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Timing of obstetrical assistance affects peripartal cardiac autonomic function and early maternal behavior of dairy cows

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HIGHLIGHTS

- Calving in a group is less stressful than calving in a maternity pen.
- Appropriately timed obstetrical assistance supports expression of maternal behavior.
- Premature obstetrical assistance results in high levels of stress during parturition.
- Premature assistance elongates SNS activation and inhibits early maternal behavior.

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ABSTRACT

Peripartal autonomic nervous system function and early maternal behavior were investigated in 79 multiparous Holstein-Friesian cows. Animals were allocated into four groups based on the technology of calving management: 1) unassisted calving in a group pen (UCG; $N = 19$), 2) unassisted calving in an individual pen (UCI; $N = 21$), 3) assisted calving with appropriately timed obstetrical assistance (ACA; $N = 20$), and 4) assisted calving with premature obstetrical assistance (ACP; $N = 19$). Heart rate, the high frequency (*HF*) component of heart rate variability (*HRV*) as a measure of vagal activity and the ratio between the low frequency (*LF*) and HF components (*LF/HF ratio*) as a parameter of sympathetic nervous system activity were calculated. Heart rate and HRV parameters were presented as areas under the curves (*AUC*) for the following periods: 1) prepartum period (between 96 h before the onset of calving restlessness and the onset of restlessness), 2) parturition (between the onset calving restlessness and delivery), and 3) postpartum period (during a 48-h period after delivery). Pain-related behaviors were recorded during parturition (i.e., the occurrence of vocalization and stretching the neck towards the abdomen) and during a 2-h observation period after calving (i.e., the occurrence of vocalization, stretching the neck towards the abdomen and the duration of standing with an arched back). Early maternal behavior was observed during the first 2 h following calving as follows: 1) latency and duration of sniffing calf's head/body, and 2) latency and duration of licking calf's head/body. No difference was found across groups in autonomic function before the onset of calving restlessness. Area under the heart rate curve was higher in ACP cows during parturition (39.6 ± 2.5 beats/min × h) compared to UCG, UCI and ACA animals (AUC = 13.1 ± 0.9 beats/min × h, AUC = 22.3 ± 1.4 beats/min × h and AUC = 25.0 ± 2.1 beats/min × h, respectively). Area under the heart rate curve did not differ across the UCG, UCI and ACA groups during the postpartum period (AUC = 65.2 ± 16.7 beats/min × h, AUC = 58.0 ± 14.2 beats/min × h and AUC = 62.9 ± 12.1 beats/min × h, respectively) but it was higher in ACP cows compared to the former groups (AUC = 269.1 ± 36.3 beats/min × h). During parturition, area under the HF curve reflected a lower vagal tone (AUC = -30.5 ± 1.6 n.u. × h) in cows with premature obstetrical assistance than in animals that calved individually without farmer assistance (AUC = 2.7 ± 0.4 n.u. × h) or with appropriately timed assistance (AUC = 3.2 ± 1.2 n.u. × h). During parturition, LF/HF ratio showed greater sympathetic activity in ACP cows than in animals from any other group. Area under the HF curve was similar across UCG, UCI and ACA cows (AUC = -232.1 ± 42.0 n.u. × h, AUC = -163.4 ± 35.6 n.u. × h and AUC = -331.4 ± 56.2 n.u. × h, respectively) during the postpartum period and was the lowest in ACP cows (AUC = -1025.6 ± 44.2 n.u. × h) reflecting a long-term stress load in the latter group. During parturition, both vocalization and stretching the neck towards the abdomen occurred more often in UCG cows than in cows from any other groups, and the incidence of both behaviors was statistically higher in ACP cows than in

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UCI and ACA animals. There were no significant differences across groups in these behaviors during the 2-h postpartum observation. UCG cows had a shorter latency and a longer duration of maternal grooming during the first 2 h following delivery compared to any other groups. UCI and ACA dams spent more time with licking the calf within the 2-h period after calving and had a shorter latency to sniff and lick the offspring compared to cows that received premature assistance. Group calving is less stressful for cows than calving in an individual pen either with or without obstetrical assistance. Calving in a group or with appropriately timed farmer assistance supports the expression of early maternal behavior and lead to a rapid postpartum recovery of the autonomic nervous system. Premature obstetrical assistance means stress for cows during parturition, leads to a prolonged postpartum recovery of the autonomic nervous system and inhibits the expression of early maternal behavior.

1. Introduction

Parturition is a natural process; however, it is a challenging and high-risk event for both the cow and her offspring. Thus, careful management during the peripartal period is crucial in terms of welfare and production. However, less time is available for individual attention in large scale farms [1], which is already occurring with the 'lost in the herd' and 'loser cow' syndromes [2]. Careful assistance can help minimize unnecessary pain and distress during calving, care must be taken when deciding on the necessity and timing of assistance during calving as unnecessary or premature intervention can cause injuries in the birth canal [3]. Based on our personal experience, assisted calvings in Holstein-Friesian cows occur in around half of the births in Hungarian large-scale farms. It is thus questionable whether the assistance given is necessary in all of the cases and whether unnecessary assistance has consequences in terms of animal stress and behavior.

Parturition a complex process triggered by the fetus, it involves alterations in autonomic nervous system (*ANS*) activity [4]. In recent years, heart rate and heart rate variability (*HRV*) have become generally accepted indicators of animal stress reflecting on the balance of the ANS [5] and therefore, they are often used in dairy cattle welfare studies [6]. Assessment of the activity of cardiac vagal tone by means of the normalized power of the high frequency (*HF*) component of HRV is increasingly used for investigating acute [7,8] and chronic [9] physiological effects of stress in dairy cows. The low-frequency (*LF*) component of HRV is closely associated with fluctuations of the peripheral vasomotor tone and reflects the 10-s periodicities, or the so-called Mayer waves, of blood pressure [10]. There are several studies reporting LF to be a poor marker of sympathetic activity in humans [11] and in dairy cattle as well [12–14] as it is influenced by baroreceptor modulation of both vagal and sympathetic pathways [11,15].

