Physiology & Behavior 139 (2015) 281–289

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Heart rate and heart rate variability in multiparous dairy cows with unassisted calvings in the periparturient period

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HIGHLIGHTS

- We investigated heart rate variability in dairy cows in the periparturient period.
- Vagal tone activity decreased before the onset of calving restlessness.
- Vagal tone increased before calving and decreased after birth.
- Autonomic nervous system activity remained altered until 4–8 h after birth.
- Duration of calving affected cardiac activity during calving and 12–24 h after birth.

ARTICLE INFO

Article history:
Received 31 May 2014
Received in revised form 10 November 2014
Accepted 12 November 2014
Available online 20 November 2014

Keywords:
Behaviour
Heart rate
Heart rate variability
Dairy cows
Unassisted calving

ABSTRACT

Behavioural changes before calving can be monitored on farms; however, predicting the onset of calving is sometimes difficult based only on clinical signs. Heart rate (HR) and heart rate variability (HRV) as non-invasive measures of autonomic nervous system (ANS) activity were investigated in Holstein–Friesian cows ($N = 20$) with unassisted calvings in the periparturient period to predict the onset of calving and assess the stress associated with calving. R–R-intervals were analysed in 5-min time windows during the following three main periods of measurement: 1) between 0 and 96 h before the onset of calving restlessness (prepartum period); 2) during four stages of calving: (I) early first stage; between the onset of calving restlessness and the first abdominal contractions; (II) late first stage (between the first abdominal contractions and the appearance of the amniotic sac); (III) early second stage (between the appearance of the amniotic sac and the appearance of the foetal hooves); (IV) late second stage (between the appearance of the foetal hooves and delivery of the calf), and 3) over 48 h following calving (postpartum period). Data collected between 72 and 96 h before calving restlessness was used as baseline. Besides HR, Poincaré measures [standard deviation 1 (SD1) and 2 (SD2) and SD2/SD1 ratio], the root mean square of successive differences (RMSSD) in R–R intervals, the high-frequency (HF) component of HRV and the ratio between the low-frequency (LF) and the HF components (LF/HF ratio) were calculated. Heart rate increased only following the onset of the behavioural signs, peaked before delivery of the calf, then decreased immediately after calving. Parasympathetic indices of HRV (RMSSD, HF_{norm} and SD1) decreased, whereas sympathovagal indices (LF/HF ratio and SD2/SD1 ratio) increased significantly from baseline between 12 and 24 before the onset of calving restlessness. The same pattern was observed between 0 and 1 h before calving restlessness. Following the onset of behavioural signs, parasympathetic activity increased gradually with a parallel shift in sympathovagal balance towards parasympathetic tone, which was possibly a consequence of oxytocin release, which induces an increase in vagus nerve activity. Parasympathetic activity decreased rapidly between 0 and 0.5 h following calving and was lower than measured during all other stages of the study, while sympathetic activity peaked during this stage and was higher than measured during any other stages. Between 0 and 4 h after calving vagal tone was lower than baseline, whereas sympathovagal balance was higher, reflecting a prolonged physiological challenge caused by calving. Vagal activity decreased, whereas sympathovagal balance shifted towards sympathetic tone with increased live body weight of the calf during the late second stage of calving,

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http://dx.doi.org/10.1016/j.physbeh.2014.11.039

suggesting higher levels of stress associated with the higher body weight of calves. All HRV indices, measured either at the late second stage of calving and between 12 and 24 h after calving, were affected by the duration of calving. Our results indicate that ANS activity measured by HRV indices is a more immediate indicator of the onset of calving than behaviour or HR, as it changed earlier than when restlessness or elevation in HR could be observed. However, because of the possible effects of other physiological mechanisms (e.g. oxytocin release) on ANS activity it seems to be difficult to measure stress associated with calving by means of HRV between the onset of calving restlessness and delivery. Further research is needed to enable more precise interpretation of the prepartum changes in HR and HRV in dairy cattle.

1. Introduction

Optimal reproduction is one of the most important aims of the dairy industry. Parturition is a natural event that involves stress and pain to the dam, therefore the period around calving is a very sensitive time for dairy cows [1]. Monitoring individual cows in the periparturient period is of great importance for decreasing neonatal losses, which is a key to maintaining profitable production on dairy cattle farms [2].

Studying clinical and behavioural signs prepartum is of particular interest in studies involving dairy cattle [3–5]. Labour is traditionally described using three stages [6,7], although there is no clear end and start to these stages, as they progress gradually [1]. The first stage begins with uterine contractions when the cow becomes restless [8,9].

Changes in behaviours associated with calving can be monitored automatically on farms [10] using sensors validated especially for the measurement of lying behaviour [11,12]. Studies on predicting the onset of calving based on non-behavioural external signs such as relaxation of the broad pelvic ligaments [13,14] or hyperplasia of the udder [15] have been carried out extensively; however, variation in the external signs was too great to obtain any valuable information [16,17].

In addition to clinical signs, several physiological markers have been used to predict the time of calving with varying results. Although there is evidence that dairy cows exhibit a distinct decrease in vaginal and rectal temperatures commencing approximately 48 h before calving [13,18,19], the detection of this decrease does not determine the onset of calving precisely [20]. A drop in P4 concentrations before calving has been detected using different on-farm tests [16,21], however, without measuring any clinical or behavioural parameters.

