



Weaning reduces body temperature and heart rate, and increases heart rate variability in ewes

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ABSTRACT

Weaning elicits strong responses in ewes and lambs, which makes it a significant welfare concern; however, limited and sometimes contradictory information is available on the physiological responses of dams at weaning. The aim of this study was to quantify the physiological changes in ewes that occur after weaning. Four days before weaning (d0), eleven lactating Rasa Aragonesa ewes received a surgically implanted subcutaneous body temperature (BT) and heart rate (HR) biollogger that had been programmed to take measurements every 15 min from d2 to d3. HR variability (HRV) variables were calculated based on the raw ECG data that were recorded every 5 min between 1700 and 1900 h. At 1130 h on the day of weaning, ewes and lambs were separated and housed in different barns. In the lactation period, ewes were fed a concentrate ration (1 kg of pellets) that was offered at 0800 h, 1 kg alfalfa hay, and barley straw ad libitum. After weaning, ewes were fed 0.5 kg barley straw, only. After weaning, BT and HR decreased gradually ($P < 0.001$), and HRV variables (SDNN and RMSSD) increased significantly ($P < 0.001$). The reductions in BT and HR in ewes after weaning seemed to be caused by the management procedures (detention of lactation and drastic reduction in feed offered) usually applied at weaning; however, the increase in HRV after weaning indicated recovery from stress, improved autonomic regulation, and welfare. HRV data collected by subcutaneous biologgers provided valuable insights into the physiological and emotional well-being of ewes at weaning.

1. Introduction

Weaning in sheep is a critical and often stressful transition for lambs and their mothers and, as a management practice, weaning marks the cessation of milk feeding and the separation of lambs from their ewes [1], which facilitates the transition to an independent, grazing-based diet. Although that practice is necessary for optimizing the health and productivity of ewes and lambs in commercial and traditional sheep farming systems, it is inherently a period of significant physiological, psychological, and social stress [2]. Understanding the dynamics of that stress is essential for improving animal welfare and productivity. Weaning disrupts the natural mother-lamb bond, an important aspect of the social structure and behaviour of sheep [3]. In wild or unmanaged conditions, weaning occurs gradually and does not occur until about 6 mo of age, which allows for a less abrupt adjustment than occurs in managed conditions [4]. In the former, weaning is controlled by the ewe through gradually limiting access to the udder, and the timing seems to

be at least partially influenced by the reduction in milk production. In common with other farmed species, however, the lamb is artificially weaned. Typically, artificial weaning on farms involves an abrupt and enforced separation, which increases the stress on both parties. To lambs, weaning involves sudden changes in nutritional source, social environment, and sense of security; however, to ewes, weaning is not merely a cessation in lactation, it is a significant social and maternal disruption [5]. The cessation of lactation causes additional physiological changes such as mammary gland engorgement and possible discomfort as milk production halts abruptly and, if not managed properly, can lead to mastitis, a painful and potentially serious condition. In addition, the stress of separation can elevate cortisol levels in ewes to a degree similar to that in their lambs [6], which can suppress immune function temporarily and delay the restoration of optimal body condition.

Dams are feed-restricted at weaning, and this adversely affect the metabolic status of dams. For instance, nutrient-restricted primiparous beef females exhibited a decrease in milk yield and mammary blood

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flow, which could lead to alterations in metabolic rates and subsequently affect body temperature (BT) and heart rate (HR) [7]. This reduced milk yield can lead to a negative energy balance, prompting the dam's body to mobilize fat reserves, which can further influence metabolic parameters such as BT and HR [8–9]. Moreover, studies on lactating rats have demonstrated that food restriction leads to a decrease in LH release, which is associated with a decline in metabolic activity [10]. This hormonal change can contribute to a reduced metabolic rate, potentially lowering both HR and BT. Additionally, maternal feed restriction has been linked to significant weight loss in lactating animals, which can also correlate with decreased metabolic rates and physiological responses [11]. In terms of specific physiological responses, energy restriction during lactation results in increased mobilization of maternal adipose tissue, which could lead to alterations in BT regulation [12]. This is particularly relevant as changes in body fat can influence thermoregulation, potentially resulting in lower BT in feed-restricted dams. Furthermore, the study by Schütz et al. highlighted that short-term feed restriction in lactating dairy cattle led to reduced milk production and metabolic disturbances, which may also manifest as changes in HR and BT [9].

