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Body temperature and heart rate variability, and their circadian rhythms in sheep as measured by biologgers

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ABSTRACT

Heart rate (HR) variability (HRV) reflects the balance between the autonomic nervous system's sympathetic and parasympathetic branches, which regulate stress and relaxation. Wearable sensors record HR and body temperature (BT), to provide data that informed decisions can be made from on animal management and welfare. The purpose of this study was to investigate daily changes in BT and HRV, recorded by subcutaneous biologgers; specifically, to quantify the 24-hour circadian rhythm in BT (°C), HR (bpm), the Standard Deviation of the R-R intervals (SDNN), and the Root Mean Square of Successive Differences (RMSSD) (ms). Five ewes were implanted with a subcutaneous biologger, configured to collect data every 5 min for 7 d. Mean (± S.E.) BT (38.06 ± 0.01), HR (111.14 ± 1.68), SDNN (34.35 ± 2.90), and RMSSD (47.95 ± 4.21) were calculated. BT and HR were highest in the day (day: 38.15 ± 0.01 and 120.71 ± 0.43; night: 37.98 ± 0.01 and 102.47 ± 0.43; $P < 0.001$), and SDNN (day: 29.30 ± 0.87; night: 37.16 ± 0.67) and RMSSD (day: 39.01 ± 1.17; night: 53.53 ± 0.94) were highest at night ($P < 0.001$). BT and HR were positively correlated ($P < 0.01$), but both negatively correlated ($P < 0.01$) with SDNN and RMSSD. BT, HR, SDNN, and RMSSD presented a 24-h circadian rhythm, with acrophases (peak activity period) for BT at 1457 h and at 1223 h for HR, but those of SDNN and RMSSD were at 0350 h and 0327 h, respectively. In conclusion, biologgers detected the diurnal rhythmicity in BT and HRV in sheep, providing an option to use these physiological measures to assess an animal's health and welfare.

1. Introduction

Heart rate (HR) variability (HRV) is a non-invasive technique for assessing stress levels that affect animal welfare (Kitajima et al., 2021). Normally in animals under stress cardiac activity is regulated by an upregulated sympathetic tone and the withdrawal of vagal tone, and is a sensitive indicator of the functional regulatory characteristics of the autonomic nervous system (ANS) (Turini et al., 2022). von Borell et al. (2007) reviewed how HRV can be used as a measure of the autonomic regulation of cardiac activity to assess stress and welfare in farm animals, and suggested that it was a promising method for evaluating stress and emotional states in animals. HR can increase in response to a reduction in vagal activity, an increase in sympathetic activity or, typically, to a combination of concurrent changes in activities within both branches. In reality, however, the interplay between the branches is quite complex. HR measurements can provide information on the net effects of all components influencing cardiac activity, but are of limited use in accurately assessing sympathovagal regulation (Tulppo et al., 1998). HRV analysis allows a more accurate and detailed assessment of the functional regulatory characteristics of the ANS. The variables most commonly used to describe HRV are the standard deviation of all interbeat intervals (SDNN), which is a variable that reflects both the vagal and sympathetic influences on HR, and the root mean square of successive R–R intervals (RMSSD), which reflects changes in the ANS that are predominantly parasympathetically (N. vagus) mediated (Mohr et al., 2002).

Studies on the use of HRV for welfare assessment in sheep are scarce. Stubsjøen et al. (2009) assessed whether changes in eye temperature, measured by infrared thermography, and HRV could quantify moderate pain in sheep. They hypothesized that a painful stimulus would cause a reduction in eye temperature because of sympathetically mediated vasoconstriction, and a reduction in HRV because of sympathetic activation. Elsewhere Stubsjøen et al. (2015) assessed HRV as an indicator of stress and pain in sheep with foot rot. Sutherland et al. (2020) observed that Romney ewes administered epinephrine via jugular catheter for 5 min had significantly ($P < 0.05$) higher BT and HR and lower RMSSD

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and SDNN than the saline group.

