivery. There was no significant difference across the periods evaluated for the concentrations of biomarkers for both mothers and lambs.

Parameters ECG	24 hours	7 days	14 days	21 days	30 days	60 days	90 days	120 days	P
HR (bpm)	204.00 ± 28.45^{a}	270.00 ± 23.28^{b}	209.00 ± 38.48^{ac}	166.00 ± 40.03^{d}	165.00 ± 28.72	164.00 ± 32.95	158.00 ± 29.39	143.00 ± 23.00	*0.000
	(132;237)	(240;321)	(155;268)	(104;238)	(122;223)	(96;209)	(104;205)	(103;179)	
RR (ms)	292.00 ± 70.00^{a}	222.00 ± 18.81^{b}	292.55 ± 61.30^{ac}	369.00 ± 96.66^{d}	552.00 ± 56.00	375.00 ± 99.83	386.00 ± 77.56	417.00 ± 67.85	*0.000
	(220;493)	(183;250)	(217;397)	(243;557)	(553;597)	(277;620)	(280;547)	(337;563)	
P (ms)	37.00 ± 6.04^{a}	40.00 ± 6.50^{b}	47.00 ± 7.33^{c}	47.00 ± 10.07^{d}	43.00 ± 9.05	38.00 ± 6.22	49.00 ± 9.06	48.00 ± 7.90	*0.000
	(30;50)	(30;50)	(35;60)	(37;63)	(30;60)	(28;47)	(33;63)	(32;60)	
PR (ms)	66.00 ± 9.02^{a}	64.00 ± 9.18^{a}	74.00 ± 9.99^{b}	77.00 ± 9.38^{c}	70.00 ± 8.82	68.00 ± 8.03	79.00 ± 9.23	80.00 ± 14.90	*0.000
	(50;87)	(50;83)	(60;95)	(63;93)	(57;80)	(55;80)	(63;93)	(52;103)	
QRS (ms)	46.00 ± 5.78	45.00 ± 6.38	43.00 ± 5.75	47.00 ± 7.90	51.00 ± 10.90	51.18 ± 11.86	49.00 ± 9.48	56.00 ± 10.06	0.192
	(40;57)	(33;57)	(30;50)	(40;63)	(37;73)	(33;77)	(33;63)	(40;77)	
QT (ms)	177.00 ± 27.97^{a}	143.00 ± 10.04^{b}	159.00 ± 28.17^{c}	159.00 ± 28.17^{d}	210.00 ± 23.13	212.00 ± 34.78	216.00 ± 28.13	228.00 ± 21.67	*0.000
	(160;258)	(127;163)	(103;197)	(103;197)	(153;257)	(173;302)	(173;260)	(187;263)	
QTc (ms)	59.00 ± 132.00^{a}	288.00 ± 51.13^{b}	294.00 ± 47.34^{c}	326.00 ± 18.90^{d}	346.00 ± 25.65	311.00 ± 105.00	347.00 ± 18.81	350.00 ± 19.55	*0.000
	(0;342)	(143;327)	(206;352)	(283;356)	(306;388)	(0;380)	(305;378)	(318;376)	
T (ms)	87.00 ± 10.02^{a}	66.00 ± 12.79^{b}	69.00 ± 12.60^{b}	89.00 ± 18.00^{c}	82.00 ± 12.08	73.00 ± 11.89	79.00 ± 10.47	76.00 ± 14.31	*0.000
	(67;100)	(47;93)	(45;93)	(63;130)	(63;100)	(57;97)	(57;93)	(57;103)	
Q (mV)	0.21 ± 0.25	0.36 ± 0.16	0.29 ± 0.22	0.26 ± 0.16	0.28 ± 0.22	0.18 ± 0.14	0.23 ± 0.12	0.20 ± 0.10	0.201
	(0;0,93)	(0,05;0,67)	(0;0,70)	(0;0,50)	(0;0,78)	(0;0,50)	(0,08;0,52)	(0,02;0,36)	
T (mV)	0.5 ± 0.18^{a}	0.31 ± 0.16^{b}	0.16 ± 0.13^{b}	0.29 ± 0.12^{c}	0.28 ± 0.15	0.25 ± 0.12	0.23 ± 0.08	0.20 ± 0.13	*0.000
	(0,31;0,76)	(0,07;0,53)	(0,03;0,36)	(0,12;0,46)	(0,10;0,64)	(0,09;0,50)	(0,10;0,33)	(0,05;0,51)	
ST	1	1	1	1	1	1	1	1	-
Axis °	115.87 ± 60.97	121.00 ± 49.86	114.37 ± 53.79	129.93 ± 4.31	127.81 ± 46.51	128.92 ± 40.80	135.76 ± 39.64	140.58 ± 46.29	0.826
	(0;180)	(44;181)	(30;180)	(30;180)	(53;180)	(36;180)	(30;169)	(10;180)	