In recent years, the development of biologgers for monitoring responses in, for example, BT, respiration, HR, HR variability (HRV), or the activity of the animals has provided a means to understanding how physiological conditions affect the resiliency of an animal to stressors. Variations in BT [13], HR [14], and HRV [15] in sheep can indicate stress, which affects welfare and productivity. Subcutaneous biologgers are particularly useful in veterinary and wildlife studies because the use of external devices often is impractical. They can measure BT, HR, and HRV in natural settings without affecting the animal's behaviour significantly, which contributes to a holistic view of its health, and increases understanding of physiological processes. Subcutaneous biologgers offer several other benefits, particularly high accuracy, comfort, and data quality. Placement directly beneath the skin reduces the external interference and signal noise that is commonly associated with surface-based monitors. In addition, those devices enable continuous and long-term monitoring, which is necessary for collecting comprehensive data over extended periods. Furthermore, subcutaneous biologgers are less prone to loss or damage than are externally affixed devices, which increases the likelihood of consistent and uninterrupted data collection [16].

Since the identification of the behavioural and physiological responses associated with weaning stress is essential for improving management practices and animal welfare, it was hypothesized that feed restriction and cessation of lactation at weaning can lead to decreased HR and BT and changes in HRV in ewes, primarily due to the metabolic stress induced by reduced nutrient intake, which affects hormonal balance, milk production, and overall physiological well-being. Under this hypothesis, the aim of this study was to quantify the physiological changes (BT, HR, and HRV) induced by weaning in ewes that were managed under intensive conditions based on measurements recorded by subcutaneous programmable biologgers.

2. Material and methods

2.1. Animals

In late Nov, eleven lactating Rasa Aragonesa ewes (59 ± 6 kg) were selected to receive a surgically implanted subcutaneous bilogger. In lactation, which lasted 45 d, ewes and lambs were kept in a communal pen (5 m \times 7 m) that had an open area (5 m \times 5 m). Ewes were fed a concentrate ration (1 kg of pellets), which was offered at 0800 h, 1 kg alfalfa hay, and barley straw ad libitum. A lamb creep pen allowed lambs to have access to additional feed concentrate that was separate from their mothers. At 1130 h on the day of weaning (day 0), ewes and lambs were abruptly separated and housed in different barns. Thereafter, ewes were fed 0.5 kg barley straw, only. Water was available ad libitum.

Four days before weaning (d0), ewes were surgically implanted with a subcutaneous T and HR bilogger (DST micro-HRT, Star Oddi, Iceland) (8.3×25.4 mm, 3.3 g) (Fig. 1).

2.2. Surgery

The biologgers were sterilized by immersing them in a 0.55 % orthophthalaldehyde solution (CIDEX-OPA, Johnson & Johnson, New Jersey, USA) for 24 h. For the surgery, ewes were put in a cradle in dorsal recumbency. A solution of povidone-iodine soap (Betadine Scrub 7.5 %, Alcon Laboratories, Inc., Fort Worth, TX) was used to prepare the skin for surgery. One ml of lidocaine hydrochloride (Anesvet, Ovejero, León, Spain) was injected subcutaneously. A subcutaneous pocket was constructed to accommodate the bilogger after an incision was made on the left thorax, just above the heart (Fig. 1). The electrodes of the bilogger were placed in contact with the muscle layer nearest to the skin, and the sensor axis was aligned with the axis of the heart. The bilogger was fastened in the pocket by a 2/0 absorbable suture (Novosyn, B-Braun, Melsungen, Germany) that was inserted through a tiny hole at the device's tip. After using two to three sutures to close the incision, aluminum spray (Aluspray, Vetoquinol, Madrid, Spain) was applied to the affected area. A similar surgical approach was used to remove the bilogger at the conclusion of the experiment.