HRV is difficult to quantify without causing stress to the animal. HRV in sheep has been monitored using Smart textile (Turini et al., 2022), disposable skin-adhesive electrodes (Konold and Bone, 2011; Kviesulaitis et al., 2018), polar heart rate monitors (a transmitter with two electrodes and a logger) (Wickham et al., 2012; Kitajima et al., 2021), a single-channel electrocardiogram (ECG) machine on a bipolar base-apex lead (Tajik et al., 2016), and a subcutaneous telemetric device that has two skin-implanted electrodes (Wassermann, 2020). Subcutaneous biologgers, however, can provide several advantages for monitoring HRV, particularly in terms of accuracy, comfort, and data quality. Direct placement of devices under the skin minimizes external interference and noise that can affect surface-based monitors and can enable continuous and long-term HRV monitoring, which is essential for capturing comprehensive data over time. Implanting devices make them less likely to be lost or damaged than externally applied devices, which increases the likelihood of consistent and uninterrupted data collection (Twiss et al., 2021). Subcutaneous biologgers are particularly useful in veterinary and wildlife studies, where external devices are often impractical. They enable the study of HRV in natural settings without significantly affecting the animal's behavior. Furthermore, they can be integrated with other physiological sensors (temperature, accelerometers) to provide a holistic view of the animal's health, enhancing the understanding of physiological interactions.

Variations in body temperature (BT) in farm animals can serve as an indicator of stress, which affects both welfare and productivity (Hahn, 1989). Normally, BT follows a circadian rhythm, being higher during the animal's active phase compared to its inactive phase (Refinetti and Menaker, 1992). The stress-induced hyperthermia response is a brief increase in body temperature (BT) triggered by stress (Bouwknecht et al., 2007). Stress-related conditions are often associated with disruptions in the circadian rhythm of BT. Therefore, studying these changes can help to determine whether a group of animals is experiencing stress, which is essential for assessing their welfare.

The aim of this study was to document the daily changes in BT, HR and HRV in sheep, and to quantify their daily interrelationships and their 24-h circadian rhythms, as recorded by subcutaneous biologgers.