ST: 1=normal, 2=depression, 3=depression; Kolmogorov-Smirnov normality test; comparison between Friedmann's test moments; * significance: $p < 0.05$; abDifferent letters superscript on the same line indicate significant difference between evaluated moments.

Table 1: Electrocardiographic parameters (mean, standard deviation, minimum, maximum) in lambs from 24 hours of birth to 120 days of age.

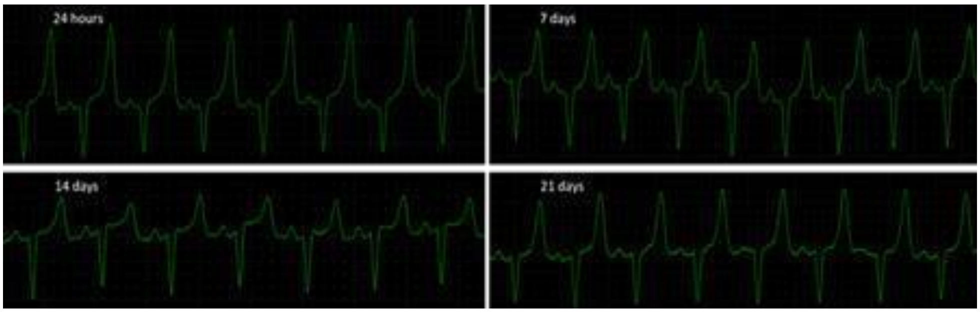

Figure 1: Electrocardiographic tracing in dorper lambs 24 hours after birth until 21 days of age.

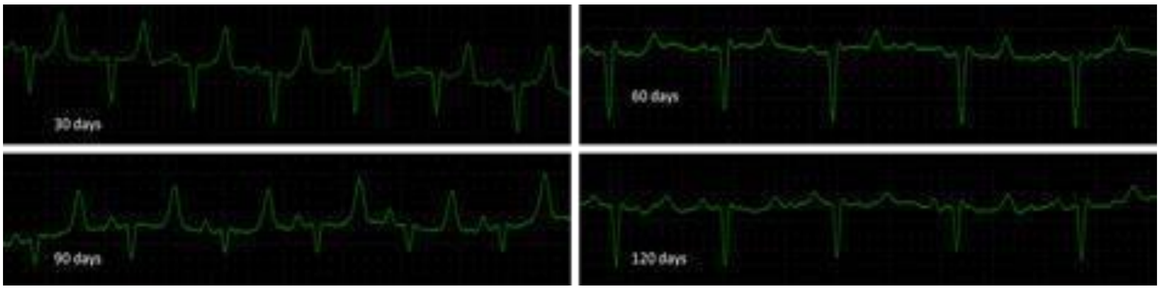

Figure 2: Electrocardiographic tracing in dorper lambs from 30 days of age to 120 days of age.

Maternal biomarker	2nd month	3rd month	4th month	5th month	P
NT-proBNP (pg/ml)	682.59 ± 366.17 (169.64;1048.40)	780.78 ± 229.19 (399.59;1048.40)	680.03 ± 374.00 (103.92;1048.40)	687.32 ± 290.98 (205.96;1048.40)	0.3254
Troponina I (µ/L)	0.01 ± 0 (0.01;0.01)	0.05 ± 0.09(0.01;0.31)	0.01 ± 0.00 (0.01;0.03)	0.02 ± 0.05 (0.01;0.19)	0.2996

Table 2: Cardiac biomarkers (NT-proBNP, troponin I) (mean, standard deviation, minimum, maximum) in Dorper ewes during pregnancy.