However, the *LF/HF ratio* [the ratio of the LF and the HF components] is an appropriate indicator of stress in domestic species [5] as it provides information on the sympathovagal balance and sympathetic activation of the ANS, even in dairy cattle [6]. We found that both HF and LF/HF parameters are better predictors of approaching calving than the onset of calving restlessness, and postpartum stress was also reflected by decreasing vagal activity parallel with a rapid sympathetic activation in multiparous cows with spontaneous calving [4]. In contrast to the numerous studies available on dystocia (difficulties at calving), only a few studies have examined the effects of any assistance provided at the time of calving. Although pain, distress and injuries caused by dystocia in individual animals receive less attention in large-scale herds [16] no research has evaluated the effect of the timing of obstetrical assistance on the stress status of cows during parturition and in the early postpartum period using physiological indicators.

Starting immediately after giving birth the dam used to nurse her calf during the first day [17]. However, in modern dairy farming systems, cow and calf are usually separated after calving, which has production efficiency and health concerns [18]. In dairy cattle, as in most other mammals, the dam licks the calf (within 90 min after calving) and some of them may eat the afterbirths (usually between 2 and 6 h after calving). These processes have roles in terms of evolution, adaptation and health; therefore, in some dairies cow and calf are kept together for the first 2 h after birth. Licking the calf has obvious advantages as an anti-predator behavior [19], ensures the continuity of the dams' entodiniomorph rumen ciliates [20], stimulates the calf to stand and is considered to essential for calves' later social behavior [17].

Expressions of early maternal behaviors and parent-offspring interactions have been studied in individual calving pens [21] and in semi-natural environments [22]. However, the effect of any assistance given at calving on the early contact between the dam and her offspring is not known so far. The few works on the associations between peripartal behavior and obstetrical condition focused mainly on behavioral changes before parturition [23,24].

The main aim of the present study was to find out whether the timing of obstetrical assistance (premature vs. appropriately timed) and the management of calving (individual vs. group calving) have an impact on cow stress status during calving and in the early postpartum period. Our second aim was to identify differences in early maternal behavior associated with the animals' obstetrical condition. For this purpose, besides heart rate we calculated ANS-related HRV parameters from the consecutive R–R intervals as indicators of animal stress. It was hypothesized that premature assistance at calving could impair cows' ANS function both during the delivery process and in the early postpartum period and affects early maternal behavior as well.

2. Materials and methods

2.1. Animals

This field study was carried out in a commercial dairy farm in Hungary with around 900 lactating Holstein-Friesian cows having a 48% prevalence rate of assisted calving in the study year. One hundred and twenty multiparous cows that calved between 15 October and 13 December 2013 were allocated for the investigation. Cows with any pharmacological treatment ($N = 2$) or with a recent history (less than two months) of clinical mastitis ($N = 6$), as well as animals suffering from lameness ($N = 10$) were excluded. Cows which were disturbed due to pharmacological treatment during parturition ($N = 5$) were not included. Fifteen cows that calved before their expected calving date were also excluded from the experiment due to the too short measurement lengths. Three animals were excluded due to malfunction of heart rate receivers. Finally, 79 cows (means ± SD; age = 5.9 ± 0.5; parity = 3.2 ± 0.3; body condition score = 3.3 ± 0.1) were involved in the experiment. Before calving, cows were kept in a pre-calving group pen, which included around 50 animals. Housing and feeding of the animals are described in our recent study [4].

2.2. Parturition management and experimental groups

According to the farm practice, cows calved in the group pen or in a separated maternity pen measured 4 × 5 m where obstetrical assistance was provided. Supervision of the dams during calving, the decision to move them into the maternity pen or to provide assistance was made by the farm personnel. Calving personnel moved cows to the separated maternity pen if the calving would have been disturbed by group mates or if assistance was required. Based on the decision of farm staff, calvings were divided retrospectively into four groups by considering the management of calving (maternity pen or group pen) and the prevalence (assisted or unassisted) and timing of obstetrical assistance (premature vs. appropriately timed) as follows: 1) unassisted calving in the group pen (UCG, $N = 19$), 2) unassisted calving in an individual pen (UCI, $N = 21$), 3) assisted calving with appropriately timed assistance (ACA, $N = 20$) and 4) assisted calving with premature assistance (earlier than in ACA cows) (ACP, $N = 19$). The ACA and ACP groups were defined taking into account recommendations on the timing of obstetrical assistance [19]. The findings from this study suggested that calving personnel should start assisting cows 70 min after amniotic sac appearance (or 65 min after feet appearance) outside the vulva. In ACA cows, the onset of obstetrical assistance ranged between 74 and 87 min (means ± SD = 81.7 ± 4.6) and between 67 and 80 min (means ± SD = 72.3 ± 4.9) after amniotic sac and fetal hooves appearance, respectively. In ACP dams, the onset of obstetrical assistance appeared earlier than recommended, ranging between 18 and 61 min (means ± SD = 36.2 ± 9.8) and between 8 and 45 min (means ± SD = 26.9 ± 12.2) after amniotic sac and hooves appearance, respectively and calving personnel started to assist mostly without notable behavioral signs of pain or distress of the cow.

The means (±SD) ages of UCG, UCI, ACA and ACP cows were 48.6 ± 2.1, 47.3 ± 2.4, 46.6 ± 2.0 and 49.7 ± 3.1 months, respectively. The mean (±SD) weights of the calves born to UCG, UCI, ACA and ACP cows were 41.2 ± 1.4, 39.3 ± 1.8, 40.2 ± 1.8 and 40.8 ± 2.1 kg, respectively. None of the focal animals required veterinary assistance at calving. In cases of assisted calvings, the start of obstetrical assistance was considered the time when at least one person assisted the cow in the calving pen using a calving rope or a calf puller, without leaving the pen for >2 min. Assisted and UCI dams were moved to the individual pen following the appearance of the amniotic sac. Unassisted cows (i.e., UCG and UCI animals) calved without any human involvement at any point during the delivery process. Newborn calves were removed from their mothers 120 min following delivery, right before the first postpartum milking. Following calf removal, the dams were kept in postpartum pens for 5 days before being introduced to the milking herd.