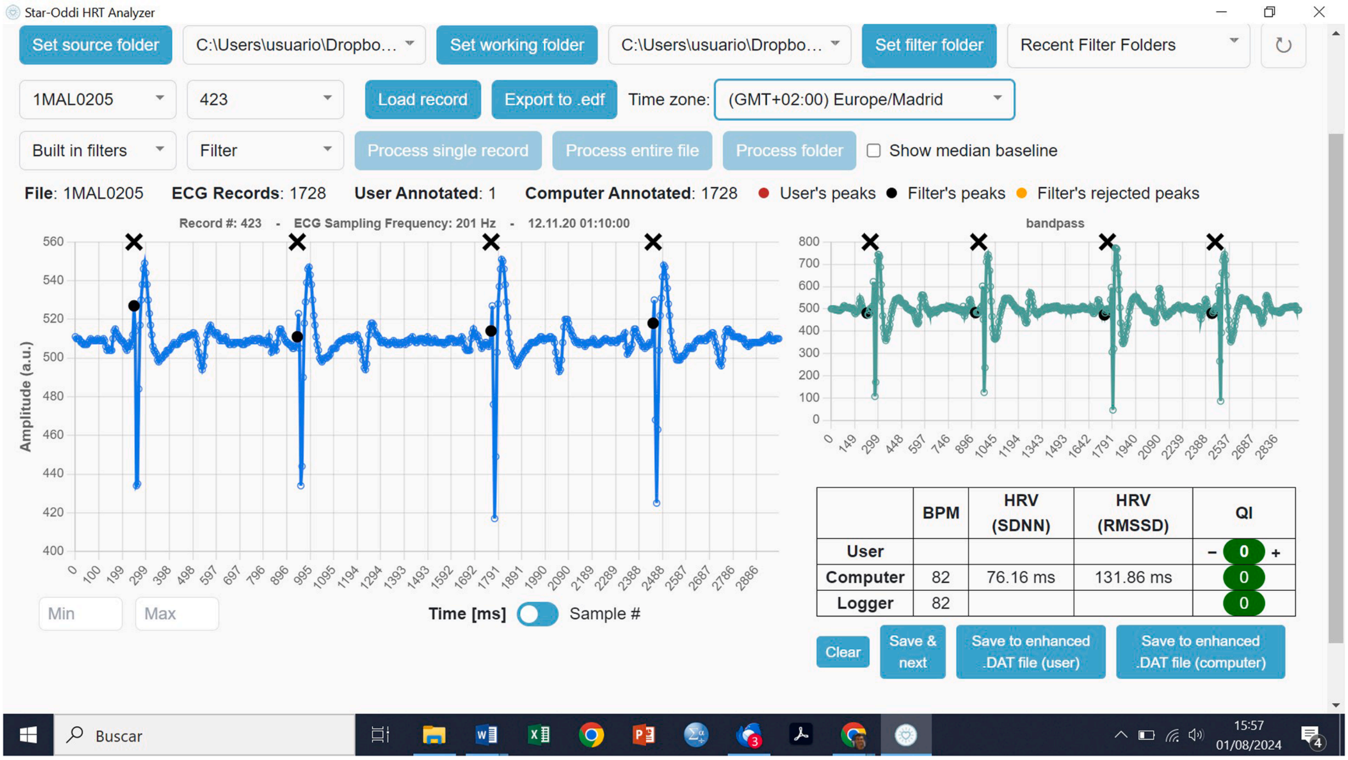
2. Material and methods

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.

2.1. Animals and biologgers

In mid-November, five lactating adult (3–5 yr old) Rasa Aragonesa ewes (65.2 ± 5.6 kg) ewes had a sub-cutaneous temperature and HR biologger (DST milli-HRT, Star Oddi, Iceland) (13 mm×39.5 mm; 12 g) implanted. They were programmed by a communication box and the Mercury software v5.83 (Star Oddi, Gardabaer, Iceland) to record data every 5 min. After 10 d, the biologgers were retrieved and the data were downloaded through the same communication box and software. Data from the first two days after implantation and the day the biologger was removed were excluded from the analysis. The biologger records HR using a leadless single-channel ECG, which records burst measurements at set time intervals and calculates the mean HR for each record. Each burst is assigned a Quality Index (QI) for validation, which is calculated by the application software.

The Star-Oddi HRT Analyzer software (Star Oddi, Gardabaer, Iceland) (Fig. 1) calculated HRV parameters based on the raw ECG data. The algorithm calculates HR, QI, and estimates HRV based on two methods, the SDNN (standard deviation of normal to normal R-R



1MAL0205

423

Time zone: (GMT+02:00) Europe/Madrid

File: 1MAL0205 **ECG Records**: 1728 **User Annotated**: 1 **Computer Annotated**: 1728

Record #: 423 - ECG Sampling Frequency: 201 Hz - 12.11.20 01:10:00

	BPM	HRV (SDNN)	HRV (RMSSD)	QI
User				− 0 +
Computer	82	76.16 ms	131.86 ms	0
Logger	82			0

Fig. 1. A representative example of a record from an individual lactating adult ewe successfully loaded into the Star-Oddi HRT Analyzer software. The peaks detected by the HRT Analyzer are indicated by black dots and an X at the top of the graph. Beats per minute (BPM), heart rate variability (HRV) variables (standard deviation of all interbeat intervals –SDNN- and root mean square of successive R–R intervals -RMSSD), and Quality Index (QI) are shown in the table.

intervals) and the RMSSD (root mean square of successive differences between normal heartbeats).

$$\mathrm{SDNN} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(\mathrm{RR}_i - \overline{\mathrm{RR}}\right)^2} \quad \overline{\mathrm{RR}} = \text{mean of RR interval}$$

$$\mathrm{RMSSD} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N-1}\left(\mathrm{RR}_{i+1} - \mathrm{RR}_i\right)^2}$$

The R-R interval represents the time between each detected heartbeat, measured from peak (R) to peak (R) on the QRS complex. The QRS complex is the ventricular contraction (systole) consisting of the Q wave, which is the first negative deviation, followed by the R wave, a positive (upward) deviation. Any negative deflection following immediately after the R portion is termed the S wave (Fig. 2).

