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Association between heart rate, heart rate variability, cortisol, glucose and electrolytes in healthy newborn calves

[*Associação entre frequência cardíaca, variabilidade da frequência cardíaca, cortisol, glicose e eletrólitos em bezerros recém-nascidos saudáveis*]

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ABSTRACT

This study aims at evaluating the effects of electrolytes, glucose and cortisol levels over heart rate (HR) and heart rate variability (HRV) in healthy newborn calves. Seventeen healthy Holstein calves were evaluated during their first month of life, and the plasma concentrations of glucose, cortisol, calcium, magnesium, inorganic phosphorus, sodium and potassium were analyzed. HRV indexes were determined in the time and frequency domains through the analysis of neonatal electrocardiogram recordings. In the first day, low blood levels of phosphorus presented a strong correlation with the HR and the increased high-frequency components of HRV. The plasma concentrations of magnesium decreased significantly throughout the 35 days, revealing a positive association with a decreasing low-frequency components of HRV at day 28. There was a strong correlation between HR, HRV indexes, some plasma electrolytes, glucose and cortisol during the studied period. Variations in the concentrations and correlations observed may be attributed to the adaptive neonatal period in calves.

Keywords: calves, electrolytes, glucose, cortisol, heart rate variability

RESUMO

Este estudo teve como objetivo avaliar os efeitos dos níveis de eletrólitos, glicose e cortisol sobre a frequência cardíaca (FC) e a variabilidade da frequência cardíaca (VFC) em bezerros recém-nascidos e saudáveis. Dezessete bezerros da raça Holandesa foram avaliados durante o primeiro mês de vida e foram analisadas as concentrações plasmáticas de glicose, cortisol, cálcio, magnésio, fósforo inorgânico, sódio e potássio. Os índices VFC foram determinados em domínios de tempo e frequência por meio da análise de gravações do eletrocardiograma neonatal. No primeiro dia, baixos níveis sanguíneos de fósforo correlacionaram-se fortemente com FC e aumento dos componentes de alta frequência da VFC. As concentrações plasmáticas de magnésio diminuíram significativamente ao longo dos 35 dias, revelando correlação positiva com a diminuição dos componentes de baixa frequência da VFC no dia 28. Houve uma forte correlação entre FC, índices de VFC, eletrólitos plasmáticos, glicose e cortisol durante o período estudado. As variações nas concentrações e correlações observadas podem ser atribuídas ao período neonatal adaptativo em bezerros.

Palavras-chave: bezerros, eletrólitos, glicose, cortisol, variabilidade da frequência cardíaca

INTRODUCTION

Heart rate variability (HRV) refers to the periodic changes in duration of cardiac cycles, which can be measured as the successive intervals between beat-to-beat intervals (Kovács *et al.*, 2014). It is based on the antagonistic oscillatory influences of the sympathetic and parasympathetic nervous system on the sinoatrial node and is used as an indicator of Autonomic Nervous System (ANS) activity in response to stress (Mohr *et al.*, 2002). Reductions in the values of the HRV indexes “standard deviation of beat-to-beat interval” (SDNN) and “root mean square of successive beat-to-beat differences” (RMSSD) reflect a shift towards sympathetic dominance, whereas increases in those values

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indicate a shift towards parasympathetic dominance (Von Borell *et al*., 2007). The spectral analysis technique may provide a tool for early detection of mutual imbalances between the two regulatory systems (Abboud and Sadeh, 1990). Low-frequency (LF nu) peaks in the spectral analysis are associated with sympathetic activity and, in part, with parasympathetic activity (Akselrod *et al*., 1985). Studies have shown the usefulness of the LF/HF ratio of the power spectrum, with increases in the ratio interpreted as a regulatory shift towards sympathetic dominance (Akselrod *et al.*, 1985; Abboud and Sadeh, 1990). High vagal tone has been linked to efficient autonomic regulatory activity, which enables an organism to increase its sensitivity and response to physiological and environmental challenges (Von Borell *et al*., 2007).