2.3. Behavioral observations

Prepartum behavior of individual cows was observed by the first and the second author with a closed-circuit camera system including two day/night outdoor network bullet cameras (Vivotek IP8331, VIVOTEK Inc., Taiwan) installed above the pre-calving group pen allowing the identification of the onset of calving restlessness and subsequent matching of the stages of the measurement (see Section 2.4 for details) and heart rate recordings. The onset of calving restlessness was established according to generally accepted behavioral predictors such as lying down frequency, tail raising and walking [23].

Behaviors reflecting pain or discomfort were observed for parturition (between the onset of calving restlessness and delivery), and during the first 2 h following calving in the group pen and in the maternity pen. The occurrence and frequency of vocalization and stretching the neck towards the abdomen were considered as signs of pain discomfort [26]. Both behaviors were normalized for 1 h. During postpartum observation, vocalization towards the newborn or alien cows was not registered. The duration of standing with an arched back was recorded for the 2 h post-partum period as indicator of abdominal pain [27]. In cases of individual calvings, animals were observed with two portable video cameras after placing cows into the maternity pen (Legria HF M36, CANON, Tokyo, Japan). Following Jensen [21], early maternal behavior was recorded during the first 2 h following calving as follows: 1) sniffing calf's head/body (muzzle in contact with, or in close proximity of, the calf's head or body) and 2) licking calf's head/body (tongue in contact with the calf's head or body). The latency and duration of sniffing and licking behaviors was calculated for each cow.

2.4. Processing of R–R interval data and observational periods

R–R intervals were recorded using a Polar Equine RS800 CX mobile recording system with a Polar T56H electrode belt with two integrated electrodes and a Polar H2 transmitter (Polar Electro Oy, Kempele, Finland). Electrodes were positioned as advised by von Borell et al. [5] and were fitted to the animals as described earlier [4] between 10 and 12 days before the expected time of calving. Animals were then released back to the pre-calving group pen. Before the recording period, a 24-h period was given to the animals to get accustomed to the equipment. Because of the limited storage capacity of the heart rate receivers (about 25,000 R–R intervals), data were downloaded in each 48 h before calving. After calving, this procedure was done immediately after the first postpartum milking when animals were in the milking parlor. Devices were removed from the cows 48 h after calving and R–R interval data were transmitted to a computer via Polar Interface. The Kubios HRV software (version 2.2, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used for HRV analysis [28]. Artifacts were corrected as described in our recent study [9]. For computing frequency domain HRV, R–R intervals were subjected to Fast Fourier Transformation (*FFT*) of power spectrum analysis. Spectral parameters included the normalized power of the HF band for representing vagal activity and the LF/HF ratio was used as a sympathetic measure. The recommendations of von Borell et al. [5] 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, the heart rate was quantified. We examined 5 min length samples of R–R interval segments as recommended for the analysis of HRV using FFT [29].

Analysis of R–R interval data was performed for the following periods: 1) prepartum period; between 96 h before the onset of calving restlessness and calving restlessness, 2) parturition; between the onset calving restlessness and delivery, and 3) postpartum period; between delivery and 48 h after birth. Time of delivery was determined as the time when the calf's hip was fully expelled from the vulva. Prepartum period included 12 measurement points as follows: 96–72, 72–48, 48–36, 36–24, 24–18, 18–12, 12–6, 6–4, 4–2, 2–1, 1–0.5, and 0.5–0 h before calving restlessness.

Four measurement points were chosen for analysis during parturition (two 5-min samples per measurement point), balanced for the duration of calving (which ranged between 72 and 243 min), including one 5-min sample at the time of the beginning of the obstetrical intervention in cases of assisted calvings. For the detailed monitoring of ANS responses to calving, postpartum period included 17 measurement points as follows: 0–0.5, 0.5–1, 1–1.5, 1.5–2, 2–3, 3–4, 4–6, 6–8, 8–10, 10–12, 12–16, 16–20, 20–24, 24–30, 30–36, 36–42, and 42–48 h after birth. For each measurement point of prepartum and postpartum periods, two 5-min samples per h were chosen for HRV analysis. For the entire recording period, 5-min samples were involved into the HRV analysis based on the following two criteria: 1) the cow is lying/standing without any disturbance from herd mates; 2) the cow has finished feeding and/or walking at least 5 min before the start of data recording. To calculate mean values for cardiac activity for measurement points, we used individual averages of 5-min samples. We excluded from analysis data that were obtained during moving cows to the maternity pens (in cases of individual calvings), and data recorded 10 min before and 30 min after the animals were tethered for data downloading.

2.5. Statistical evaluation

All statistical analyses were performed in the R 3.0.2 statistical environment and language [30]. Data were tested for constant variance (Levene's test) and the Shapiro–Wilk test was used for testing normal distribution. A log transformation was used before analysis of the frequency domain measures of HRV (HF and LF/HF ratio). Then, repeated measures ANOVA was followed for the evaluation of cardiac autonomic activity separately for calving groups. Statistical significances were

Table 1
Duration and frequency of pain-related behaviors (means ± SEM) of the dam observed between the onset of calving restlessness and delivery (parturition), and during the first 2 h postpartum.

Pain related behaviors	Calving groups[1]				Statistics	
	UCG	UCI	ACA	ACP	$F_{3,78}$	P
During parturition						
Vocalization (1/h)	8.6 ± 1.1[a]	1.2 ± 0.2[b]	1.28 ± 0.8[b]	2.4 ± 0.8[c]	26.2	0.002
Stretching the neck towards the abdomen (1/h)	12.8 ± 2.5[a]	3.3 ± 0.3[b]	2.8 ± 0.4[b]	3.5 ± 0.6[c]	35.6	0.001
During 2 h following calving						
Vocalization (1/h)	1.2 ± 0.5	1.8 ± 0.4	1.7 ± 0.3	1.4 ± 0.7	6.7	NS
Stretching the neck backwards (1/h)	1.7 ± 0.3	1.9 ± 0.6	1.8 ± 0.4	2.0 ± 0.8	4.8	NS
Arched back (min/h)	*	*	1.2 ± 0.6	2.1 ± 1.4		

F-statistics are based on results from the ANOVA. Statistical significances between groups are based on Tukey's test. Means with different superscripts within a row are significantly different ($P < 0.05$). NS = non-significant.