The sampling rate was 200 Hz, which is the frequency recommended for small ruminants. Each record is either 600 samples normal or 1500 samples long ECG. The duration of the ECG measurement is proportional to the sampling frequency used (3 s for normal ECGs and 7.5 s for long ECGs). The long ECG option is especially useful in this software when recording very low heart rates, since it guarantees minimum heart rates based on this sampling frequency.

This is used to ensure that the HR does not surpass the lower limits of the detected HR. The algorithm that calculates the QI (Star Oddi, 2024) is a two-step process; initially, it searches the record for QRS waves and calculates each R-R interval. If there is < 20 % variability within the R-R intervals, the grade is set to QI $= 0$ (good). If there is no R-R interval, QI $= 3$ (poor), and HR is calculated as 2 bpm. If HR is above (600 bpm) or below (20 bpm) a certain defined threshold, QI $= 3$. Any data that did not meet those criteria were subjected to step two of the algorithm. In that step, each potential R wave is given a grade based on various traits; e.g., amplitude and "sharpness". Two thresholds are identified based on the lowest and highest grade; i.e., the lower-level threshold (LLT) and the higher-level threshold (HLT). If the thresholds overlap (LLT $\geq$ HLT), all potential R waves are of a similar grade, which are used to calculate beats per minute (bpm), and the QI is set to 1 (good). That is typical for good quality recordings that have an arrhythmia or > 20 % variation among R-R intervals within a single ECG recording. Otherwise, potential R-waves graded above the HLT are used in the calculation, and the QI is still set to 1 (good), except if one or more potential R-waves are graded between the LLT and HLT. In that case, the rating is considered somewhat ambiguous, and the QI is set to 2 (fair). If only a single R-wave is above the HLT, the QI is set to 3 (poor), and HR 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.

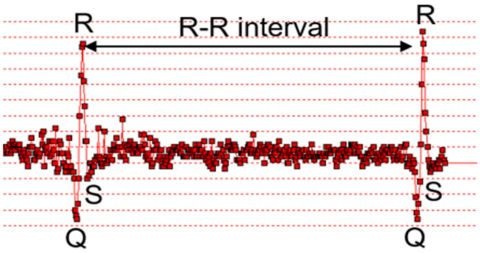


Fig. 2. Explanation of the QRS complex of an electrocardiogram. The R-R interval represents the time between each detected heartbeat, measured from peak (R) to peak (R) on the QRS complex. The QRS complex consists of the Q wave, which is the first negative deviation, followed by the R wave, a positive (upward) deviation. Any negative deflection following immediately after the R portion is termed the S wave (source: Star-Oddi HRT Analyzer software Manual).

Ewes were placed in a communal pen (5 m × 7 m), with an open area (5 m x 5 m) (latitude 41.633920, longitude −0.858790), and were fed barley straw ad libitum and 0.45 kg/d per ewe concentrate once per day at 08:00 h, which provided 2.0 Mcal of metabolizable energy and 12 % crude protein. Water was available ad libitum. Ewes were in the first month of lactation, and lambs were kept permanently with their mothers. Max, mean and min ambient temperatures at the location of the experiment in November are 14.8, 10.6 and 6.3 °C, mean rainfall 30 mm, and relative humidity 73 %.

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 hours. Ewes were then placed in a cradle in dorsal recumbency for the procedure. The skin was prepared for surgery using a povidone-iodine soap solution (Betadine Scrub 7.5 %, Alcon Laboratories, Inc., Fort Worth, TX), and 1 ml of local anesthetic (lidocaine hydrochloride, Anesvet, Ovejero, León, Spain) was injected subcutaneously. An incision was made on the left thorax, directly above the heart, and a subcutaneous pocket was created to hold the biologger. The biologger was positioned with the sensor axis parallel to the heart's axis (Fig. 3), and the electrodes in contact with the muscle layer closest to the skin. A 2/0 absorbable suture (Novosyn, B-Braun, Melsungen, Germany) was used to secure the biologger in the pocket by threading the suture through a small hole at the tip of the device. The incision was then closed with 2–3 sutures, and the area was sprayed with aluminum spray (Aluspray, Vetoquinol, Madrid, Spain). At the end of the study, the biologger was removed using a similar surgical procedure.