2.3. Biologgers

Biologgers were set up with a communication box through the Mercury software v5.83 (Star Oddi, Gardabaer, Iceland). Data were captured from D-2 to d3 (weaning = d0) every 15 min. In order to save memory, HR variability (HRV) characteristics were estimated based on raw ECG data collected every 5 min between 1700 and 1900 h. Four days following weaning, the biologgers were retrieved and the data were downloaded via the communication box and software. The bilogger measures HR through a leadless single-channel ECG, taking burst readings at predetermined intervals, and calculating the mean HR for each record. The software calculates a Quality Index (QI) for each burst.

Based on the raw ECG data, the Star-Oddi HRT Analyzer program (Star Oddi, Gardabaer, Iceland) computed HRV parameters. The program calculates HR, QI, and HRV based on the following two methods: the SDNN (standard deviation of normal to normal R-R intervals) and the RMSSD (root mean square of consecutive deviations between normal heartbeats).

$$SDNN = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (RR_i - \overline{RR})^2} \quad \overline{RR} = \text{mean of RR intervals}$$

$$RMSSD = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N-1} (RR_{i+1} - RR_i)^2}$$

The R-R interval is the time between each detected heartbeat, measured from peak to peak (R) on the QRS complex. The QRS complex is the cardiac contraction (systole) that begins with the Q wave, a negative deviation, and ends with the R wave, a positive (upward) deviation. The S wave is any negative deflection that occurs immediately following the R.

The sample frequency was 200 Hz, which is recommended for small ruminants [17]. The algorithm defining the QI is a two-step [18]; initially, it scans the recording for QRS waves and calculates each R-R interval. If there is more than one R-R interval in the recording and less than a 20 % variability between any R-R interval, the grade is set to QI = 0 (great). If there is no R-R interval, the grade is QI = 3, and HR is calculated as 2 bpm. If HR is calculated above or below a certain defined threshold, the grade is QI = 3. In most cases, HR graded QI = 3 should be ignored since it cannot be considered reliable. If there is exactly one R-R interval, and if the R-R interval consumes approximately 45 % of the



Fig. 1. Biolloggers (DST micro-HRT, Star Oddi, Iceland; 8.3×25.4 mm, 3.3 g) implanted subcutaneously in eleven Rasa Aragonesa ewes, and some pictures of the surgical procedures used to implant them subcutaneously in the animals.

sampling duration, the QI is set to QI = 0 (great). Anything that falls outside the aforementioned description goes on to step two of the algorithm. In this step, each potential R wave is given a grade that depends on various traits, for example, amplitude and "sharpness". Two levels are calculated based on the lowest and highest grade. They can be called lower-level threshold (LLT) and higher-level threshold (HLT). If the levels overlap ($LLT \geq HLT$), that means all potential R waves are of similar grade. Then they are all used for the calculation of the BPM, and the QI is set to QI = 1 (good). This is typical for good quality recordings that have an arrhythmia or >20 % variation between any R-R interval inside a single ECG recording. Otherwise, only potential R-waves graded above the HLT are used for calculation. The QI is still set as QI = 1 (good), except if one or more potential R-waves are graded between the LLT and HLT. Then the rating is considered somewhat ambiguous, and

the QI is set to QI = 2 (fair). Finally, if only a single R-wave is above the HLT, the QI is set as QI = 3 (poor), and heart rate is calculated as 1 bpm.

In all cases, HR that graded QI = 2 or 3 were excluded from the analyses because they cannot be considered reliable. For each ECG record, the HRT Analyzer software calculates HRV as SDNN and RMSSD. At the end of the software procedures, the data can be downloaded to a computer as a ".HRV" file, and transformed for other analyses.

2.4. Statistical analysis

Mean (\pm S.E.) BT, HR, and HRV (SDNN and RMSSD) were calculated at hourly intervals for each day of the experiment. A Paired Samples t-Test was used to detect significant differences in BT, HR, and HRV in the days before and after weaning (d0). Pearson correlation coefficients

between BT, HR and SDNN and RMSSD measured concurrently were calculated.