[1] UCG = unassisted calving in a group pen ($N = 19$), UCI = unassisted calving in an individual pen ($N = 21$), ACA = assisted calving with appropriately timed assistance ($N = 20$), ACP = assisted calving with premature assistance ($N = 19$).

* No occurrences of behavior (0.0 ± 0.0).

calculated for mean heart rate, HF and LF/HF ratio between all time points of measurement (means ± SEM). For this purpose, Tukey's *post-hoc* test was used. The level of significance was set at $P < 0.05$.

For the evaluation of the effects of obstetrical intervention in terms of cow welfare, the cardiac autonomic activity of calving groups was compared. For this purpose, HRV parameters were calculated as area under the curve (*AUC*), which reduced the number of statistical comparisons across calving groups. Using this method, the statistical analyses can be simplified by transforming the multivariate data into univariate space [31], especially in our case, when the numbers of repeated measurements were high (35 measurement points). Maximum heart rate and LF/HF ratio and minimum HF were chosen as short-term indicators of ANS function. Long-term measures included time to return to baseline (the time interval required to return to baseline from the delivery of the calf) and AUC. For time to return to baseline a 5% threshold value was established from baseline. AUCs, maximums, minimums, and times to return to baselines were calculated for each individual and the averaged values were used for comparisons across groups. All parameters were calculated for prepartum, parturition and postpartum periods. To determine AUC, we used a trapezoid method, which was described by Lay et al. [32] as follows:

$$\text{AUC} = \Sigma[(C_n + C_{n+1})/2 \times h - \text{BASELINE}]$$

where 'C' is a value of a cardiac parameter at a given time point, 'h' is the time in hours between the two C-values, and 'baseline' is the mean value of heart rate, HF and LF/HF for the first three measurement points (calculated from the first 12 R–R interval samples, between 96 and 36 h before the onset of calving restlessness). To determine AUC for parturition, the duration of individual parturitions was used. Postpartum AUC was determined for the period of time to return to baseline. Data were tested for normal distribution (Shapiro–Wilk test) and for equality of error variances with the modified robust Brown-Forsythe Levene-type test based on the absolute deviations from the median. AUCs, maximums and minimums (the latter in the case of HF) were compared across groups with the Kruskall–Wallis test. Pairwise comparisons between means were performed by the Wilcoxon rank sum test with Bonferroni adjustment. The level of significance was set at $P < 0.05$.

Counts of behavioral events related to pain and discomfort were normalized to a frequency per hour for both parturition (between the onset of calving restlessness and delivery) and the first 2 h post-partum. After verifying normality of data (Kolmogorov–Smirnov test) for both behaviors and for the durations of standing with an arched back (in case of post-partum observations) an ANOVA was followed and Tukey's *post-hoc* test ($P < 0.05$) was used to compare means between groups. Parameters of early maternal behavior (i.e., latency and duration of sniffing and licking the calf) were determined the ANOVA and pairwise comparison of means between groups was carried out with Tukey's *post-hoc* test ($P < 0.05$).

3. Results

3.1. Behavior around calving

Between the onset of calving restlessness and delivery, both vocalization and stretching the neck towards the abdomen occurred more often in UCG cows than in cows from any other groups (Table 1). Although the incidence of both behaviors was statistically higher in ACP cows than in UCI and ACA animals during parturition, the occurrence of these behaviors were low in all groups calved in the maternity pen. There were no significant differences across groups in vocalization and stretching the neck towards the abdomen during the 2-h post-partum observation (Table 1). Within the first 2 h after delivery, ACP cows spent slightly more time with standing with an arched back than animals from the other groups, however, the incidences and duration of this behavior in the other groups were too low to enable statistical comparisons to be made.

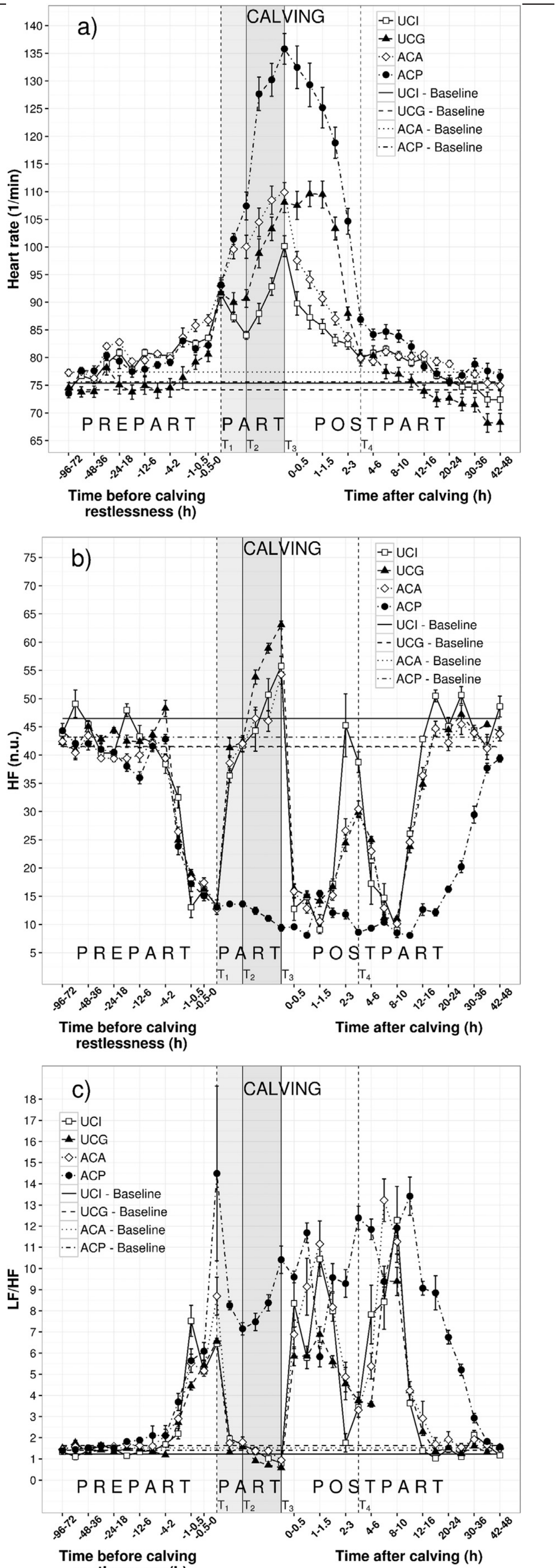
Cows in UCG group had a shorter latency to sniff and lick their calf during the first 2 h following delivery as well as a longer duration of sniffing and licking the calf compared to any other groups (Table 2).