2.3. Statistical analysis

BT, HR and HRV (SDNN and RMSSD) (mean ± S.E.) were calculated at hourly intervals for 7 d (D3 to D9). An ANOVA confirmed whether

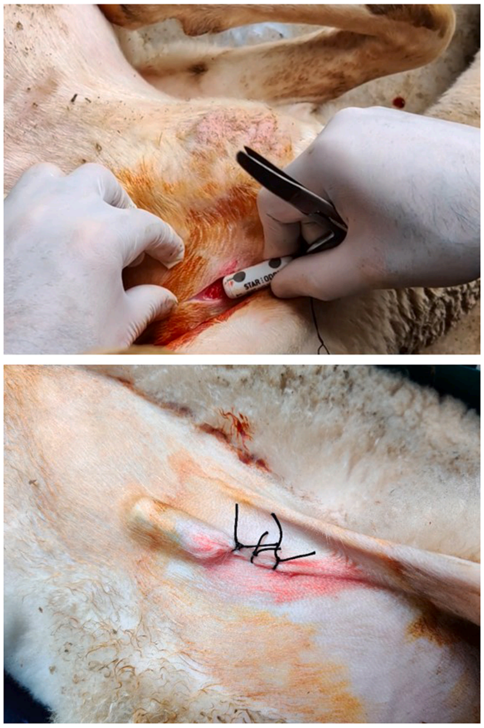


Fig. 3. Surgical procedures used to implant biologgers (DST milli-HRT, Star Oddi, Iceland) (13 mm×39.5 mm; 12 g) in five lactating adult ewes, which recorded heart rate every 5 min.

there were significant differences in BT, HR and HRV between day (0800–1800 h) and night (1900–0700 h). Pearson correlation coefficients between BT, HR and SDNN and RMSSD measured concurrently were calculated.

Circadian rhythms in body temperature (BT), heart rate (HR), and heart rate variability (HRV) were analyzed by fitting the time-series data from each sheep to a 24-hour cosine curve using the Cosinor online platform (Molcan, 2019). For each variable in each individual, the MESOR (Midline Estimating Statistic of Rhythm, representing the average value around which the 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 this model 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, typically set to 24 hours for circadian data, and φ is the acrophase.

Results were expressed as mean ± S.E.M.

3. Results

Of the 9576 records that were downloaded from the five biologgers, 85 % had Q0 ($n = 6590$) or Q1 (1543). The remaining 15 % were graded Q2 ($n = 1155$) or Q3 ($n = 288$), and excluded. For each of the five ewes, the proportion of records that were Q0 +Q1 was 66.5 %, 73.8 %, 84.5 %, 95.7 %, and 97.7 %.

Mean (± S.E.) BT, HR, SDNN, and RMSSD were 38.06 ± 0.01 °C, 111.14 ± 1.68 bpm, 34.35 ± 2.90 ms, and 47.95 ± 4.21 ms, respectively. The mean changes in the four variables throughout the day, based on the 5-min intervals is shown in Fig. 4, and the hourly changes in are shown in Fig. 5. BT was highest at 1500 h, HR at 0800 h, and SDNN and RMSSD at 0700 h, although all variables differed significantly ($P < 0.001$) between day and night. Mean BT and HR were significantly ($P < 0.001$) higher in the day (38.15 ± 0.01 °C and 120.71 ± 0.43 bpm) than it was at night (37.98 ± 0.01 °C and 102.47 ± 0.43 bpm), and SDNN and RMSSD were significantly ($P < 0.001$) higher at night than they were in the day (SDNN: 29.30 ± 0.87 vs. 37.16 ± 0.67 ms; RMSSD: 39.01 ± 1.17; vs. 53.53 ± 0.94 ms, for day and night, respectively). BT and HR were significantly correlated ($P < 0.01$), but both were negatively correlated with SDNN and RMSSD (Table 1).