To calculate and compare the circadian rhythms in BT, HR, and HRV, mean values of these variables were calculated for the days before and the day of weaning (d-2 to d0), and after weaning (d1 to d3), and were analyzed by fitting the time-series data from each sheep to a 24-hour cosine curve using the Cosinor online platform [19]. For each variable in each individual, the MESOR (Midline Estimating Statistic of Rhythm, the average value around which a variable fluctuates), amplitude (the difference between the peak and the mean value of the wave), and acrophase (the time of peak activity) were calculated. A P-value of < 0.05 was used to confirm the fit of the time series to a 24-hour rhythm. The standard cosinor model fits a cosine function to the time series data with the following equation:

$$y(t) = M + A \cos(2\pi t / T + \varphi),$$

where $y(t)$ is the value of the time series at time t , M is the mesor, A is the amplitude, T is the period of the rhythm, which typically is set to 24 h for circadian data, and φ is the acrophase.

3. Results

Of the 1379 records that were downloaded from the 11 biologgers to estimate HRV, 61 % had Q0 ($n = 585$) or Q1 ($n = 252$). The remaining 39 % were graded Q2 ($n = 455$) or Q3 ($n = 87$), and, therefore, were excluded from the analysis. For each of the eleven ewes, the proportion

of records that were Q0 or Q1 was 42 %, 70 %, 79 %, 82 %, 94 %, 39 %, 41 %, 73 %, 44 %, 77 % and 55 %.

BT and HR decreased significantly between the days before and the days after weaning (Fig. 2), with significant differences ($P < 0.01$) among d-2, d-1 and d0 compared with d1, d2 and d3. Except for the day of weaning, BT and HR followed the same diurnal pattern every day of the study, although the magnitudes differed. BT peaked at 0400–0500 h and again before dusk, and HR peaked around 0800 h. On the day of weaning, BT and HR peaked at noon, about 30 min after the animals were separated. For the HRV variables (SDNN and RMSSD) measured in from 1700 to 1900 h, there were no significant differences among days from d-2 to d0, but were significantly higher ($P < 0.001$) from d1 to d3 after weaning (Table 1) than they were on the days before weaning.

BT and HR were significantly correlated ($P < 0.001$), and both were negatively correlated with SDNN and RMSSD ($P < 0.001$) (Table 2).

Table 1

Mean (\pm S.E.) standard deviation of SDNN (ms) and RMSSD (ms) in 11 ewes two days before and three days after weaning (d0), recorded by subcutaneous biologgers (in the same row, a,b indicate significant differences at $P < 0.01$).

	d-2	d-1	d0 weaning	d1	d2	d3
SDNN	27.3 \pm 2.3 ^a	40.2 \pm 6.5 ^a	38.3 \pm 4.5 ^a	118.8 \pm 17.4 ^b	113.0 \pm 14.4 ^b	104.3 \pm 11.9 ^b
RMSSD	31.7 \pm 2.6 ^a	49.8 \pm 8.7 ^a	49.7 \pm 5.9 ^a	179.8 \pm 26.0 ^b	167.3 \pm 20.5 ^b	153.7 \pm 16.5 ^b