During the neonatal period, newborn animals undergo changes needed for adaptation to extra-uterine life. Some hematological blood parameters can determine the level of environmental adaptation and, therefore, the welfare of the animals (Mohri *et al*., 2007). Biochemical tests evaluate the internal condition of the body, the function of different organs and the course of metabolic changes in the body (Kaneko *et al*., 1997). The main morphological and functional changes occur during the first week of life for calves (Knowles *et al.*, 2000). When an animal perceives a threat to homeostasis, there is a quick response by the sympathetic-adrenal medullary system (SAM), which involves the central nervous system (CNS). These neural pathways activate the release of epinephrine by the adrenal medulla and norepinephrine by the peripheral sympathetic nerves. The hypothalamic–pituitary–adrenocortical (HPA) stress-response system mediates a long-term, sustained response with the involvement of major adrenocortical hormones, such as glucocorticoids and mineralocorticoids (Manteuffel, 2002).

In calves, the blood concentrations of many constituents change dramatically with age, particularly during the first week of life (Blum, 2006). Plasma concentrations of cortisol decrease during the first week of life in neonate calves and decrease temporarily after intake of colostrum, milk or milk replacer (Hadorn *et al*., 1997). Glucocorticoids are an essential component of many physiological functions, including stress, metabolism, and immunity. Despite this, there are no studies in bovines correlating the levels of electrolytes, glucose or cortisol with heart rate or heart rate variability. Theoretically, disturbances in the electrolyte levels could alter HRV in a sick animal but not in a healthy one. Therefore, this study aims to evaluate the effects of electrolytes, glucose and cortisol concentrations over heart rate and heart rate variability in apparently healthy newborn calves.

MATERIALS AND METHODS

This study was conducted in a dairy farm located near the city of Botucatu, São Paulo State, Brazil, on 17 clinically healthy Holstein calves born after full term (9 males and 8 females), which were monitored for 35 days after birth. The calves were kept in a sheltered outdoor pen on a conventional calf rearing program. This project was approved by the Ethics Committee on Animal Use - CEUA (Code: 89/2014), and informed consent was given by the owner of the farm.

Neonatal electrocardiogram (ECG) recordings were taken weekly after birth until the calves were 35 days old. Blood samples were taken after 10-15 minutes of ECG recordings.

Blood samples were taken from the calves from birth until they were 35 days old. A blood sample was taken within 24h (1= first sampling) and after 7 (2= second sampling), 14 (3= third sampling), 21 (4= forth sampling), 28 (5= fifth sampling) and 35 (6= sixth sampling) days. In newborn calves, blood samples were collected after ECG recording by jugular venipuncture. The samples were then centrifuged at 3.000 × g for 8min, and aliquots of plasma were stored at 20°C until assayed.

Plasma concentrations of glucose, calcium (Ca^{++}), magnesium (Mg^{++}) and inorganic phosphorus (P) were analyzed with a UV spectrophotometer and sodium (Na^{+}) and potassium (K^{+}) concentrations by flame-photometry.

The plasma concentration of cortisol was determined with a radioimmunoassay using a commercially available kit (INMUNOTECH s.r.o Czech Republic) following the manufacturer's instruction. The intra-assay coefficient of variation was 19%, and the inter-assay coefficient of variation was 24%. The minimum detectable concentration was 0.3nmol/L.

Neonatal ECG measurements were made with the Televet 100 recording system (version 4.2.3; Kruuse, Marslev, Denmark). Two square inches of hair was shaved at the sites where the self-adhesive pads were to be attached. The Televet 100 device was attached to an elastic band around the thorax, and the electrodes were attached to the coat with self-adhesive pads. The red and black electrodes were placed on the right side of the lower part of the thorax, and the green and yellow electrodes were placed on the left side in the precordial region. The electrodes were connected to the Televet 100 recording device and the data via Bluetooth to a computer.