Biomarker/Animal	NT-proBNP sheep (pg/ml)	NT-proBNP lamb (pg/ml)	Troponin I sheep (µ/L)	Troponin I lamb (µ/L)
2 hours after birth	609.98 ± 327.96 (137.54;1048.40)	69.72 ± 71.44 (0.00;206.04)	0.02 ± 0.04 (0.01;0.16)	2.26 ± 6.11 (0.07;19.60)
8 hours after birth	530.46 ± 279.65 (150.85;1048.40)	187.02 ± 232.16 (24.77; 803.26)	0.04 ± 0.09 (0.01;0.32)	0.90 ± 1.86 (0.08;6.04)
12 hours after birth	512.83 ± 239.90 (229.66;1048.40)	235.28 ± 308.51 (0.00;991.57)	0.03 ± 0.05 (0.01;0.19)	1.15 ± 2.83 (0.05;9.15)
7 days	400.76 ± 278.45 (126.98;1048.40)	225.64 ± 278.50 (32.10;754.30)	0.01 ± 0.00 (0.01;0.02)	0.04 ± 0.03 (0.01;0.10)
14 days	502.84 ± 306.03(126.98;1048.40)	159.77 ± 100.17 (12.89;270.91)	0.01 ± 0.00 (0.01;0.02)	0.03 ± 0.01 (0.01;0.06)
21 days	486.41 ± 218.71(276.73;1048.40)	124.18 ± 68.72 (41.56;268.77)	0.01 ± 0.00 (0.01;0.01)	0.08 ± 0.10 (0.01;0.33)
30 days	490.49 ± 394.38(140.83;1303.60)	454.74 ± 797.16 (80.59;2706.20)	0.01 ± 0.00 (0.01;0.01)	0.04 ± 0.05 (0.01;0.17)
60 days	477.09 ± 339.54(88.11;1048.40)	294.54 ± 250.34 (29.05;933.93)	0.01 ± 0.00 (0.01;0.01)	0.05 ± 0.13 (0.01;0.45)
90 days	594.02 ± 721.96(31.74;2405.10)	309.05 ± 178.73 (115.98;761.92)	0.01 ± 0.00 (0.01;0.02)	0.03 ± 0.02(0.01;0.10)
120 days	885.97 ± 732.85 (91.46;2689.40)	306.34 ± 293.05 (75.72;1054.70)	0.01 ± 0.01 (0.01;0.04)	0.02 ± 0.02 (0.01;0.08)
P	0.3254	0.3264	0.2996	0.3281

Table 3: Cardiac biomarkers (NT-proBNP, troponin I) (mean, standard deviation, minimum and maximum) in Dorper ewes and lambs after calving up to 120 days.

Discussion

In our study, after 21 days, there was a reduction in the HR in lambs, which may be due to the beginning of the balance of activity of both branches of the ANS in this phase. Siimes, et al. in their study in lambs <30 days and >30 days old, reported that although the proportional change in general Heart Rate variability (HRV) indexes after beta-adrenergic block was positively associated with age, all responses to (HRV) were significantly lower in older lambs, which may be due to SNA maturation [16]. Thus, the authors concluded that in young neonate lambs, both divisions of the ANS regulate HRV, and subsequently, there is a predominance of vagal activity due to ANS maturation.

In the present study, the predominant rhythm during all phases of development was the sinus, and Atmaca, et al. found a predominance of the same rhythm mentioned in Angoran goats in the age groups of 1 and 2 years old [17]. There was a difference between the moments evaluated in the electrocardiographic parameters, with the duration of the RR and PR intervals inversely proportional to the HR, which decreased from 21 days, as previously mentioned; the average RR interval gradually increased starting from the 21st day after birth. Koether et al., in their study evaluating electrocardiograms in lambs of the Bergamasca breed up to 35 days of age, reported a gradual increase in the RR interval and a concomitant reduction in HR, where similar to our results, these parameters underwent changes as the lambs developed [18].