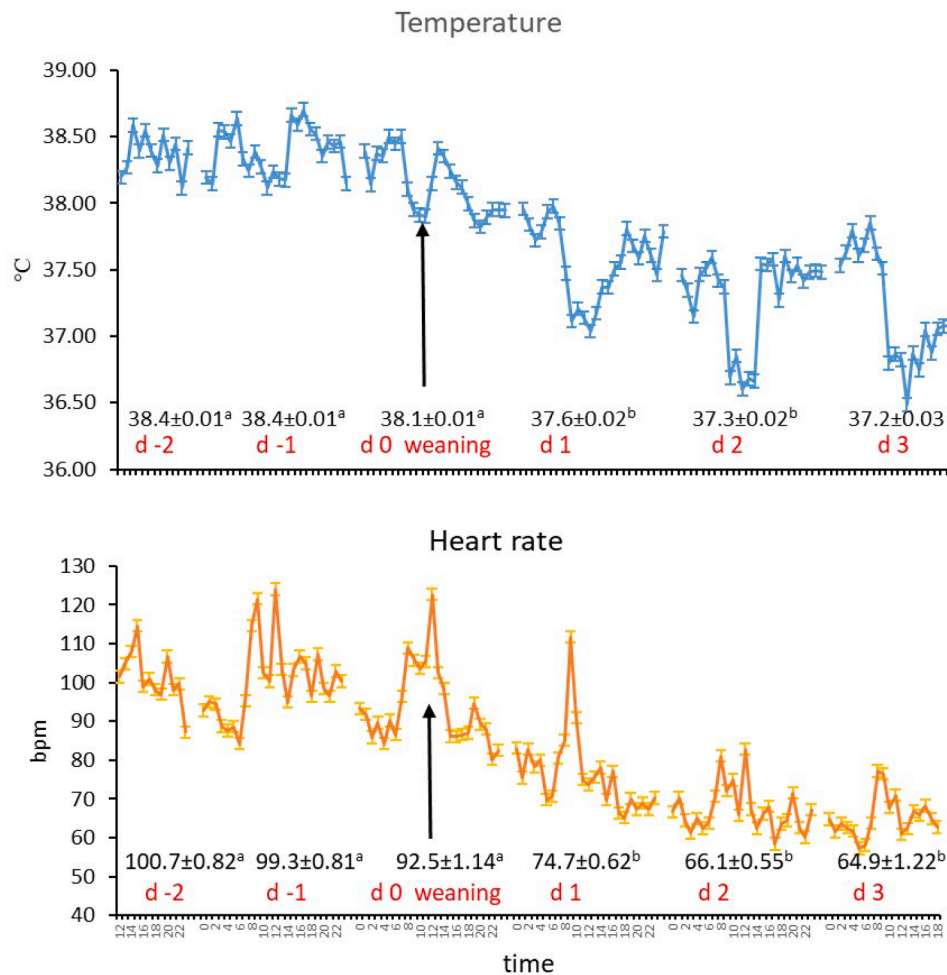


Fig. 2. Hourly mean (\pm S.E.) body temperature and heart rate in eleven ewes two days before and three days after weaning (d0), recorded by subcutaneous biologgers (a, b indicate significant differences $P < 0.01$).

Table 2

Matrix of correlations among body temperature (BT), heart rate (HR), and SDNN and RMSSD, in 11 ewes two days before and three days after weaning, recorded by subcutaneous biologgers (***) $P < 0.001$.

	BT	HR	SDNN	RMSSD
BT				
HR	0.367***			
SDNN	-0.130***	-0.435***		
RMSSD	-0.148***	-0.454***	0.988***	

BT and HRV exhibited 24-h circadian rhythms. Fig. 3 shows the cosinor curves of those variables for the mean of the previous days to weaning and the mean of the days after weaning. Before weaning, ewes presented significantly ($P < 0.01$) higher MESOR and Amplitude in BT and HRV (Table 3), and different Acrophases than they did after weaning.

4. Discussion

In our study, 61 % of the HR records from the eleven ewes were graded as QI = 0 or 1, and were included in the analysis. Among the eleven ewes, the proportion of the records that graded as QI = 0 or 1 ranged between 39 % and 94 %. In a previous study, which used the same brand of bilogger, although a larger model to better suit an adult sheep [20], 85 % of the 9576 records had Q0 or Q1, and the proportion of records that were $Q \leq 1$ ranged 67–98 %. Muller et al. [21] assessed the effects of device orientation and electrode placement on the quality

of the data from rainbow trout that were collected by biologgers of the same brand (albeit smaller) and found that the orientation of the electrodes had a significant effect on data quality but, as long as the device was positioned close to the heart, the electrodes were in contact with the musculature, and double sutures were used, electrode orientation did not affect data quality. This variability indicates that electrode orientation and the size of the devices likely influence whether high-quality HR recordings are obtained in sheep, which might have contributed to the differences among individuals. Although the devices were implanted following a standardized procedure (with electrodes positioned to contact the muscle layer nearest the skin), slight rotations of the biologgers might have occurred, particularly because the devices used in our experiment might have not been the most appropriate size given the size of the animal. In addition, some animals were more active than others, which might have displaced the electrodes from their initial orientation.

In our study, data from the subcutaneous biologgers indicated that, immediately after weaning, ewes experienced dramatic changes in BT, HR, and HRV. Lactating ewes had higher body temperatures than did non-lactating ewes because of the metabolism involved in maintaining milk production [22]. Furthermore, the high plasma prolactin levels in lactating ewes elevate heat loads [22–23], and prolactin can modulate some mechanisms of heat dissipation and heat production involved in homeothermy [24]. Thus, probably, the reduction in BT after weaning was not caused by weaning *per se* as a stressor, but by the cessation of milk production and the reduction in prolactin secretion.