A software was used for HRV analysis. In agreement with the generally accepted recommendations for HRV analysis in animals, a 5-min interval of each recording was used to determine the HRV indexes (Tarvainen and Niskane, 2008). The HRV indexes analyzed were the time and frequency domain indexes. In the time domain, heart rate (HR), beat-to-beat interval (RR), square root of the variance of RR intervals (SDNN), and square root of the mean squared differences of successive RR intervals (RMSSD) were assessed. SDNN reflects all cyclic components of the variability in recorded series of RR intervals. RMSSD was used as an estimate of high-frequency variations in short-term RR recordings. The studied parameters of the frequency domain were low frequency measured in normalized units (LF nu), high frequency measured in normalized units (HF nu), and LF/HF ratio. LF was used to assess sympathetic modulations while HF was used to assess parasympathetic modulations. The LF/HF ratio was used to assess the balance between sympathetic activity and vagal tonus. Divergent respiratory frequencies in calves were taken into consideration by setting the limits of the HF band to 0.3Hz (lower limit) and 0.8Hz (upper limit) (Von Borell *et al.*, 2007).

The statistical analysis was conducted using the SPSS software (SPSS 21) for Windows. Kolmogorov–Smirnov tests were used to assess normality for each variable. Analysis of variance (ANOVA) for repeated measurements was used to analyze significant changes. The Bonferroni test was used for the post hoc analysis of each endocrine parameter, biochemical parameter, heart rate, and heart rate variability indexes at all sampling times. In addition, a paired t-test was used to compare sampling stages with the first sampling. Heart rate (HR) was introduced as a covariate when screening the studied variables for partial correlations between the electrolyte ion levels, glucose levels, cortisol levels and heart rate variability indexes. Pearson's correlation was used to determine possible associations between heart rate and biochemical and endocrine parameters. Linear regression analysis was conducted to assess the relationship between plasma concentrations of electrolytes, glucose, and cortisol with HRV indexes. Results are expressed as mean (± SEM). Statistical significance was set at $P< 0.05$.

RESULTS

Means, standard error of the mean, maximum and minimum concentration of glucose, cortisol, and electrolytes for the newborn calves are shown in Table 1. Sampling time had a significant effect on the potassium, magnesium, phosphorous, calcium and cortisol levels, while the sodium and glucose levels presented no significant changes during the first month of life.

On the first week of life, calcium concentrations showed a downward trend from the first day (10.3±0.9mg/dL) throughout the entire month until day 35, when the lowest value (8.7±1.2mg/dL) was registered with statistical significance ($P\leq 0.01$). Calcium levels presented a significant decline in day 21 (9.4±0.9mg/dL). Sampling time had a significant effect on magnesium concentrations. At birth, mean magnesium concentrations were 2.4±0.4mg/dL. Starting at day 14, magnesium presented a downward trend (2.0±0.2mg/dL) with statistical significance until day 35 (1.8±0.3mg/dL). During the first day of life, the mean concentration of phosphorus was 6.5±1.2mg/dL. On the second week, a significant increase was noted in the concentrations of this electrolyte (7.4mg/dL), followed by a significant decline to 6.9mg/dL at day 35 (Table 1).

Table 1. Blood plasma concentrations of electrolytes, glucose and cortisol (x ± SEM, minimum and maximum) in newborn Holstein calves at six moments during the first 35 days of life (n= 16 to 18)