The amplitude of the T-wave in our study decreased at 14 days of age and increased 24 hours after delivery up to 7 days of age. Our results are similar to the study by the aforementioned authors, where the amplitude of the T-wave decreased on the 7th and 14th days compared to at birth and increased from the 21st day, remaining high until the 35th day. After delivery and at 7 days of age, which may be due to the changes and development that occur in the ventricular masses, consequently contributing to the period of ventricular repolarization.

In the studies by Koether, et al. the authors also reported that during neonatal development, the P-waves gradually presented with less amplitude and a longer duration due to an increase in atrial size [18]. The highest results for the amplitude of the T-wave were compiled 24 hours after delivery and at 7 days of age, which may be due to the changes and development that occur in the ventricular masses, consequently contributing to the period of ventricular repolarization.

We believe that fluctuations in the measurement of electrocardiographic parameters occur at the expense of complexity and constant haemodynamic changes in the transition between neonatal and youth, as well as into adulthood. In children, electrocardiographic changes are frequent during the first year of life, particularly in the neonatal period (from the 1st to the 30th day), reflecting the anatomical and physiological changes that occur in the preterm period and/or shortly after birth [19]. During the first month of life, there is an acute phase of circulatory and respiratory adaptation in neonates of most species. At this time, there are multiple variations in circulatory and respiratory parameters compared to adults, particularly during the period immediately after birth [20].

The progressive elevations in the electrocardiographic parameters in the lambs in our study can also be attributed to development with an increase in cardiac mass. Considerable changes in some electrocardiographic parameters occur between middle and late gestation in the human foetus (particularly the increased P- and T-wave durations, PR, QRS and QTc intervals), and reduced changes occur during the last gestational period. It is proposed that these changes are due to the growing myocardial mass during development, which creates a larger surface area that the electrical signal must cross [21]. Thus, the cardiac mass influences cardiac electrical conduction.

The durations of the QRS complex in the lambs in the present study were higher (45-50 ms) than the QRS durations found in Dorper ewes in the study by Ker and Web (average 40 ms) [22]. The authors reported that as the myocardial changes progressed, the QRS durations were greater than 60 ms. For the duration of the P-wave, our results were superior to those found by Mohan et al. (average P-wave duration 42 ms) in goats and higher than those also found in goats by Pradhan et al. (40 ms), but both authors used the conventional method for recording the ECG, we believe that the computerized method (used in the present study) compiles longer durations of the electrocardiographic waves [23,24].

We observed that the QTc interval 24 hours after delivery was relatively shorter than at seven days of age, where from that age onwards, it steadily increased. Koether, et al. reported that the QTc interval increased significantly up to 35 days of age [18]. It is also emphasized that, according to Antolic, et al., cardiac tissue undergoes changes due to cardiac development throughout late pregnancy; the dynamics of the foetal cardiac action potentials and the conduction system are mature in the last 10 days of pregnancy, suggesting that cardiac ion channels are present and mature at this time [21].

We observed that for the electrocardiographic parameters in lambs, there is a predominance of a QS pattern during development in the DII derivation. Kumar, et al., reported that in Muzaffarnagari ewes, a QRS complex rS pattern predominated in the aVL lead, QR in leads II, III and aVF, and qRs in leads I and aVR [25]. According to these authors, this variability in QRS complexes may be due to differences in the topographic anatomy of the heart within the chest, the position of the heart in relation to the limbs and the activation mechanism of the ventricles. Thus, studies are also needed to investigate and standardize the patterns of the QRS complex in the different breeds of sheep, as well as in different age groups in adult animals, since in the present study, the QS pattern predominates during development.

We also believe that there is an individual variation for cardiac electrical conduction, which is influenced by environmental parameters (stress, manipulation), endogenous, requiring further studies to verify if there are differences between devices from differentmanufacturers, as well as computerized and conventional electrocardiography, such as cited by Camacho, et al., where the authors found notable differences in the computerized method, which presented average values for the duration of the P-wave and the QRS complex above the reference values recommended for the conventional method [26].

For the cardiac electrical axis after 21 days, we observed a predominance of a deviation to the right of the heart, since the values observed during the following periods were approximately +129° and +140°. We believe that this occurs due to the greater development of the left cardiac mass during the neonatal period, since after birth, there is an increase in pressures on the left side of the heart due to the closure of the ductus arteriosus [27].