Information on changes in HR in lactating sheep after weaning has been limited. In dairy cows, HR decreases after dry-off, which occurs

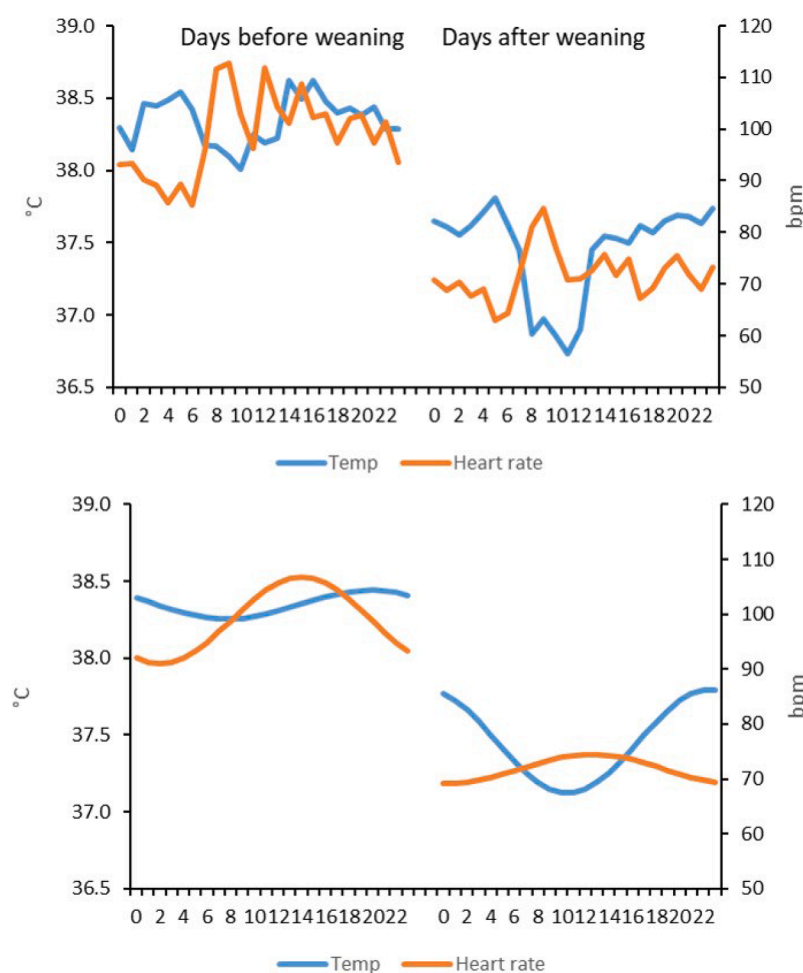


Fig. 3. Mean 24-h temperature and heart rate (upper panel), and the corresponding cosinor curves of the 24-h activity rhythms (lower panel) of eleven ewes two days before and three days after weaning (d0), recorded by subcutaneous biologgers.

Table 3

Mean (\pm S.E.) MESOR, amplitude, and acrophase in body temperature ($^{\circ}$ C) and heart rate (bpm) in 11 ewes two days before and three days after weaning, recorded by subcutaneous biologgers (in the same column, a,b indicate significant differences $P < 0.05$).

	MESOR		Amplitude		Acrophase	
	Temperature	Heart rate	Temperature	Heart rate	Temperature	Heart rate
Before weaning	38.4 \pm 0.1 ^a	98.8 \pm 0.9 ^a	0.09 \pm 0.01 ^a	7.91 \pm 0.01 ^a	19:46 h ^a	13:59 h ^a
After weaning	37.5 \pm 0.1 ^b	71.8 \pm 0.7 ^b	0.03 \pm 0.01 ^b	2.64 \pm 0.01 ^b	22:31 h ^b	12:32 h ^b