Ewes exhibited 24-h circadian rhythms in BT, HR, SDNN and RMSSD (Fig. 5), with a MESOR of 38.06 °C, 111.13 bpm, 34.34 ms, and 47.94 ms, respectively, and amplitudes of 0.13 °C,12.72 bpm, 7.36 ms, and 11.18 ms, respectively. The acrophases of BT, HR, SDNN, and RMSSD were at 1457 h, 1223 h, 0350 h, and 0327 h, respectively.

4. Discussion

The ewes in our study showed a circadian rhythm in BT, HR and HRV and did not exhibit any adverse effects from having had a biologger implanted subcutaneously.The use of these particular biologgers of various sizes has been validated in numerous mammal species, as sheep (Fuchs et al., 2019), reindeer (Trondrud et al., 2021), cattle (Palacios et al., 2021), pigs (Lorbach et al., 2021), several wild species (Wild Vervet Monkeys, McFarland et al., 2024; wild boars, Ruf et al., 2023; brown bear, Thiel et al., 2022), broiler chickens (Khan et al., 2023), and fish species (Olive Flounder, Koo et al., 2024; Gilthead Seabream Broodstock, Cabrera-Álvarez et al., 2024; Atlantic salmon, Zrini and Gamperl, 2021; Brown trout and Perch, Norling, 2017; Bowfin and Largemouth bass, Doherty et al., 2022; Salmon, Westerberg et al., 1999),