Parameter	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Sodium	114.3±1.5	118.3±1.5	116.8±1.6	115.5±1.6	114.4±2.6	120.2±2.4
(mEq/L)	101-125	102-128	103-127	93-128	102-142	102-142
Potassium	3.7±0.08[a]	4.0±0.1[b]	3.8±0.05[a]	3.6±0.09[a]	3.5±0.1[a]	3.6±0.1[a]
(mEq/L)	3-4	3-5	3-4	3-4	3-4	3-4
Glucose	124.6±8.7	139.4±7.5	149.7±9.4	139.8±10.7	122.3±8	132.2±6.1
(mg/dL)	57-176	97-236	102-216	73-229	74-193	86-180
Magnesium	2.4±0.1[a]	2.0±0.06[ab]	2.0±0.05[bc]	1.9±0.04[bcd]	1.8±0.07[e]	1.8±0.09[be]
(mg/dL)	2-3	2-3	2-2	2-2	1-2	1-3
Phosphorus	6.5±0.3[a]	7.4±0.4[d]	7.8±0.4[d]	7.4±0.3[d]	6.9±0.3[ab]	6.9±0.2[abc]
(mg/dL)	5-9	5-10	5-9	5-9	5-8	5-8
Calcium	10.3±0.2[a]	10.3±0.3[a]	9.5±0.2[a]	9.4±0.22[ab]	8.8±0.2[ab]	8.7±0.3[ab]
(mg/dL)	9-12	9-12	8-11	8-11	7-10	6-10
Cortisol	32.7±6.4[ab]	18.9±4.9[ab]	9.5±2.2[a]	16.8±5.2[ab]	17.3±3.7[ab]	16.4±4.4[b]
(nmol/L)	5.8-72	5.7-60	1.7-34	1-62	2.6-45	3.1-59

Bonferroni test: Within each row, different superscripted letters (a, b, c, d, e) indicate a significant difference, P< 0.05; x ± SEM; standard error of the mean; N: number of animals.

The concentration of sodium in blood plasma was 132.1mEq/L after birth, increasing to 138.3mEq/L in the second week of life. On the third week, a moderate increase in sodium concentrations (149.7mEq/L) was noted, followed by a decline to 132.2mEq/L on day 35. The changes observed were not statistically significant.

Mean levels of potassium (7.4±1.5mEq/L) presented a statistically significant (P≤ 0.05) upwards trend on day 7. The plasma concentrations of cortisol were high during the first 24 hours of life (32.7±6.4nmol/L). From the first day, a statistically significant decrease was noted in cortisol levels, reaching 9.5±2.2nmol/L at day 14 (Table 1).

Table 2 presents the mean values for heart rate (HR) and the heart rate variability (HRV) indexes. The time domain indexes included are the standard deviation of beat-to-beat interval (SDNN) and the root mean square of successive beat-to-beat differences (RMSSD), while the frequency domain indexes included are low frequency measured in normalized units (LF nu), high frequency measured in normalized units (HF nu) and LF/HF ratio of newborn calves from the first 24 hours to 35 days old.

Table 2. Heart rate variability indexes (x ± SEM, minimum and maximum) at six moments during the first 35 days of life in newborn Holstein calves (n= 16 to 18)

HRV Index	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
RR (ms)	471±15[ab]	441±15[a]	460±21[abc]	461±24[ab]	460±16[abcd]	502.3±18[d]
	310-576	312-584	302-617	351-660	378-591	378-591
HR (bpm)	130±5[ab]	139±5[b]	135±6[ab]	137±7[ab]	134±5[ab]	123±5[a]
	104-195	103-193	98-198	90-171	101-164	98-158
RMSSD (ms)	18.4±6.3[b]	18.1±6.1[ab]	21.4±4.5[ab]	16.6±5.5[a]	23.8±7.0[ab]	28.4±9.8[ab]
	2-98	8-100	4-81	5-94	7-91	6-131
SDNN (ms)	34±4.5	33±5.1	34±4.4	38±7.6	35.4±6.1	47.1±9.8
	11-90	13-79	14-64	14-112	11-95	9-135
LF (nu)	87±3[ab]	83±3.9[a]	82±3.9[ab]	90±2.7[b]	82.1±4.5[ab]	80.5±4.4[ab]
	65-99	45-98	55-99	55-99	29-99	51-97
HF (nu)	31±5.9	29±6.1	36±5.2	28.1±4.7	36.9±6.5	37.5±6.8
	2-79	2-84	4-73	1-58	5-95	2-87
LF/HF (ratio)	6±1.7	6.1±1.6	4.1±1.2	6.09±1.4	5.33±1.5	3.79±1.2
	1-26	1-28	0.9-21	1-21	0.8-18	1-18

Bonferroni test: Within each row, different superscripted letters (a, b, c, d) denote a significant difference, P< 0.05; x ± SEM: standard error of the mean.