Table 2
Duration and frequency of maternal behaviors (means ± SEM) of the dam observed for the first 2 h postpartum.

Behavioral parameters	Calving groups[1]				Statistics	
	UCG	UCI	ACA	ACP	$F_{3,78}$	P
Latency of lick calf head/body (min)	2.5 ± 1.0[a]	6.8 ± 1.8[b]	8.5 ± 2.3[b]	27.4 ± 4.9[c]	41.0	0.002
Latency of sniff calf head/body (min)	1.6 ± 0.8[a]	4.3 ± 1.2[b]	4.8 ± 1.4[b]	22.6 ± 5.8[c]	37.7	0.003
Duration of lick calf head/body (min/h)	52.8 ± 2.5[a]	40.3 ± 6.6[b]	38.7 ± 5.4[b]	15.1 ± 3.5[c]	36.2	0.012
Duration of sniff calf head/body (min/h)	5.6 ± 1.4[a]	3.3 ± 0.6[b]	2.8 ± 0.4[bc]	2.0 ± 0.5[c]	22.4	0.030

F-statistics are based on results from the ANOVA. Statistical significances between groups are based on Tukey's test. Means with different superscripts within a row are significantly different ($P < 0.05$).

[1] UCG = unassisted calving in a group pen ($N = 19$), UCI = unassisted calving in an individual pen ($N = 21$), ACA = assisted calving with appropriately timed assistance ($N = 20$), ACP = assisted calving with premature assistance ($N = 19$).



Unassisted dams and dams for those appropriately timed assistance was provided spent more time with licking the calf within the 2-h period after calving and had a shorter latency to contact with the offspring compared to cows that received premature assistance (Table 2).

3.2. Heart rate and HRV

Changes in heart rate, HF and LF/HF ratio (mean ± SEM) during the peripartal period are shown in Fig. 1a–c separately for calving groups. An evolution over 4 h before calving restlessness can be observed for heart rate (Fig. 1a); however, it increased above baseline only after the onset of calving restlessness for all calving groups (UCG: $P = 0.016$, UCI: $P = 0.002$, ACA: $P = 0.001$, ACP: $P < 0.001$). During parturition, heart rate increased progressively in ACA and ACP groups, while after calving restlessness a slight decrease was observed for UCI cows. Heart rate peaked at the time of birth in all groups and, except for UCG cows where it remained elevated for 1–1.5 h after calving, decreased progressively until moving cows to the postpartum pen.

There was no difference across groups regarding maximum heart rate and areas under the heart rate curves during prepartum period (Table 3). Area under the heart rate curve was similar in UCG and ACA cows during parturition ($P = 0.875$), but it was greater in these animals than in UCI cows ($P = 0.008$ and $P = 0.033$, respectively). Heart rate AUC was greater in ACP animals than in any other calving groups during parturition ($P < 0.001$ for all comparisons). Maximum heart rate was similar in the UCG and ACA groups ($P = 0.950$), and it was higher in ACP cows than in all the other groups ($P < 0.001$ in all comparisons) during parturition. Heart rate AUC did not differ across the UCG, UCI and ACA cows during parturition ($P = 1.000$ for all comparisons), but it was higher in ACP cows than in animals from the former groups ($P = 0.001$, $P = 0.022$ and $P = 0.014$, respectively). Maximum heart rate was higher in ACP dams than in any other group in the postpartum period ($P < 0.001$ in all comparisons). Heart rate returned to baseline in the UCI, ACA and ACP groups within a similar time, but heart rate required less time to return to baseline for UCG cows ($P < 0.001$ for all comparisons).

Between 96 and 2 h before calving restlessness, HF was constant, with minor alterations for all groups. HF decreased between 2 and 1 h before calving restlessness from baseline in all groups (UCG: $P = 0.002$, UCI: $P = 0.026$, ACA and ACP: $P < 0.001$), reaching the lowest peak before the onset of calving restlessness (Fig. 1b). Following calving restlessness, HF increased progressively above baseline for UCG, UCI and ACA cows until the time of calving ($P < 0.001$ in all groups); however, in ACP cows it continued to decrease and remained below baseline until 48 h after calving (Fig. 1b). After calving, an abrupt fall was observed for HF in the UCG, UCI and ACA groups. From 1.5 h after calving, HF increased gradually, but following the introduction of cows into the postpartum pens, it decreased again until 10 h after calving in UCG, UCI and ACA dams. For the remainder of the postpartum period, a gradual rise in HF was observed for all groups, which was moderate in ACP cows.

No difference was found across groups in minimum HF and area under the HF curve before the onset of calving restlessness (Table 4). HF AUC was greater in UCG cows compared to UCI cows ($P = 0.011$), reflecting a higher vagal tone of cows that calved in a group. HF AUC and minimum HF were lower in ACP cows compared to the other

Fig. 1. Heart rate (a), the high frequency (HF) component of HRV (b) and the ratio between the low frequency (LF) and HF components (LF/HF) (c) before, during and after parturition in dairy cows with unassisted and assisted calvings. UCG = unassisted calving in a group pen ($N = 19$), UCI = unassisted calving in an individual pen ($N = 21$), ACA = assisted calving with appropriately timed assistance ($N = 20$), ACP = assisted calving with premature assistance ($N = 19$). T1 indicates the onset of calving restlessness. T2 indicates the beginning of obstetrical assistance in ACA and ACP groups (● and ◇) and T3 is defined as the moment of birth. T4 indicates the time of introducing the dam into the postpartum pen, following the first milking. PREPART = prepartum period, PART = parturition, POSTPART = postpartum period. Data are given as means ± SEM of non-transformed data.