Calving is physically challenging, causes considerable distress to cows [9] and was ranked as one of the most painful conditions experienced by cattle [22]. In animals, the parasympathetic branch of the autonomic nervous system (ANS) plays a key role in regulating heart rate (HR) in response to stress [23–25]. The non-invasive measurement of HR and heart rate variability (HRV), i.e. the short-term fluctuations in successive cardiac interbeat intervals, has increasingly been used for the assessment of pain in calves [26,27] and cows [28,29]. Measurement of cardiac vagal tone by means of the root mean square of successive differences (RMSSD) in consecutive R–R intervals and the high-frequency (HF) component of HRV has been found useful in numerous studies investigating stress in dairy cattle under different physiological conditions [30]. Reduced vagal tone was found in cows subjected to waiting after parlour milking with non-voluntary exit [31], during milking in a novel milking environment [32] and in calves exposed to external stress or pathological loads [33].

The HF component is generally recognised to reflect parasympathetic modulation of the heart influenced by baroreceptor input of vagal receptivity [34,35]. Briefly, decreases in the values of HF reflect a shift towards sympathetic dominance, while increased values indicate a shift towards vagal activity. The low-frequency component (LF) is thought to be closely associated with fluctuations of the peripheral vasomotor tone and reflects the 10-s periodicities, or so-called Mayer waves, of blood pressure [36,37]. LF has been used as a stress indicator in dairy cattle [30], but in most cases it was found to be a poor marker of sympathetic activity [33,38,39] as it is influenced by baroreceptor modulation of both vagal and sympathetic pathways [34,40].

The LF/HF ratio provides essential information on the state of sympathovagal balance in dairy cattle [30]. An increase in the LF/HF ratio is interpreted as a regulatory shift towards sympathetic dominance [41].

To calculate the geometric means of HRV, non-linear Poincaré plots have also been used in dairy cattle studies [31,42] for assessing the vagal regulation of cardiac dynamics. For a recent review on the use of HRV for stress assessment in dairy cattle, see Kovács et al. [30].

To date, prediction of calving based on continuous and detailed monitoring of the ANS in parallel with the animal's behaviour has not been done.

The present study had a dual purpose. First, we investigated HR and HRV parameters to test their usefulness in predicting the onset of calving by the assessment of stress-related changes in ANS activity associated with parturition. The second objective was to identify the impact of certain circumstances of calving (time of day at birth, body condition of the cow, live body weight of the calf, duration of calving) on the animal's cardiac activity 1) between the appearance of the foetal hooves and delivery of the calf and 2) between 12 and 24 h after calving.

2. Materials and methods

2.1. Animals

Our study was conducted as part of a larger research project on metabolic, behavioural and physiological aspects of parturition at the Prograg Agrárcentrum Ltd. in Ráckeresztúr, Lászlópuszta, Hungary, which has a herd of 900 Holstein–Friesian cattle.

A total of thirty-five multiparous cows that calved between October and December 2013 were selected from clinically healthy animals for this study. Three animals that had assisted calving as well as two cows, which were disturbed during parturition (one by her group mates and another due to pharmacological treatment), were not included. Three of the cows that calved before their expected calving date were also excluded from the experiment due to too short measurement lengths. One cow was excluded because she started to calve during data downloading. Three cows that calved in standing position were also excluded as it is well known that HRV is different in standing and lying posture in cattle [39]. Two animals with postpartum health problems (one with retained placenta and one that suffered from downer cow syndrome) were also excluded from the study. Finally, 20 cows (means $\pm$ SD; parity $= 3.4 \pm 1.2$; BCS $= 2.8 \pm 0.3$, locomotion score: 1.6 ± 0.2) were included with spontaneous calving that required no calving assistance or other procedures.

2.2. Selection of animals

From approximately 4 weeks before calving, cows were kept in a 35 m $\times$ 20 m group pen including 60–70 animals, bedded with deep straw. Animals were checked twice a day (at 7:00 a.m. and 6:00 p.m.). During each observation, cows were first visually inspected from a distance for signs of raised tail or a suddenly enlarged, tense udder. Then, after entering the pen, each cow was examined physically in standing position. Criteria used for the selection of the experimental animals included 1) enlargement of the vulva, 2) tenseness and filling of the teats, 3) changes in the quantity and viscosity of vaginal secretions, and 4) relaxation of the sacrosciatic ligaments. Any signs detected on the cows were recorded and compared to previous records during each check. When more than one of the above clinical signs existed, individual cows were moved from the group pen into a 15 m × 10 m separated experimental area having 3.5 m high solid sides made of wood for HRV measurements (Fig. 1). According to retrospective calculation, this was on average 5 days and 12 h before calving. The size of the group kept in the experimental pen was usually between 5 and 12 animals, which could be clearly observed simultaneously (see details in Section 2.3). The composition of the experimental pen was dynamic as cows left the pen to calve, while others got in, but at least five experimental animals were in the experimental pen when the last cow of the block calved in a separate maternity pen, which allowed visual contact between the dam and her herd mates. After calving, cows were left in the maternity pen with their calf for 30 min and then kept in a postpartum group pen for 3 days before being moved to the milking herd.

2.3. Data collection

R–R intervals were recorded continuously using a Polar Equine RS800 CX mobile recording system (Polar Electro Oy, Kempele, Finland) with two integrated electrodes in the measuring belt and a specific transmitter. Transmitters and the two electrodes were positioned as advised by von Borell et al. [25] in their review. To optimise conductivity and to minimise electrical resistance, electrode sites were covered with ample ultrasound transmission gel (Aquaultra Blue, MedGel Medical, Barcelona, Spain).

Because calving is often characterised by an increase in position changes by the cow [5,9], the electrode belt was protected against external impacts by a self-designed girth which contained a pocket for the HR monitor. The girth was strapped around the cow's thorax, immediately behind the forelimbs. This procedure was done before cows were placed into the experimental area. Before data collection was started, animals were allowed an adaptation period of one day to get accustomed to the equipment. Because of the limited storage capacity of the HR receivers (about 25,000 R–R intervals), data were downloaded in each 48 h before calving in a restraining cage placed into the experimental area. After the first postpartum milking, a 10-min period was provided for this procedure, during which the animals were fixed in the milking parlour. During all times of data downloading the electrodes were covered with extra gel.