In the first hours of life, beat-to-beat interval (RR) and heart rate were 128bpm and 483 (ms) respectively. Heart rate increased after 24 hours, later decreasing to 122bpm at day 35, which is a statistically significant change. For the time domain indexes, RMSSD presented statistically significant changes while SDNN did not. High frequency (HF nu) and LF/HF ratio did not present significant changes during the 35 days. Low frequency presented a statistically significant downward trend during the 35 days.

This study registered correlations between heart rate, phosphorus concentrations and sodium concentrations on the first day after birth (r= 0.55; P= 0.02), after one week (r= 0.53; P= 0.03) and at day 21 (r= 0.53; P= 0.03). A strong negative association between heart rate and cortisol (r= -0.62; P= 0.02) was noted at day 28. There were no records of correlations between calcium, magnesium, potassium or glucose concentrations with heart rate during the first month of life (Table 3).

Table 3. Pearson's correlation between heart rate, endocrine and biochemical parameters in newborn Holstein calves at different six moments during the first 35 days of life

Parameter	Unit	Moment	*r*	*p*-value
Sodium	mEq/L	Day 7	0.53	0.03
Sodium	mEq/L	Day 21	0.53	0.03
Cortisol	nmol/L	Day 28	-0.62	0.02
Phosphorus	mg/dL	Day 1	0.55	0.02

r: coefficient of correlation*;* P< 0.05

A strong correlation between calcium, phosphorus, sodium, magnesium, cortisol concentrations and HRV indexes was observed during the first month of life. In the first day, the relationship between cortisol and the root mean square of successive beat-to-beat differences (RMSSD) presented a coefficient of correlation of r= -0.97 and significance of P= 0.004. The correlation between calcium and phosphorus concentrations and the square root of the variance of RR intervals (SDNN) presented coefficients of correlation and significance r= -0.97, P= 0.005 and r= 0.78, P= 0.03 respectively.

Sodium concentrations presented a strong correlation with high and low frequencies at day 7 (r= 0.85; P= 0.007; r= -0.71; P= 0.04) and magnesium concentrations presented a strong negative correlation with the HRV index low frequency (r= -0.86*;* P= 0.01) at day 28 (Table 4). In neonates, no correlations were observed between the HRV indexes and cortisol, glucose or electrolyte concentrations at days 14, 21, or 35, and no correlation was observed between the endocrine or biochemical parameters with LF/HF ratio during the study.

Table 4. Correlations between the heart rate variability (HRV) indexes, endocrine and biochemical parameters in newborn Holstein calves at different moments

Parameter	Unit	Heart Rate Variability indexes	Moment of sampling	*r*	*p*-value
Calcium	mg/dL	SDNN (ms)	Day 1	0.97	0.005
Sodium	mEq/L	Low frequency (nu)	Day 7	-0.71	0.04
Sodium	mEq/L	High frequency (nu)	Day 7	0.85	0.007
Cortisol	nmol/L	RMSSD (ms)	Day 1	-0.97	0.004
Phosphorus	mg/dL	High frequency (nu)	Day 1	0.78	0.03
Magnesium	mg/dL	Low frequency (nu)	Day 28	-0.86	0.01

r: Coefficient of correlation; RMSSD: Root mean square of successive beat-to-beat differences; SDNN: square root of the variance of RR intervals; nu: normalized units; ms: milliseconds.

On the first day of life, there was a strong correlation between the root mean square of successive beat-to-beat differences (29±7.3ms) and high concentrations of cortisol (32.7±6.4nmol/L), as well as high calcium concentrations (116.7±1.8mg/dL) with the minimal values for high frequency (31±5.9nu) and low concentrations of phosphorus (6.5±0.3mg/dL) with low values for high frequency (31±5.9nu). During the first week, the heart rate (bpm) presented significant differences, revealing strong associations with high levels of sodium (118.3±1.5mEq/L) and minimal values for low and high frequencies measured in normalized units (83±3.9; 31±6.1nu).