In our study, 85 % of the HR records from the five ewes graded as QI

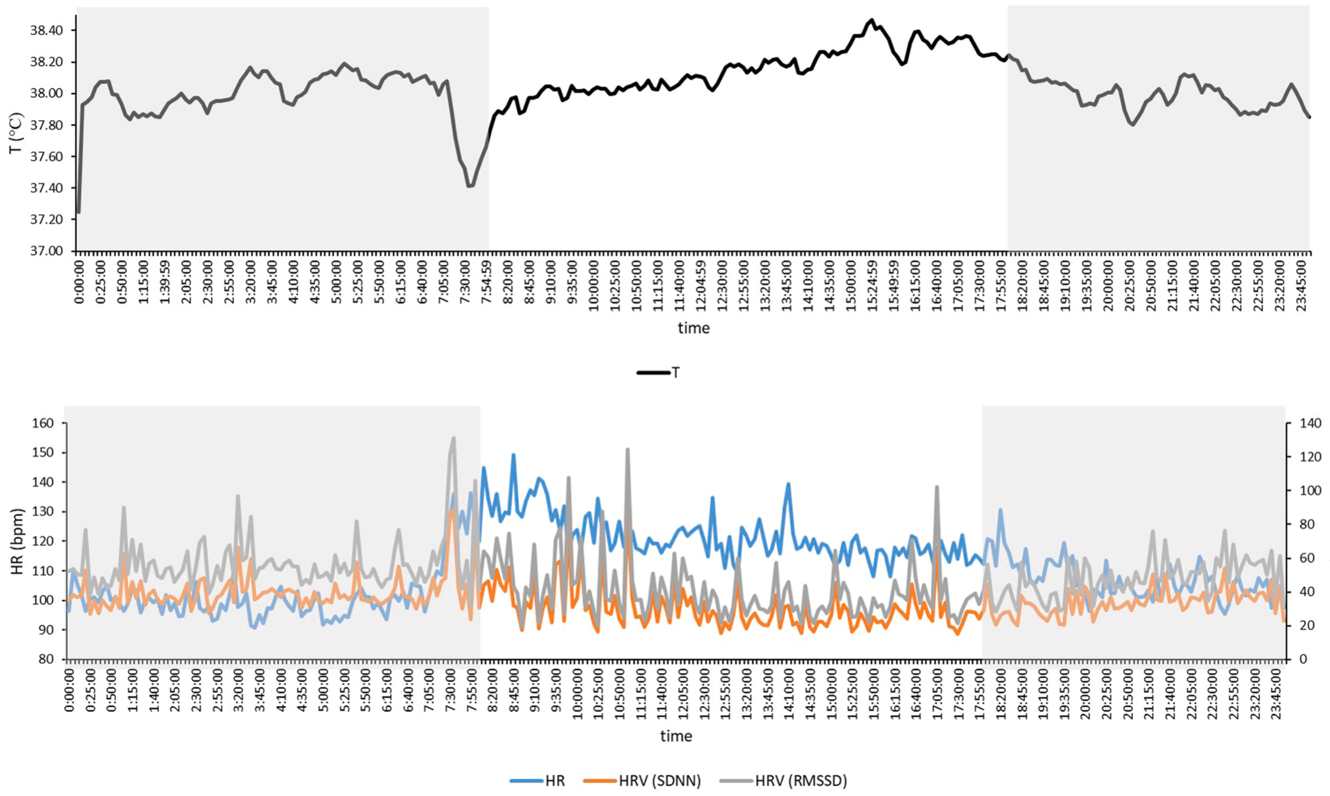


Fig. 4. Mean 24-h body temperature (BT), heart rate (HR) and HR variability (HRV) as measured by the standard deviation of all interbeat intervals (SDNN), and the root mean square of successive R–R intervals (RMSSD) of five lactating adult ewes that had received a subcutaneous biologger (DST milli-HRT, Star Oddi, Iceland) that was programmed to record data every 5 min (gray areas represent night).