2.4. Behavioural observations

The posture and behaviour of the cows were video-recorded with a closed-circuit camera system including two day/night outdoor network bullet cameras (Vivotek IP8331, VIVOTEK Inc., Taiwan) installed above the experimental pen in a way that gave the best possible view of the animals. Start and end points of the different periods and stages of the measurement were noted for each animal continuously, allowing subsequent matching of the individual's behaviour and her HR recordings. For ease of visual identification, cows were marked with numbers on their hind legs and backs simultaneously with attaching the HR devices.

Cows were moved from the experimental area to a maternity pen after 40 min of more than one of the signs of calving restlessness being present. We started to move individual animals only when they were in standing posture. The beginning of the time of moving was noted for each cow to allow later exclusion of HRV data recorded 10 min after moving the animals. The signs of calving restlessness were established as follows: increase in the number of lying bouts; increase in the number of activities (e.g. standing, walking); increase in the frequency of tail raises while standing; staring at the abdomen; licking the ground; searching for a hiding place; increase in the frequency of urination and/or defecation. A pre-calving, control observation was also made for each cow for the same behavioural patterns, 3–4 days earlier than the calving observation.

2.5. Periods and stages of measurement

Following the traditional 3-stage way description of labour which is based on both behavioural (calving restlessness) and internal changes (e.g. dilation of the cervix, uterine contractions) it would have been difficult to determine which stage of labour a cow was in before calving. Therefore, we decided to divide prepartum periods of our measurement based on only clearly visible behavioural and clinical signs. Due to the high variation in the duration of calving between individual cows (ranging between 40 and 316 min), the start (the onset of calving restlessness defined as T_01) and the end points (the moment of birth defined as T_02) of this period were fixed and the time lag between T_01 and T_02 was defined as the period of *calving* (Fig. 2). The four main stages of calving are presented in Table 1.

The first main period was then defined as a time lag lasting 96 h before T_01, hereinafter called *prepartum period*. It included 8 stages: 96–72 h, 72–48 h, 48–36 h, 36–24 h, 24–12 h, 12–6 h, 6–1 h and 1–0 h before T_01. The third main period started at T_02 and lasted 48 h after calving; it is hereinafter called *postpartum period*. This main period included 8 stages as follows: 0–0.5 h (between delivery of the calf and removal of the calf from the cow), 0.5–1 h (between calf removal and the first milking of the cow), 2–4 h (following the first milking of the cow), 4–8 h, 8–12 h, 12–24 h, 24–36 h and 36–48 h after calving. During each stage of the prepartum and postpartum periods one sample per hour was chosen for later HRV analysis.

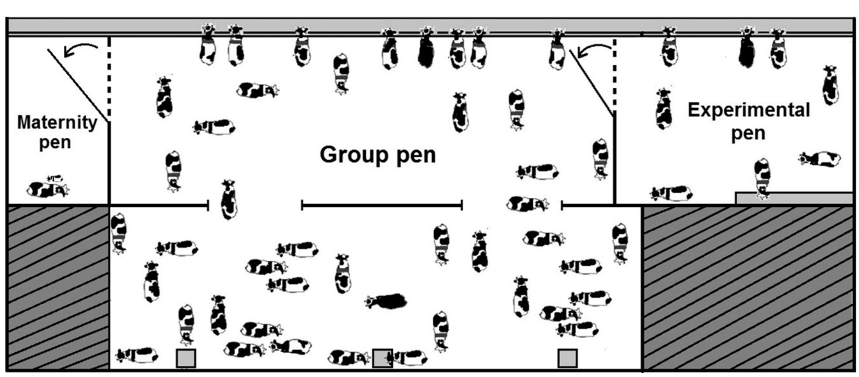

Fig. 1. The spatial arrangement of areas involved in the study.

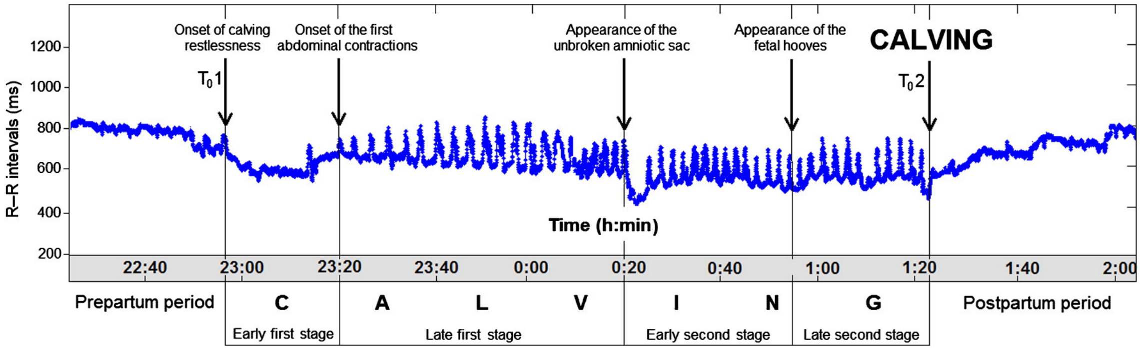

Fig. 2. R–R interval data presented from an experimental animal (using the Kubios 2.1 HRV analysis software). The decrease in R–R-intervals is clearly visible following the onset of calving restlessness ($T_0 1$). Following the onset of abdominal contractions, a periodicity can be observed in the alterations of R–R intervals. During the *late first stage* of calving every period lasted 60–80 s, whereas during the *early* and *late second stages* of calving this interval shortened to 40–50 s. Following calving, a rapid increase in R–R intervals (a decrease in heart rate) can be observed.