DISCUSSION

A high plasma concentration of calcium was observed after birth, followed by a significant decrease. It is important to highlight that the plasma calcium concentration in newborn calves is not directly dependent on maternal calcium concentrations. These high concentrations are probably the result of the high demands of growing calves since calcium is responsible for stimulating osteoblast growth and skeletal development (Szenci *et al*., 1994). On the first day after birth, the high physiological levels of calcium correlated strongly with a low standard deviation of beat-to-beat intervals, indicating a reduced HRV, which might be interpreted as stress load. It is possible that high levels of calcium results in decreases in the HRV indexes. The effects of calcium on the heart rate may be attributed to the modulation of cardiac muscle excitability (Edwards, 1993). After the first week, calcium levels presented a downwards trend until day 21, when it reached significant blood concentrations. The levels of phosphorus reached the highest plasma concentration during the second week and then started to decline until day 35. In the first day, the low blood levels of phosphorus were strongly correlated with heart rate and an increased high-frequency parasympathetic index, indicating a shift towards vagal activity and cardiac modulation.

Magnesium concentrations presented a significant decrease throughout the 35 days. It is hypothesized that these changes are a consequence of enhanced magnesium utilization (along with calcium and phosphorus) (Blum and Hammon, 2000). On day 7, the magnesium concentrations started a significant decline until day 28. At this point, the analysis registered a positive association with a decreased low-frequency sympathetic index, indicating cardiac modulation with parasympathetic dominance.

The concentrations of the main extracellular electrolyte (Na^+) were stable during the entire study. During the first week, the sodium concentrations presented correlations with high and low frequencies, corresponding to the activity of sympathetic and parasympathetic components and indicating cardiac and respiratory modulation after birth with a predominance of vagal activity. The HF component is generally recognized to reflect parasympathetic modulation, corresponding to respiratory modulation and being an indicator of the action of the vagus nerve on the heart (Vanderlei *et al*., 2009). Decreased HF values reflect a shift towards sympathetic dominance, while increased values indicate a shift towards vagal activity. During the first week, the heart rate increased significantly, presumably due to rising demands on the cardiovascular system with adaptation to extra-uterine life (Van Reenen *et al*., 2005).

These results have shown that healthy calves regulate electrolyte homeostasis efficiently and are likely to modulate HRV through its effects on heart excitability.

In this study, the plasma concentrations of cortisol were high on the first day, presenting a strong association with the mean squared differences of successive RR intervals (RMSSD) and reflecting the activity of the parasympathetic (vagal) branch of the autonomous nervous system. RMSSD reflects alterations in the autonomic nervous system (ANS) that are mediated predominantly by vagal tonus (Malik and Camm, 1990). Cortisol concentrations decreased during the first month of life, showing declines with the first intake of colostrum, milk or milk replacer (Herosimczyk *et al*., 2011). The higher glucose concentration immediately after birth may be related to increased levels of corticosteroids during parturition or colostrum intake. Zanker *et al*. (2001) reported that glucose levels were higher than the reference range from birth until day 83 or 120. Gluconeogenesis is essential to cover the glucose requirements in neonates, and both glucagon and cortisol stimulate the process, being essential for glucose homeostasis in calves (Drackley *et al*., 2001). This study did not observe correlations between glucose and cortisol levels.

CONCLUSIONS

This study established a strong correlation between HR, HRV indexes with the levels of some blood plasma electrolytes, glucose and cortisol in newborn calves at different times.

Variations in concentrations and this strong correlation may be attributed to functional development during the adaptive period in calves, and the changes observed in this animal category are necessary for the adaptation to the extra-uterine life. This study contributes to the knowledge regarding the adaptation processes.

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