In our study, there were no differences across the periods evaluated for the concentrations of maternal cardiac biomarkers and lambs; however, we observed high concentrations of the maternal NT-proBNP biomarker in the third gestational month, which may also be due to foetal development and consequently a greater volume overload and activation of SRAA. Studies of the hearts of foetuses in sheep have revealed marked changes in the myocardium during the last third of pregnancy. In addition to changes in the development of vascularization, myocytes, myofibrils and mitochondrial content, there is a marked increase in the number of cardiomyocytes in the heart as well as a transition from mononucleated cells from myocytes to binucleate (terminally differentiated) [28]. In the hearts of sheep, the transition from mononucleated to binucleated cells begins at approximately 100 days of gestation (at term 145 days) in such a way that 70% of cardiomyocytes are binucleated or terminally differentiated [10].

The concentration of NT-proBNP did not differ during the periods evaluated in lambs; however, we observed high concentrations when compared to concentrations in adult humans under the age of 75 years, where it is usually less than 125 pg/ml. Thus, after birth, the biomarker concentrations were lower than in successive periods. High concentrations of natriuretic peptides in foetuses compared to adults indicate that the natriuretic peptide system is important in controlling the cardiac volume in foetuses [29]. Plasma concentrations of the aminoterminal portion of ANP and NT-proBNP in healthy newborns revealed a strong increase in the first days of life, suggesting that ANP and BNP have important physiological roles during the change from the foetal to the neonatal circulation [30].

In a study by Markovic-Sovtic, et al., the plasma concentration of NT-proBNP was higher in umbilical cord blood than in maternal blood, implying that the transplacental transfer of natriuretic peptides does not occur [31]. The plasma NT-proBNP is very high during the first days of life in healthy newborns, presumably due to the transition from the foetal to the neonatal circulation. After this period, NT-proBNP decreases rapidly until the end of the first week of life and then it decreases slowly until the end of the neonatal period. The maturation of renal function seems to play an important role in decreasing NT-proBNP after the second day of life. However, in our study, the concentrations of biomarkers were high as development progressed.

The concentrations of the biomarker troponin I did not differ between the periods evaluated for mothers and lambs in the present study, but the elevated values 2 hours after birth in lambs may be due to the haemodynamic changes that occur mainly at birth, such as duct closure among arteries and elevation of the pressure in the left ventricle. During development in lambs, even with the progression of cardiomyocytes, there is no myocardial injury. Circulating cardiac troponin I (cTnI) is the "gold standard" for the noninvasive diagnosis of myocardial injury in people and small animals. The measurement of cTnI in blood proved to be useful in the early diagnosis of myocardial injury and it has mostly replaced the biomarkers previously used, such as Creatine Kinase (CK) and its specific CEC isoenzyme (CK-MB). Elevated cTnI concentrations can be detected in humans within 3-4 hours after acute ischaemic myocardial injury, with a peak concentration occurring after 48 to 72 hours. Detectable troponin concentrations persist for up to 2 weeks before being reduced to preinjury levels [32].

Thus, we observed that despite the countless haemodynamic changes that occur during development and the gestational period, there is no myocardial injury in this phase, even with the multiplication of cardiomyocytes. SRAA activity also changes with growth and events during pregnancy, contributing to volume overload, which may be to the detriment of the renal maturity process as well as to the endogenous hormonal influences as the neonate develops and due to volume overload by the release of angiotensin into the circulation that occurs during pregnancy.

This study have some limitations. We did not find differences between the respective evaluated moments; however, this fact can be attributed to the small size of the samples (10 animals), since we observed that both the concentrations of NT-proBNP and troponin I were high in the first hours after delivery and we don’t have a control group, that could contribute to investigate the normal levels of biomarkers in sheep.

Conclusion

The HR in lambs is high in the neonatal period, but after 21 days, there is a reduction, which illustrates the beginning of the balance of ANS activity between the vagal and sympathetic components in this age group. ECG is easily used in sheep, and as development progresses, there are changes in cardiac electrical conduction. During development, there seems to be no myocardial injury despite the numerous physiological changes that occur during the neonatal stage.