2.6. HRV analysis

Devices were removed from the cows 48 h after calving and data were transmitted to a computer via Polar Interface for further analysis. The Kubios HRV software was used for HRV analysis [43].

Ectopic heartbeats were eliminated and artefacts were corrected. Using the custom filter of the programme, R–R intervals differing from the previous R–R interval by more than 30% were identified as artefacts. In addition, a visual inspection of the corrected data was performed to edit any artefact still existing. Parameters in frequency and time domains were calculated as follows. For computing frequency-domain HRV the R–R interval data were subjected to Fast Fourier Transformation (FFT) of power spectrum analysis. We examined equal time periods of 5 min as recommended for the analysis of HRV using FFT in earlier reviews [25,41]. In accordance with earlier studies on adult cattle [33,39], spectral parameters included the normalised power of the high-frequency (HF) band for representing vagal activity and the relative power of the low-frequency (LF) band and HF component (LF/HF ratio) for measuring the sympathovagal balance. The recommendations of von Borell et al. [25] were considered by setting the limits of the spectral components as follows: LF: 0.05–0.20 Hz and HF: 0.20–0.58 Hz. In the time domain, besides HR (beats/min) we quantified RMSSD (ms), which is used to estimate short-term beat-to-beat variations representing vagal regulatory activity [44] and is performed for stress assessment in dairy cows [26,31,45].

For graphical representation of the correlation between successive R–R intervals, where each interval in the time series ($R–R_{i+1}$) is plotted against its successor ($R–R_i$), standard deviation 1 (SD1) and 2 (SD2) were calculated by Poincaré plot analysis according to our recent study [31]. SD1 describes short-term HRV generally caused by parasympathetic activity and SD2 describes long-term variability [46,47], whereas their ratio (SD2/SD1 ratio) reflects sympathovagal balance [42].

For each stage of the prepartum and postpartum periods, R–R interval samples were chosen for HRV analysis during lying posture. Because cows remained recumbent from the onset of abdominal contractions until birth, it was possible to analyse data segments that were recorded while cows were lying during calving (Table 1).

Data of three animals recorded during the early first stage were deleted from the analysis as these animals did not spend enough time lying to allow correct evaluation of HRV. Any practice that increased prepartum standing time was avoided. Since HR and HRV are also affected by physical activity [48], continuous bouts of lying were defined as starting 3 min after a cow had lain down and at least 5 min after finishing rumination. In order to eliminate influences on HRV by the environment, we only considered parts of the R–R data segments 5 min after any kinds of disturbance (sudden noise, presence of people, handler walking close by) or any particular activity or visible social interaction. We excluded from the analysis data that were obtained 10 min before and 30 min after the animals were tethered for data downloading. During the entire sampling period an average of 148 samples per cow were analysed. The average value calculated from individual baselines of each cow measured between 96 and 72 h before calving restlessness was used as a baseline for HR and all HRV parameters. To calculate average values representing cardiac activity during the remaining stages of the periparturient period we used also individual averages of 5-min samples. Fig. 2 presents an illustrative display of R–R interval data around calving.

2.7. Statistics

Statistical analysis was performed using the general linear model (SPSS version 18.0, Chicago, Illinois, USA) with penalised quasi-likelihood. The residuals of the model were inspected graphically for distribution and homogeneity of variances by the Kolmogorov–Smirnov test. Since data were not normally distributed, HR and HRV parameters (dependent variables) were subjected to logarithmic transformations prior to analysis. Covariates [time of day at birth (h:min), body condition score (ranging between 2 and 3.5), live body weight of the calf

Table 1
Description of the four main stages of calving based on the clinical signs.

	Stages of calving	Definition	Number of R–R samples
First stage of calving			
1	Early first stage	Between the first behavioural signs of calving restlessness and the first abdominal contractions[a]	1–2
2	Late first stage	Between the onset of abdominal contractions and appearance of the amniotic sac	1–3
Second stage of calving			
3	Early second stage	Between the appearance of the amniotic sac[b] and appearance of the foetal hooves	2–3
4	Late second stage	Between appearance of the foetal hooves and delivery of the calf[c]	2–4

[a] The first time when the cow is lying on her side, or partially on her side, and the abdominal muscles contract and release in a rhythmic motion.
[b] The unbroken amniotic sac appears outside the vulva.
[c] The calf's hips are fully expelled from the cow.

(kg), duration of calving (min)] were added to the model as fixed factors. For the evaluation of the effects of covariates on the various parameters of HRV, two stages of parturition were chosen: (1) the late second stage of calving (i.e., between appearance of the foetal hooves and delivery of the calf) and (2) between 12 and 24 h after calving. The model included HR and HRV parameters as dependent variables. The covariate time of day was consistently entered into the model first. After the time of day, live body weight of the calf was entered into the model, because the effects of cow condition and calf body weight were to some extent confounded and the effect of live body weight on the cardiovascular system of the calf was considered more informative. Other potential covariates (condition of the dam, duration of calving) were entered after body weight of the calf.

Due to equipment failure, HR and HRV data recordings between 0.5 and 1 h after calving for one cow were missing from the analysis. Pairwise differences between the 20 stages of the main periods [before T_01 (prepartum period), between T_01 and T_02 (calving), and after T_02 (postpartum period)] including the baseline period (i.e., between 72 and 96 h before calving restlessness) were tested by the Bonferroni post-hoc test separately for HR and HRV parameters averaging all 5-min R–R interval samples for individual cows in each stage of measurement. A value of $P < 0.05$ was considered significant. These averages were also used for graphical presentation of the results, which are given as means ± SEM.

3. Results

3.1. Changes in HR and HRV around calving

Changes in HR and HRV parameters during calving and in the prepartum and postpartum periods are summarised in Fig. 3.