Table 3
Maximum heart rate and area under the heart rate curve (AUC) for three stages of the peripartal period in dairy cows.

Heart rate parameters[2]		Calving groups[1]				Statistics	
		UCG	UCI	ACA	ACP	$\chi^2(3)$	P
$AUC_{PREPART}$	beats/min × h	152.9 ± 12.4	174.0 ± 18.3	148.9 ± 20.1	182.9 ± 36.3	5.87	NS
$Max_{PREPART}$	beats/min	83.1 ± 1.2	83.9 ± 0.5	86.8 ± 1.5	85.2 ± 1.9	9.84	NS
AUC_{PART}	beats/min × h	22.3 ± 1.4^a	13.1 ± 0.9^b	25.0 ± 2.1^a	39.6 ± 2.5^c	38.14	0.002
Max_{PART}	beats/min	108.3 ± 1.8^a	100.1 ± 1.9^b	113.9 ± 2.0^a	135.8 ± 2.7^c	42.89	0.003
$AUC_{POSTPART}$	beats/min × h	58.0 ± 14.2^a	65.2 ± 16.7^a	62.9 ± 12.1^a	269.1 ± 36.3^b	17.25	0.006
$Max_{POSTPART}$	beats/min	112.3 ± 2.3^a	92.2 ± 1.9^b	98.7 ± 1.6^b	134.6 ± 3.2^c	51.77	0.001
Time to return to baseline	h	20.7 ± 0.3^a	22.9 ± 0.4^b	22.4 ± 0.5^b	24.7 ± 0.9^b	50.12	0.008

χ^2-statistics are based on the Kruskall–Wallis test. Statistical significances between calving groups (means ± SEM) are based on the Bonferroni-adjusted Wilcoxon rank sum test. Means with different superscripts within a row are significantly different ($P < 0.05$). NS = non-significant.
[1] UCG = unassisted calving in a group pen ($N = 19$), UCI = unassisted calving in an individual pen ($N = 21$), ACA = assisted calving with appropriately timed assistance ($N = 20$), ACP = assisted calving with premature assistance ($N = 19$).
[2] Areas under the heart rate curves (AUC) and maximum heart rate are calculated for PREPART (between 96 h before the onset of calving restlessness and the onset of calving restlessness), PART (between the onset of calving restlessness and delivery of the calf), and POSTPART periods (for the period of time to return to baseline after birth).

calving groups during parturition ($P < 0.001$ for all comparisons for both parameters, Table 4). Area under the HF curve was similar in the UCI and ACA groups during the postpartum period ($P = 1.000$), but it was greater in UCG animals than in the former groups ($P = 0.034$ and $P = 0.012$, respectively). HF AUC was lower in ACP cows than in UCG, UCI and ACA dams during parturition ($P = 0.006$, $P = 0.005$ and $P = 0.001$, respectively) and vagal tone had the lowest peak in ACP cows during the postpartum period as reflected by minimum HF values ($P < 0.001$ in all comparisons). Like heart rate, HF required a shorter time to return to baseline levels in UCG, UCI and ACP cows than in ACP dams ($P < 0.001$ for all comparisons), and the time to return to baseline was similar across the former groups (Table 4).

The LF/HF ratio, which usually changes oppositely to HF, increased above baseline in all groups from 2 h before calving restlessness (UCG: $P = 0.002$, UCI: $P = 0.014$, ACA: $P = 0.001$, ACP: $P = 0.012$) and stayed elevated during the prepartum period until the onset of calving restlessness for all groups (Fig. 1c). During parturition, the LF/HF ratio decreased in the UCG, UCI, and ACA groups below baseline until birth, while in ACP cows a characteristic fall after calving restlessness was followed by a gradual increase from the beginning of obstetrical assistance (see T2 on Fig. 1c) until the time of calving. Following delivery of the calf, the LF/HF ratio increased in UCG, UCI and ACA dams and was higher than baseline until 1.5–2 h after calving (UCG: $P = 0.004$, UCI: $P = 0.008$, ACA: $P < 0.001$). After introducing cows into the postpartum pens, LF/HF increased again in these animals until 8–10 h postpartum and then returned to baseline levels within 6 h. In ACP cows, the LF/HF ratio remained elevated until 36 h after birth without pronounced dips and peaks.

Maximum LF/HF ratio and area under the LF/HF ratio curve reflected no difference in sympathetic activity across groups before the onset of calving restlessness (Table 5). During parturition, LF/HF ratio AUC was similar in UCI and ACP cows ($P = 0.772$), and in cows calved in group, it was lower than in the former groups ($P = 0.004$ and $P = 0.009$, respectively). LF/HF ratio AUC was higher in ACP cows compared to the other groups during parturition ($P < 0.001$ for all comparisons, Table 5). There was no difference in maximum LF/HF ratio across the UCG, UCI and ACA cows during parturition ($P = 1.000$ for all comparison), but it was higher in ACP cows compared to the former groups ($P < 0.001$ in all comparisons). Area under the LF/HF curve was similar across the UCG, UCI and ACA groups during the postpartum observation ($P = 0.73$ for UCG–UCI, $P = 1.000$ for UCI–ACA, and $P = 0.31$ for UCG–ACA) and it was greater within the first 48 h after calving in ACP dams than in the former groups ($P < 0.001$ for all comparisons). Maximum LF/HF ratio was the lowest in UCG cows across all groups after calving ($P = 0.042$ from UCI, $P = 0.007$ from ACA, and $P < 0.001$ from ACP animals, respectively) (Table 5). A similar time interval was required for the LF/HF ratio to return to baseline in UCG, UCI and ACA cows ($P = 1.000$ for all comparisons), which was shorter than in ACP dams ($P < 0.001$ for all comparison).