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Author Contributions

Conceptualization, M.L.G. L and S.B.C.; methodology, D.O.L.F. and M.C.G.; formal analysis, A.S.C.A. and M.C.F.L.; investigation, R.A.P.; resources, A.L.H.A. and R.T.T.; writing—original draft preparation, A.S.C.A.; writing—review and editing, A.S.C.A.; visualization, M.H.T.; supervision, M.H.T. and S.B.C.; project administration, M.L.G.L.; funding acquisition, M.L.G.L. laboratory analysis: V.M.C., H.D.M.G., E.O and F.F.S. All authors have read and agreed to the published version of the manuscript.

Competing Interests

The authors declare they have no competing interests.

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Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was conducted according to the animal well-being guidelines and approved by the Ethics Commission on Animal Use (CEUA, Comissão de Ética no Uso de Animais) of the School of Veterinary Medicine and Animal Science at Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu Campus, under protocol CEUA-0174/2016. The owners of the animals consented to the experimental plan and to the procedures performed. An Ethics Committee comprised of 8 veterinarians, 2 professors of Animal Surgery and Reproduction, 2 professors of Veterinary Clinic, 2 professors of Animal Production and Preventive Veterinary Medicine and 2 Professors of Animal Improvement and Nutrition of the School of Veterinary Medicine and Animal Science at Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu Campus; 2 biologists and 2 professionals in the field of quantitative methods from the same institution. The project was conducted in accordance with the precepts of Law No. 11.794, October 8, 2008, Decree No. 6.899, July 15.2009, and the rules issued by the National Council for the Control of Animal Experimentation-CONCEA. The sheep used in this experiment belong to Sítio Vale Verde, Cabanha Estrela do Vale.

Consent for Publication

Not applicable.

References

1 Abreu DC, Feldman J, Deccache W, Goldwasser GP (2004) Significant elements of the electrocardiogram in newborns and infants: its clinical uses. Rev SOCERJ. 17:257-261.

2 Mattos SS (1997) Physiology of Fetal Circulation and Diagnosis of Functional Changes in the Fetal Heart. Arq Bras Cardiol 69:1-3.

3 NogueiraSSS,FariaEG,SousaMG (2010)Avaliaçãodoeletrocardiograma em cãese gatosneonatos. Revcienteletrônica medvet 8:101-107.

4 Huang Y, Hunyor SN, Jiang L, Kawaguchi O, Shirota K, et al. (2004) Remodeling of the chronic severely failing ischemic sheep heart after coronary microembolization: functional, energetic, structural, and cellularresponses. Am JPhysiolHeartCirc Physiol286:2141-2250.

5 Borestein N, Bruneval N, Behr L, Laborde F, Montarras D, et al. (2006) An ovine model of Chronic Heart Failure: Echocardiographic and Tissue DopplerImagingCharacterization. J CardSurg 21:50-56.

6 Borestein N, Bruneval N, Behr L, Laborde F, Montarras D, et al. (2006) An ovine model of Chronic Heart Failure: Echocardiographic and Tissue DopplerImagingCharacterization. J CardSurg 21:50-56.

7 Power JM, Tonkin MA. (1999) Large animal models of heart failure. Aust N Z J Med 29:395-402.

8 Rabbani S, Ahmadi H, Fayazzadeh E, Sahebjam M, Boroumand MA, et al. (2008) Development of an ovine model of myocardial infarction. Anz J Surg 78:78-81.

9 Locatelli P, Olea F, Lorenzi A, Salmo F, Janavel GLV, et al. (2011) Reference values for echocardiographic parameters and indexes of left ventricular function in healthy, young adult sheep used in translational research: comparison with standardized values in humans. Int J Clin Exp Med 4:258-264.

10 Sandgren J, Scholz TD, Segar JL (2015) ANG II modulation of cardiac growth and remodeling in immature fetal sheep. Am J Physiol Regul Integr Comp Physiol 308:965-972.

11 Brescia EM (2006) Evaluation of the irma technique for measuring vasoactivepeptides(ANP and BNP) in fetal blood. Thesiswork 1-9.