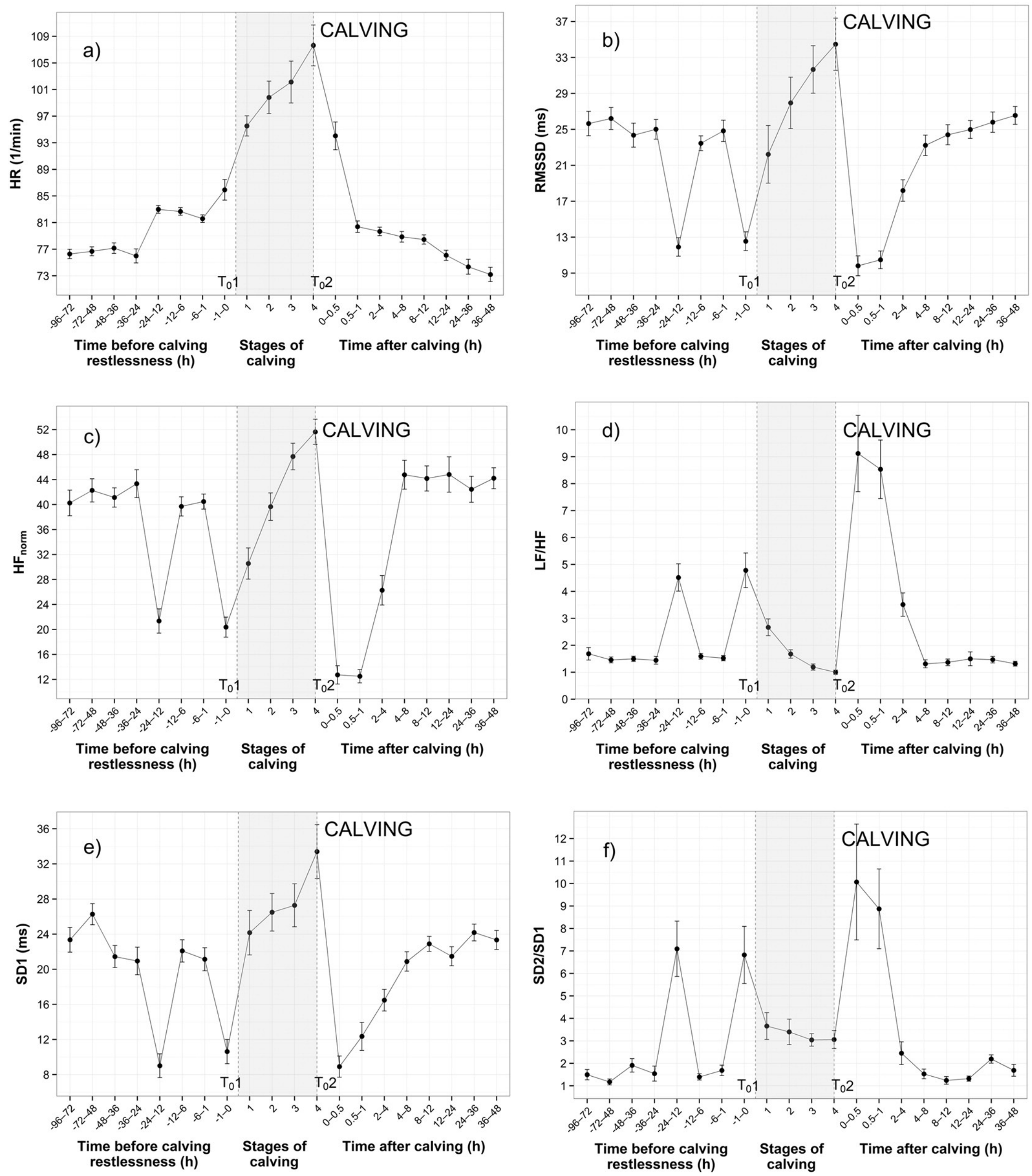

Fig. 3. (a) Heart rate (beats per min), (b) root mean square of successive R–R differences (RMSSD, ms), (c) high-frequency component (HF_{norm}), (d) ratio of low-frequency (LF) and HF components (LF/HF) and Poincaré measures, (e) SD1 (ms) and (f) SD2/SD1 in dairy cows ($N = 20$) with unassisted calving in the periparturient period. Data are means ± SEM. The dashed line vertical to the *X* axis indicates the time of onset of calving restlessness (T_01) and T_02 is defined as the moment of birth. Numbers between T_01 and T_02 indicate the stages of calving (marked with grey on the graphs) as follows: 1 = between the first behavioural signs of calving restlessness and the first abdominal contractions; 2 = between the onset of the first abdominal contractions and appearance of the amniotic sac; 3 = between appearance of the amniotic sac and appearance of the foetal hooves; 4 = between appearance of the foetal hooves and delivery of the calf.

Table 2
Changes in heart rate (HR) and heart rate variability parameters[a] during the late second stage of calving (between appearance of the foetal hooves and delivery of the calf) in Holstein–Friesian cows ($N = 20$) in relation to covariates.

Cardiac parameter	Median ± MAD	Time of day at birth	Live body weight of the calf	Condition of the cow	Duration of the calving
HR (min^{-1})	73.1 ± 1.4	ns	↑23.0**	↑18.6*	ns
RMSSD (ms)	24.8 ± 1.1	ns	ns	ns	↑8.3**
HF_{norm}	44.0 ± 3.8	ns	↓17.4***	ns	↑16.9***
LF/HF	1.4 ± 0.2	ns	↑1.8**	↑1.6*	↓1.5*
SD1 (ms)	20.7 ± 1.2	ns	↓7.8**	ns	↑8.7**
SD2/SD1	1.3 ± 0.1	ns	↑1.7*	↑1.5*	↓2.1**

Statistics are based on the output for the generalised linear model (GLM) based on log-transformed dependent cardiac variables. ↑/↓: parameter increases/decreases with increase in covariates. Descriptive statistics are based on the individual's median ± median absolute deviation (MAD) data.
Statistical significance: ns: non-significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
[a] RMSSD = root mean square of successive R–R interval differences, HF_{norm} = normalised power of the high-frequency band, LF/HF = the ratio between the low-frequency (LF) and the HF band, SD1 = standard deviation of instantaneous R–R variability measured from axis 1 in the Poincaré plot, SD2 = standard deviation of long-term continuous R–R variability measured from axis 2 in the Poincaré plot, SD2/SD1 = the ratio between SD2 and SD1.