4. Discussion

The economic and production concerns of bovine parturition are extensively studied but the welfare aspects of calving are less well understood. We addressed this gap in knowledge by studying the parameters of HRV as physiological markers of stress in multiparous cows underwent spontaneous calvings [4]. However, in several cases, the dam needs human assistance during parturition and the intervention itself, although necessary, may lead to additional pain [33]. In the present work, we aimed to identify differences in stress load and in the expression of early maternal behavior between cows that received farmer assistance at calving and cows with spontaneous calving. Earlier investigations generally compared the progress of the delivery process and the outcomes of calving in assisted and unassisted cows [24,25,

Table 4
Maximum high frequency (HF) and area under the HF curve (AUC) for three stages of the peripartal period in dairy cows.

HF parameters[2]		Calvin groups[1]				Statistics	
		UCG	UCI	ACA	ACP	$\chi^2(3)$	P
$AUC_{PREPART}$	n.u. × h	−134.4 ± 36.1	−182.5 ± 17.8	−153.8 ± 16.2	−195.2 ± 37.8	18.24	NS
$Min_{PREPART}$	n.u.	14.8 ± 0.3	11.4 ± 0.7	14.5 ± 0.6	13.6 ± 0.8	4.77	NS
AUC_{PART}	n.u. × h	4.1 ± 1.1^a	2.7 ± 0.4^b	3.2 ± 1.2^{ab}	−30.5 ± 1.6^c	56.63	0.001
Min_{PART}	n.u.	13.2 ± 0.3^a	13.3 ± 0.1^a	12.6 ± 0.9^a	7.4 ± 0.7^b	31.44	0.007
$AUC_{POSTPART}$	n.u. × h	−163.4 ± 35.6^a	−232.1 ± 42.0^b	−331.4 ± 56.2^b	−1025.6 ± 44.2^c	49.89	0.008
$Min_{POSTPART}$	n.u.	9.2 ± 0.3^a	8.2 ± 0.6^a	8.0 ± 0.5^a	6.8 ± 0.3^b	13.39	0.004
Time to return to baseline	h	30.4 ± 0.5^a	29.8 ± 0.4^a	30.1 ± 0.5^a	44.1 ± 0.6^b	27.34	0.005

χ^2-statistics are based on the Kruskall–Wallis test. Statistical significances between calving groups (means ± SEM) are based on the Bonferroni-adjusted Wilcoxon rank sum test. Means with different superscripts within a row are significantly different ($P < 0.05$). NS = non-significant.
[1] UCG = unassisted calving in a group pen ($N = 19$), UCI = unassisted calving in an individual pen ($N = 21$), ACA = assisted calving with appropriately timed assistance ($N = 20$), ACP = assisted calving with premature assistance ($N = 19$).
[2] Areas under the HF curves (AUC) and maximum HF are calculated for PREPART (between 96 h before the onset of calving restlessness and the onset of calving restlessness), PART (between the onset of calving restlessness and delivery of the calf), and POSTPART periods (for the period of time to return to baseline after birth).

Table 5
Maximum values of LF/HF ratio and area under the LF/HF curve (AUC) for three stages of the peripartal period in dairy cows.

LF/HF parameters[2]		Calving groups[1]				Statistics	
		UCG	UCI	ACA	ACP	$\chi^2(3)$	*P*
$AUC_{PREPART}$		14.7 ± 0.9	16.2 ± 0.7	15.2 ± 1.8	21.6 ± 4.6	38.79	NS
$Max_{PREPART}$		5.9 ± 0.3	7.4 ± 0.4	6.6 ± 0.3	7.4 ± 0.5	6.308	NS
AUC_{PART}		0.13 ± 0.09^a	0.58 ± 0.13^b	0.50 ± 0.17^b	6.92 ± 1.34^c	60.64	0.005
Max_{PART}		6.6 ± 0.8^a	6.4 ± 0.3^a	8.9 ± 1.1^a	19.1 ± 3.6^b	36.31	0.006
$AUC_{POSTPART}$		50.7 ± 6.0^a	79.3 ± 13.5^a	87.3 ± 12.3^a	235.4 ± 22.4^b	48.78	0.001
$Max_{POSTPART}$		10.7 ± 0.6^a	13.9 ± 1.3^b	16.1 ± 1.4^b	15.7 ± 1.6^b	22.73	0.005
Time to return to baseline	h	30.7 ± 0.3^a	30.0 ± 0.3^a	30.4 ± 0.3^a	38.4 ± 0.4^b	33.11	0.003

χ^2-statistics are based on the Kruskall–Wallis test. Statistical significances between calving groups (means ± SEM) are based on the Bonferroni-adjusted Wilcoxon rank sum test. Means with different superscripts within a row are significantly different ($P < 0.05$). NS = non-significant.

[1] UCG = unassisted calving in a group pen ($N = 19$), UCI = unassisted calving in an individual pen ($N = 21$), ACA = assisted calving with appropriately timed assistance ($N = 20$), ACP = assisted calving with premature assistance ($N = 19$).

[2] LF/HF = the ratio between the low (LF) and high frequency (HF) components of HRV. Areas under the LF/HF curves (AUC) and maximum LF/HF are calculated for PREPART (between 96 h before the onset of calving restlessness and the onset of calving restlessness), PART (between the onset of calving restlessness and delivery of the calf), and POSTPART periods (for the period of time to return to baseline after birth).

34]; however, results may have been confounded by the decisions made by the stockmen as to when assistance was necessary. In the present work, we differentiated assisted calvings based on the recommendations on the timing of obstetrical assistance [25].