12 Santhekadura PK, Kumar DP, Seneshaw M, Mirshahi F, Sanyal AJ (2017) The multifaceted role of natriuretic peptides in metabolic syndrome. Biomed Pharmacother 92:826-835.

13 SantemaBT,ChanMMY,Tromp J, Dokter M, vanderWal HH, et al. (2019) The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patientswithheart failure . ClinResCardiol109:331-338.

14 SinghJSS,BurrellLM,Cherif M, SquireIS,ClarkAL, at al.(2017) Sacubitril/valsartan:beyond natriuretic peptides.Heart103:1569-1577.

15 Cameron VA, Ellmers LJ (2003) Minireview: Natriuretic Peptides during Development of the Fetal Heart and Circulation. Endocrinology 144:2191-2194.

16 Siimes ASI, Valimaki IAT, Antila KJ, Julkunen MKA, Metsala TH, et al. (1990) Regulation of Heart Rate Variation by the Autonomic Nervous System in NeonatalLambs. PediatricRes27:1-9.

17 Atmaca N, Simsek O, Emre B (2014) Some electrocardiographic values of Angora goats. Vet Fak Derg 61:15-19.

18 Koether K, Ulian CMV, Lourenço MLG, Gonçalves RS, Sudano MJ, et al. (2016) The normal electrocardiograms in the conscious newborn lambs in neonatal periodanditsprogression.BMCPhysiology 16:1-12.

19 Yiallourou SR, Sands SA, Walker AM, Horne RSC (2011) Maturation of heart rate and blood pressure variability during sleep in term-born infants. Sleep 35:177-186.

20 Brockmeier K, Nazal R, Sreeram N (2016) The electrocardiogram of the neonateandinfant.JElectrocardiol 49:814-816.

21 Antolic A, Wood CE, Keller-Wood M (2017) Use of radiotelemetry to assess perinatal cardiac function in the ovine fetus and newborn. Am J PhysiolRegulIntegrCompPhysiol313:660-668.

22 Ker J, Webb EC (2005) Electrocardiographic surrogates of structural myocardial alterations in the Dorper sheep heart. Onderstepoort J Vet

Res72:273-277.

23 Mohan NH, Niyogi D, Singh HN (2005) Analysis of normal electrocardiograms of Jamunapari goats. J Vet Sci 6:295-298.

24 Pradhan RR, Mahapatra APK, Mohapatra S, Jyotiranjan T, Kundu AK (2017) Electrocardiographic reference values and configuration of electrocardiogram waves recorded in Black Bengal goats of different age groups. Vet World 10:1020-1025.

25 Kumar V, Hoque M, Saxena AC, Bodh D, Dey S (2017) Electrocardiographic reference values of Muzaffarnagari sheep. Indian J Small Rumin 23:219-225.

26 Camacho AA, Júnior PD, Pascon PE, Teixeira AA (2010) Comparison between conventional and computerized electrocardiography in cats. Arq Bras Med Vet Zootec 62:765-769.

27 Dawes GS, Mott JC, Widdicombe JG (1955) The patency of the ductus arteriosus in newborn lambs and its physiological consequences. J Physiol 128:361-367.

28 Chalhoub M (2001) Ultrasonographic profile of embryonic / fetal growth of sheep from the 21st to the 41st day of gestation. Rev Bras Health Prod 2:65-68.

29 Merz WM, Gembruch U (2014) Old tool – new application: NT-proBNP in fetal medicine. Ultrasound Obstet Gynecol 44:377-385.

30 Afif EL-Khuffash, Eleanor Molloy (2009) The use of N-Terminal-Pro-BNP in preterm infants. Int J Pediatr 2009:175216.

31 Markovic-Sovtic G, Kosutic J, Jankovic B, Bojanin D, Sovtic A, et al. (2014) N-terminal pro-brain natriuretic peptide in the assessment of respiratory distress in term neonates. Pediatr Int 56:373-377.

32 Varga A, Schober KE, Holloman CH, Stromberg PC, Lakritz J, et al. (2009) Correlation of serum cardiac troponin i and myocardial damage in cattle with monensin toxicosis. J Vet Intern Med 23:1108-1116.