Heart rate tended to be higher from 24 to 12 h before the onset of calving restlessness but increased above baseline levels ($P < 0.01$) only after the first behavioural signs of restlessness (in the early first stage of calving) and then peaked during the late second stage of calving (107.5 ± 12.4, difference from baseline: $P < 0.001$, Fig. 3a). Between 0 and 0.5 h after calving, the HR stayed elevated above baseline (98.3 ± 7.6, difference from baseline: $P < 0.001$), then it decreased suddenly between 0.5 and 1 h following calving, and for the remainder of the postpartum period it did not differ from the baseline.

There were no significant changes in parasympathetic (RMSSD, HF_{norm} and SD1) or sympathovagal indices (LF/HF ratio and SD2/SD1 ratio) of HRV between 96 and 24 h prior to calving restlessness (Fig. 3b–f). Between 24 and 12 h before calving restlessness RMSSD, HF_{norm} and SD1 decreased significantly (−117%, −105%, −143%, respectively, $P < 0.001$ for each parameter) and returned to baseline between 12 and 1 h before calving restlessness. In contrast, LF/HF ratio and SD2/SD1 ratio were higher than the baseline values (+163%; $P < 0.01$ and +367%, $P < 0.001$, respectively). The same pattern was observed between 0 and 1 h before the onset of calving restlessness for each HRV parameter (Fig. 3b–f).

Following the first behavioural signs of calving restlessness RMSSD, HF_{norm} and SD1 increased progressively ($P < 0.001$ as compared to 0–1 h before calving restlessness for each parameter), but only after the onset of the first abdominal contractions did they rise significantly above the baseline. RMSSD and HF_{norm} reached baseline levels during the late second stage of calving, whereas SD1 rose more rapidly and reached the baseline level already during the early first stage of calving. During the early and late second stages of calving, RMSSD, HF_{norm} and SD1 continued to increase and peaked before delivery of the calf, exceeding the baseline ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively).

During the early first stage of calving, the LF/HF and SD2/SD1 ratios decreased from the values measured before the onset of calving restlessness (Fig. 3d, f). Following the onset of the first abdominal contractions (late first stage of calving) the LF/HF and SD2/SD1 ratios returned to baseline and stayed there until birth.

RMSSD, HF_{norm} and SD1 dropped rapidly between 0 and 0.5 h following calving (−231%, −323% and −242%, respectively) and were lower than in all other measured stages of the study periods (difference from baseline: $P < 0.001$ in all cases). The LF/HF and SD2/SD1 ratios peaked during this stage (at values 643% and 225% higher than those obtained during the late second stage of calving, respectively) and were significantly higher than the values measured during any other stages ($P < 0.001$ in both cases).

With the exception of SD1, which was higher ($P < 0.05$), none of the HRV parameters measured between 0.5 and 1 h after calving differed from those found in the previous stage of the postpartum period.

RMSSD, HF_{norm} and SD1 increased gradually following calf removal and returned to baseline between 4 and 8 h after calving (e.g. they did not differ statistically from the baseline values). During the remainder of the postpartum period HF_{norm} slightly exceeded the baseline value (Fig. 3b, c, e). In contrast, the LF/HF and SD2/SD1 ratios decreased during that period, then returned to baseline levels between 4 and 8 h after calving, and were subsequently balanced for the remainder of the measurements.

3.2. Effect of covariates on HR and HRV during the late second stage of calving

Covariates other than time of day at birth were statistically significant for at least 3 cardiac parameters during the late second stage of calving (Table 2).

Table 3
Changes in heart rate (HR) and heart rate variability parameters[a] between 12 and 24 h after calving in Holstein–Friesian cows ($N = 20$) in relation to covariates.

Cardiac parameter	Median ± MAD	Time of day at birth	Live body weight of the calf	Condition of the cow	Duration of the calving
HR (min^{-1})	102.7 ± 4.5	ns	ns	↑12.4*	ns
RMSSD (ms)	34.2 ± 2.8	↑4.1*	ns	ns	↑8.3**
HF_{norm}	50.0 ± 1.9	ns	↓13.1**	ns	↑14.7***
LF/HF	1.0 ± 0.1	ns	↑1.2*	↑1.0*	↓1.5*
SD1 (ms)	32.1 ± 3.4	ns	↓6.4**	ns	↑7.3**
SD2/SD1	2.9 ± 0.2	ns	↑1.4*	↑1.3*	↓1.7**

Statistics are based on the output for the generalised linear model (GLM) based on log-transformed dependent cardiac variables. ↑/↓: parameter increases/decreases with increase in covariates. Descriptive statistics are based on the individual's median ± median absolute deviation (MAD) data.
Statistical significance: ns: non-significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
[a] RMSSD = root mean square of successive R–R interval differences, HF_{norm} = normalised power of the high-frequency band, LF/HF = the ratio between the low-frequency (LF) and the HF band, SD1 = standard deviation of instantaneous R–R variability measured from axis 1 in the Poincaré plot, SD2 = standard deviation of long-term continuous R–R variability measured from axis 2 in the Poincaré plot, SD2/SD1 = the ratio between SD2 and SD1.