One of our main findings could be the benefit of group calving in terms of parturition stress and early maternal behavior. Although some evidence exists that cows prefer seclusion to calve in semi-natural environments [22], in this study areas under the curves, minimum maximum values calculated for vagal and sympathovagal measures of HRV indicated that group calving has a positive impact on the dam's autonomic function during parturition and in the early postpartum period. The lower intensity and duration of sympathetic activation in UCG cows than in UCI and ACA animals suggest that group calving is less stressful for cows than calving in the maternity pen either with or without assistance. This phenomenon might have resulted from management factors, as placing animals from the group pen into the maternity pen disturbed UCI and ACA cows, which was reflected in their ANS activity. According to an early study, stockmen's continuous presence can lead to reduced cervical dilatation and associated stress [35], which might have also mirrored by HRV of dams calved with farmer supervision and assistance.

Cows that received obstetrical assistance before the optimal time were characterized with extremely low vagal tone and increased sympathetic activation during parturition as was shown by areas under the curves, minimum HF values and maximum LF/HF ratios. In the other groups, consistently with our earlier finding on HRV of cows with unassisted calvings [4], vagal tone increased progressively from the onset of the first behavioral signs of calving restlessness. Based on earlier findings on strong relationships between increased vagal tone and oxytocin secretion [36], depressed vagal activity during parturition was presumably associated with premature stretching of the birth canal in ACP cows, which resulted in a more intensive stress response inhibiting oxytocin release during the delivery process. Interestingly, in contrast to ACA animals, vagal activity decreased and LF/HF ratio increased right after the onset of calving restlessness in ACP cows. Although the reason of this phenomenon could not be appropriately identified, one explanation for our finding could be that ACP cows were usually moved into the maternity pen right after the onset of calving restlessness, if it was identified by farm personnel. Driving animals between the onset of calving restlessness and the start of obstetrical assistance might have caused additional stress for ACP cows, whereas ACA dams were driven to the calving pen closer to the time of calving.

Despite their increased vagal and decreased sympathetic activity during parturition, UCG cows performed higher levels of pain-related behaviors between the onset of calving restlessness and delivery compared to cows that calved in the maternity pen, irrespectively from the obstetrical condition of the latter ones. The reason for this phenomenon can be the continuous human observation of these animals during parturition. According to Stafford and Mellor [37], animals may be more likely to hide pain-related behaviors when they see people watching.

In the present study, ANS-related HRV measures showed pronounced differences in stress levels across groups during the postpartum period. A significant increase of sympathovagal balance and a reduced vagal tone were followed by a pronounced recovery response of the ANS in all groups except for ACP cows after introducing dams in the postpartum pen (Fig. 1b and c). The prolonged decline of vagal tone and elevated sympathetic activity in ACP animals were indicated by areas under the curves and times to return to baseline levels for HF and LF/HF ratio reflecting a sympathetic predominance until 20–24 h after calving.

The slower postpartum recovery of the ANS function in ACP cows than in dams from the other groups proves the existence of high levels of pain in cows underwent difficult calvings within 24 h following delivery [38]. Similar vagal and sympathetic activity was observed for UCI and ACA cows suggesting that appropriately timed assistance has no serious impairment regarding animal stress after calving.

Although ANS activity indicated the lowest levels of stress in UCG cows during the postpartum period, within the first 1–1.5 h after delivery, heart rate remained elevated in these animals. In this group, we observed herd mates to sniff and lick the newborn following birth, which was also reported by other authors [22,39]. In our study, elevated heart rates after calving in UCG cows were presumably resulted from increased physical activity associated with the more intensive licking the calf than in cows from any other groups (see later) or from the presence of alien cows, which showed interest in the offspring. In these cases, dams behaved aggressively, drove other cows away or were agitated; however, this was not reflected in increased sympathetic activity in UCG animals. When focusing on the first 2–3 h after delivery, heart rate showed a decreasing tendency, however, remained above baseline in all groups. Although the role of the expulsion of the placenta in elevated postpartum heart rates was not studied in the present paper, this phenomenon could be explained with releasing the afterbirths and associated physical activity. In our recent study we proved that increased physical activity is associated with higher heart rates in dairy cows [40].

During the first 2 h following delivery, the occurrence of pain-related behaviors was rare, irrespectively from the dams' obstetrical condition. The lack of differences observed between groups for vocalization and stretching the neck towards the abdomen is likely to be associated with the anticipation resulted from the presence of the newborn or with the continuous human observation which might have masked the expression of behavioral reactions reflecting pain after calving. A shortcoming of our study could be that pain-related behaviors were observed only for 2 h following birth, nevertheless, the spatial arrangement of the postpartum pen did not allow the appropriate video recording of the dams' behavior. In accordance with our finding, earlier observations did not find differences in pain-related behaviors between assisted and non-assisted dams [41].

In agreement with findings made on other species [42,43], we found important differences between assisted and unassisted dams in early maternal behavior. The shorter latency and duration to lick their calf in cows that calved in the group pen without any human intervention during the delivery process suggest the possible benefits of group calving over individual calving in terms of maternal grooming. The lack of differences in the onset of licking or sniffing the calf and in the duration of maternal grooming between UCI and ACA groups suggests that calving with human assistance has no serious effect on parent-offspring interaction if the assistance is timed properly. The impaired onset and quantity of licking and sniffing the calf could be a result of postpartum pain in cows with premature obstetrical assistance, which inhibited the expression of early maternal behavior.

Our results emphasize that the appropriate time for intervention is paramount for postpartum ANS recovery and expression of early maternal behavior. The profound negative impact that premature obstetrical assistance has on peripartal ANS function and on the expression of early maternal behavior should make calving management a higher priority on dairy farms. Thus, professionals must focus on the appropriate timing of obstetrical assistance by reducing stress to improve cow welfare. The positive effects of group calving on peripartal wellbeing and postpartum maternal behavior should be also considered. Based on the present findings, physiological indicators of stress such as HRV may reveal opportunities for improvements in calving management.

5. Conclusions

Calving in a group has benefits over calving in an individual pen in terms of animal stress and early maternal behavior. Calving with appropriately timed assistance causes no serious impairment in cow welfare during parturition and in the first 48 h after calving. Premature obstetrical assistance results in high levels of stress during parturition, prolonged postpartum recovery of the ANS and depressed maternal behavior.

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