because a reduction in nutrient intake suppresses milk production. A decrease in rumen fill causes a reflexive slowing of HR, primarily because of an increase in parasympathetic tone [25]. Furthermore, cows that had dried-off under a silage diet or a straw-based diet exhibited a reduction in HR, but the reduction was significantly greater in the latter than it was in the former, which occurred in the ewes in our study. Rumsey and Bond [26] reported that rectal T, respiratory rate, and HR in beef heifers and steers were significantly lower after 48 h of feed and water deprivation, but they returned quickly to a physiologically normal state after the resumption of feed and water. In goats, a food-restriction used to terminate milk production produced a persistently depressed HR and reduced mean and systolic blood pressures at night [27]. It is likely that the reduction in HR after weaning in the ewes in our study was caused by a reduction in food consumption rather than from weaning *per se*.

In our study, the significant increase in the HRV in ewes after weaning is more difficult to explain. An increase in HRV is associated with an increase in parasympathetic activity [28], which suggests that ewes were leaving the acute stress phase, which is characterized by sympathetic dominance, into a calmer, more relaxed state. Thus, the increase in HRV following the initial stress of weaning suggests that ewes are adapting successfully to the new social and environmental conditions, and reflects the ability of the animals to cope and recover from the stressor [29]. Generally, an increase in HRV is a positive welfare indicator because it suggests that the animal is no longer experiencing high levels of chronic stress [15]. In one study, lactating and non-lactating cows did not differ significantly in the time or frequency domain characteristics of HRV [30]. If ewes exhibit a significant increase in HRV after weaning, probably, it is not because of a cessation in lactation but, rather, the effectiveness of management practices intended to reduce stress. For example, gradual weaning, enriched environments, or the presence of familiar conspecifics can facilitate quicker adaptation and recovery.

The process of weaning in ewes is a critical period that induce not only physiological changes but also psychological and social stress. This stress is primarily attributed to the abrupt separation from the lambs, which disrupts the established maternal bond and alters the social dynamics within the flock. Research indicates that abrupt weaning is associated with various physiological and behavioral changes in ewes, which serve as indicators of stress [1,31]. The stress response can manifest in behaviors such as increased locomotion and alertness, while simultaneously decreasing resting and feeding behaviors [1,31–32]. The abrupt cessation of lactation can lead to a decrease in serum protein concentrations, reflecting the physiological stress response [33].

In our study, BT and HR exhibited a circadian rhythm and were significantly correlated throughout the experiment (see also [20]). In that study, HRV was negatively correlated with BT and HR in sheep. The correlation between HRV and HR is both a physiological and a mathematical phenomenon [34], but the physiological dependence of HRV on HR is governed by the autonomic nervous system, and an increase in parasympathetic nervous system activity lowers HR and increases HRV. Sutherland et al. [35] reported that eye temperature tended to be higher and HRV lower in sheep that had been given epinephrine in an infusion period than it was in sheep that had received saline. In our study, the management and housing systems used on our farm, especially the timing of concentrate feed, influenced the 24-h circadian rhythms in BT and HR in ewes. BT was lowest just before feeding and increased

thereafter before peaking immediately after feeding. Feeding time serves as a zeitgeber (time cue) for BT and HR, which was demonstrated by Mohr and Krzywanek [36], who observed distinct peaks in BT and HR that coincided with the timing of food presentation.

5. Conclusion

In conclusion, the reduction in BT and HR exhibited by ewes after weaning appeared to be a consequence of the management procedures (detention of lactation and drastic reduction in feed offered) that are applied commonly at weaning. The increase in HRV after weaning was a positive sign that indicated recovery from stress, better autonomic regulation, and improved welfare. An analysis of HRV based on the data collected by subcutaneous biologgers provided valuable insights into the physiological and emotional well-being of ewes at weaning. Research into those effects can inform welfare-friendly practices and management strategies that reduce the stress associated with weaning in livestock.

Ethics statement

The Ethics Committee for Animal Experiments at the University of Zaragoza approved all of the procedures performed in the study. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

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CRediT authorship contribution statement

José A. Abecia: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Francisco Canto:** Writing – review & editing, Methodology, Investigation. **Irene Viola:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Isabella Manenti:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Paola Toschi:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Silvia Miretti:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that might have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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