Heart rate, LF/HF ratio and SD2/SD1 ratio increased, whereas HF_{norm} decreased with higher live body weight of the calf. The condition of the cow affected only HR and sympathetic HRV indices (LF/HF ratio and SD2/SD1 ratio), which were higher in individuals with higher body condition scores during the late second stage of calving (Table 2).

RMSSD, HF_{norm} and SD1 were higher in cows with a longer duration of calving, while sympathovagal balance decreased with increased length of calving (lower LF/HF ratio and SD2/SD1 ratio), while the duration of calving had no effect on HR.

3.3. Effect of covariates on HR and HRV between 12 and 24 h after calving

The time of day (the moment of birth) affected only RMSSD between 12 and 24 h after calving, which increased when the time of calving was later. Heart rate, LF/HF ratio and SD2/SD1 ratio tended to be higher in cows with higher body condition scores ($P < 0.05$), whereas RMSSD, HF_{norm} and SD1 were not influenced by the condition of the cows in the postpartum period (Table 3). Vagal activity measured both in frequency- (lower HF_{norm}) and Poincaré-domains (lower SD1) decreased, while sympathovagal balance increased (higher LF/HF ratio and SD2/SD1 ratio) in cows that had calves with higher live body weight (Table 3).

Between 12 and 24 h after calving, all parameters apart from HR were influenced by the duration of calving. Consistent with the results obtained during the late second stage of calving, vagal activity increased (higher RMSSD, HF_{norm} and SD1) while sympathovagal indices decreased (lower LF/HF ratio and SD2/SD1 ratio) in cows with a longer duration of calving.

4. Discussion

The prepartum behavioural patterns of dairy cows have been studied extensively, which suggests that they have the potential to provide important information on the progress of parturition [5,8,9]; however, there is a lack of works exploring whether, in addition to the animal's behaviour, physiological indicators of stress can also be used for predicting the onset of calving. Therefore, the present study was designed to investigate changes in ANS activity on the basis of HRV before and following the onset of calving restlessness. Experimental and field studies provide strong evidence that the evaluation of HRV is useful for the assessment of cardiac autonomic regulation in farm animals [25]. Until now, studies on the changes of HRV parameters in dairy cattle have been scarce as long-term data recording might pose difficulties under field and unrestrained conditions in loose-housed large animals [30].

Our study provided new data on ANS activity using HRV parameters determined in the periparturient period in dairy cattle. HRV parameters measured between 24 and 12 h and between 0 and 1 h prior to calving restlessness indicated a decrease in vagal tone and a shift towards stronger dominance of the sympathetic branch of the ANS. Several authors have found a significant reduction of vagal activity in cattle exposed to acute stress [27,31,32,39,45]; however, they also reported an increased HR parallel to reduced parasympathetic activity. The lack of HR changes between 12 and 24 h and between 0 and 1 h before the onset of calving restlessness suggests that HR responses are not a direct result of impaired ANS activity and that a decrease in parasympathetic tone is not necessarily accompanied by elevated HR. Our results support earlier findings suggesting that HR provides information only on the net effects of all components affecting cardiac activity and is of limited use for accurately assessing sympathovagal regulation [35,49,50].

There may be several reasons for the impaired fluctuation in cardiac autonomic tone observed between 12 and 24 h before calving restlessness. One explanation could be the calf's intrauterine dislocation in preparation for parturition and the onset of the first uterine contractions parallel to cervical dilatation. It must be noted that the time frame of the decrease in vagal tone was not consistent with any stress-related behaviours or unusual environmental effects existing during this stage.

A reduced vagal tone was found with a parallel rise in sympathovagal indices and without any changes in HR between 0 and 1 h before the onset of the first behavioural signs. Our finding confirms an earlier statement suggesting that the wellbeing of animals might be impaired even when signs of stress are not obviously visible [51]. This decline in vagal tone was possibly indicative of acute visceral pain associated with the first uterine contractions and dilation of the cervix, which was only later reflected in restlessness behaviour. Although specific pain responses were not measured in this study, an earlier report supports our hypothesis according to which in women the intensity of pain experienced during childbirth correlates with cervical dilation [52].

Since strong physiological loads are known to cause a decrease in vagal activity according to the polyvagal theory of Porges [53], the lowest level of vagal activity was expected during the early and late second stages of calving. However, in the present study, a gradual elevation in parasympathetic tone and a shift in sympathovagal balance towards parasympathetic activity were found from the onset of calving restlessness until birth. The elevated parasympathetic activity was possibly a consequence of the highly increased maternal oxytocin levels which were observed earlier in association with uterine contractions and cervical dilatation in cows when the foetus entered the birth canal [54,55]. During the stage of expulsion, the hooves and the nose of the calf stretched the cervix, further stimulating the release of oxytocin and initiating the abdominal press [56]. In humans, oxytocin release induces an increase in vagal nerve activity [57]; therefore, it can be assumed that the increased concentrations of maternal oxytocin were sufficient to override the expected effect of physiological load associated with the expulsion of the calf on the functioning of the cow's ANS.

Nevertheless, in accordance with the findings of a recent study on cows with unassisted calving [58], animals became recumbent from the start of the first abdominal contractions and remained in this position until birth, and maximum HR was registered during the late second stage of calving. Most of the restlessness behaviours were observed during the early first stage of calving and became less frequent after the movement of cows to the maternity pen, and the animals made no attempts to escape. In addition, all animals involved in this study calved without any assistance or presence of people. Therefore, we concluded that elevated HR during calving is not due to isolation or disturbance by handlers; rather, it is simply an indicator of increased physical activity during calving.

Based on our results, the measurement and interpretation of stress associated with calving seem to be difficult by HRV analysis because of the possible confusing effects of a complex cascade of physiological events involved in parturition (e.g. oxytocin release and increased physical activity due to expulsion of the calf).

It is possible that the measurement of cortisol concentrations could lead to a more detailed evaluation of stress; however, serial blood sampling around parturition seriously hampers HRV data collection due to the disturbance caused to the animals. Furthermore, according to recent studies ANS measurements have advantages over measuring HPA activity when investigating responses to stress, as they provide more immediate information [29,59]. Due to the rigorous sampling regime for HRV analysis only the R–R data recorded during lying were analysed, thus a more intensive sampling was also impossible during calving for a more precise evaluation of ANS activity, since animals were more active after the onset of calving restlessness than before that.

Decreased parasympathetic activity and the shift of sympathovagal balance towards sympathetic tone between 0 and 1 h after calving are suspected to be related to the degradation of released oxytocin rather than being the result of significant stress load. The heart rate confirms this hypothesis, since it returned to baseline following calf removal (between 0.5 and 1 h after calving) and was consistent with that found in an earlier study on cow–calf separation using multiparous dairy cows [60].

It is worth mentioning that increased sympathetic tone was paralleled by a sudden decline in HR after calving. Decreased HR was possibly driven by the reduced physical activity [61], as following birth the contractions of the uterus expulse the foetal membranes without any abdominal press [62,63], the latter playing a dominant role only in foetal expulsion [56].

It should also be noted that although data were collected while the cows were lying, a state in which sympathetic tone is normally very low, animals had high levels of activity before sampling due to licking their calf which may have raised the sympathetic tone after calf removal. Our results suggest that calving has a prolonged effect on ANS activity. HRV parameters returned to baseline only between 4 and 8 h after calving, indicating a persistent reduction of vagal tone and a shift in sympathovagal balance towards sympathetic tone. Since homeostasis can be defined as an autonomous state characterised by increased vagal activity [64], our results revealed a greater general challenge to the cardiovascular system of cows in the first 4–8 h of the postpartum period than thereafter. This might be associated with pain associated with the uterine contractions after calving, which play a key role in foetal membrane expulsion and generally occur within 8 h after birth [6].

The present study has demonstrated that HRV parameters, but not HR, were highly sensitive indicators of the duration of calving. A prolonged duration of calving was associated with a higher vagal tone and a decrease in sympathovagal indices both during the late second stage of calving and between 12 and 24 h in the postpartum period. Since oxytocin is released in an episodic manner in conjunction with uterine and abdominal contractions [65], higher parasympathetic activity measured during the late second stage of calving might be the result of a higher concentration of released oxytocin in animals with a more prolonged calving. A similar pattern was observed between 12 and 24 h postpartum. Whether the latter finding is the linear consequence of the former or it has a more complex background still remains unclear, it seems that prolonged calving has a supportive effect on ANS function as was reflected by higher vagal activity in the affected animals.

As expected, higher body weight of the calves reduced the parasympathetic tone and increased the dominance of sympathetic tone during the late second stage of calving, reflecting a stronger physiological challenge. This is inconsistent with the findings of an earlier study in which RMSSD and HF were lower in the presence of higher levels of pain caused by laminitis in horses [66]. Our results also suggest the opposing effect of stress on the possible action of oxytocin resulting in increased vagal tone during calving.

As expected, sympathetic tone was higher in cows with a higher body condition score either during the late second stage of calving and between 12 and 24 h in the postpartum period. This suggests that calving is more challenging for animals with higher body condition scores. Time of day at birth was the only covariate with no influence on any of the HRV parameters during the late second stage of calving. Time of day at birth had an effect only on RMSSD between 12 and 24 h after calving and, as it had no effect on any other parameters, we supposed that the time of day at birth is irrelevant for evaluating postparturient cardiac activity.

Although our study was unable to determine the levels of stress and pain before parturition, it has become clear that after calving a rapid decrease in vagal tone occurred with a parallel increase in sympathetic nerve activity. Our results may indicative the presence of pain between 0 and 1 h before the onset of calving restlessness, since earlier studies reported on extensive interactions between neural structures involved in pain sensation and ANS control [67]. However, further research is needed to provide an accurate explanation for the changes occurring in ANS activity in the prepartum period. These studies may help to gain some insight into the physiological mechanisms underlying bovine parturition, which may be useful in evaluating the levels of stress and pain associated with unassisted calving.

5. Conclusions

Unassisted calving is associated with characteristic alterations in the ANS regulation of the cardiovascular system both in the prepartum and postpartum periods. We found a decrease in parasympathetic activity between 12 and 24 h and during the last hour before the onset of behavioural signs of calving restlessness. Although the physiological background of this phenomenon seems to be complex, we can conclude that changes in HRV are more immediate predictors of the onset of calving than behaviour or HR, as they occurred before the onset of calving restlessness. Between the first behavioural signs of calving restlessness and the delivery of the calf, increased vagal activity was accompanied by a parallel decrease in sympathetic activity. Between 4 and 8 h after calving, we observed a postpartum recovery in ANS functioning. The duration of calving had the most prominent effect on HRV parameters both during the late second stage of calving and between 12 and 24 h after birth. An increased live body weight of the calf resulted in a decrease of parasympathetic tone during the late second stage of calving, suggesting that expulsion of a larger calf induced higher levels of stress for cows with unassisted calvings in this period.

Acknowledgements

The authors would like to thank Ferenc Bodó, the owner of the farm for supporting the study, Dr. Szabolcs Boldizsár for his valuable technical assistance and the farm staff of Prograg Agrárcentrum Ltd. at Ráckeresztúr, Lászlópuszta for taking care of the animals during the experimental period. We are indebted to Dr. András Székely for correcting the English. The study was partly supported by the GOP — 1.1.1-11-2012-0153 project and the Research Centre of Excellence — 8526-5/2014/TUDPOL project.

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