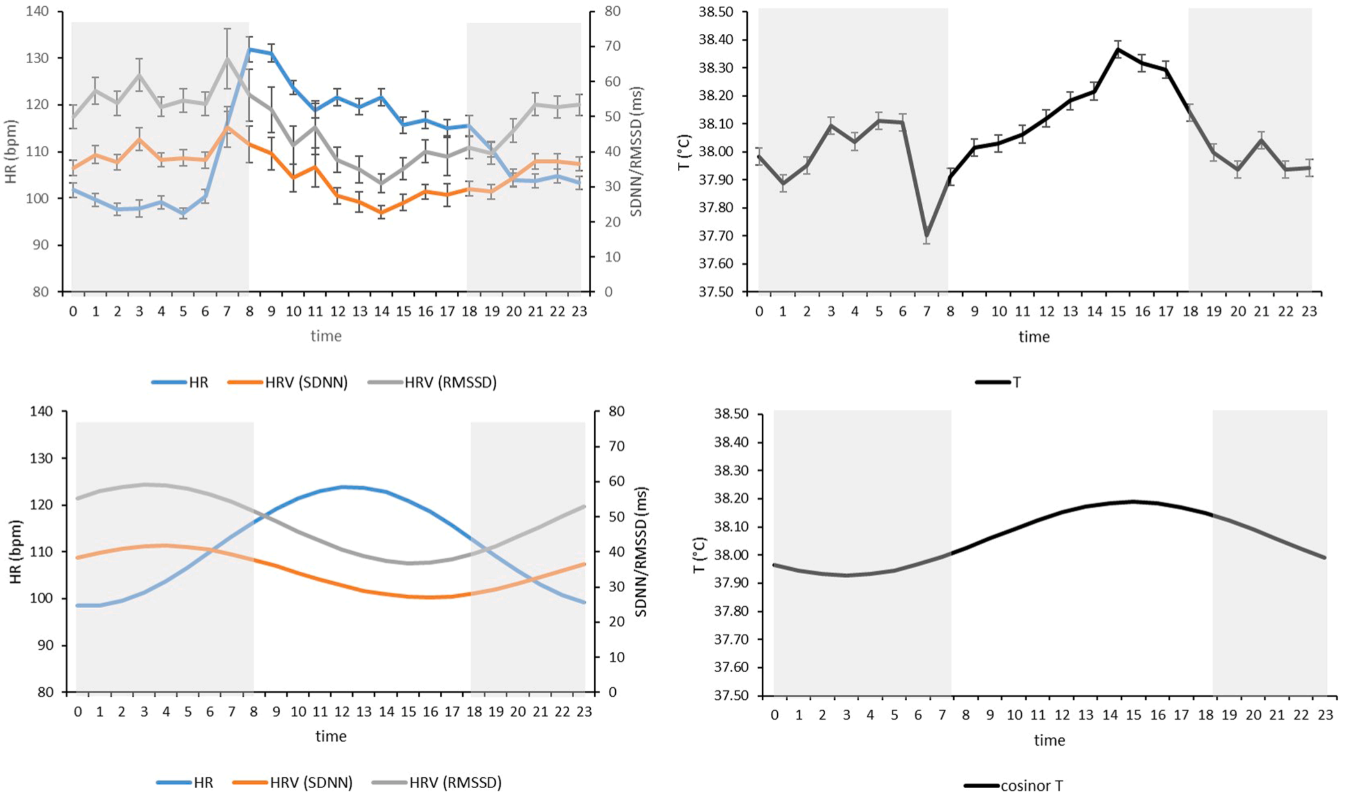


Fig. 5. Mean (± SE) hourly body temperature (BT), heart rate (HR) and HR variability (HRV) as measured by the standard deviation of all interbeat intervals (SDNN), and the root mean square of successive R–R intervals (RMSSD) (upper panel), and the corresponding cosinor curves of a 24-h activity rhythm (lower panel) of five lactating adult ewes that had received a subcutaneous biologger (DST milli-HRT, Star Oddi, Iceland) that was programmed to record data every 5 min (gray areas represent night).

Table 1
Matrix of correlation between body temperature (BT), heart rate (HR) and HR variability (HRV) as measured by the standard deviation of all interbeat intervals (SDNN), and the root mean square of successive R–R intervals (RMSSD) of five lactating adult ewes that had received a subcutaneous biologger (DST milli-HRT, Star Oddi, Iceland) that was programmed to record data every 5 min (**, P < 0.01).

	BT	HR	SDNN	RMSSD
BT		0.117 * *	−0.101 * *	−0.107 * *
HR	0.117 * *		−0.140 * *	−0.123 * *
SDNN	−0.101	−0.140		0.984 * *
RMSSD	−0.107 * *	−0.123 * *	0.984 * *	

= 0 or 1 and, therefore, were included in the analysis. Muller et al. (2020) evaluated the effects of device orientation and electrode placement in trout on the quality of the data gathered by the same brand of (albeit smaller) biologgers, and concluded that the orientation of the electrodes was critical, in rainbow trout; however, the orientation of the electrodes did not have an effect on data quality trout as long as the device was placed as close to the heart as possible, electrodes were in contact with musculature, and double sutures were used. Among the five ewes used in our study, the proportion of records that graded QI = 0 or 1 ranged from 66.5 % to 97.7 %, which suggests that, in this species, the orientation of the electrodes probably was essential in obtaining optimal records of HR, which might have caused the variability among individuals. Considering that in the present work devices were inserted following a standard procedure (electrodes in contact with the muscle layer closest to the skin) it is likely that this variability could be due to slight rotations of the biologgers due to, for instance, to a higher activity of a particular animal, dislocating the original position of the electrodes.

BT, HR and HRV exhibited a circadian rhythm, as we reported in sheep using the same biologgers (Abecia et al., 2021, 2022). The management and housing system likely had a direct impact on the body temperature (BT) and heart rate (HR) of the ewes, whose circadian rhythms were influenced by the timing of concentrate feeding. Body temperature reached its lowest point just before feeding and began to rise afterward, peaking immediately post-feeding. Feeding time acted as a zeitgeber (time cue) for both BT and HR, as demonstrated by Mohr and Krzywanek (1995), who reported that BT and HR showed distinct peaks at the time of food presentation. HRV also exhibited a circadian rhythm, with higher values during the day and lower values at night, a pattern observed in other species such as dogs (Hasegawa et al., 2024), cattle during summer (Kovács et al., 2016), pigs (Kuwahara et al., 2004), and humans (Sammito et al., 2016). In a systematic review of studies that evaluated HRV variables over a 24-h period in humans almost all of the studies detected a circadian rhythm in the HRV variables increased at night with a peak in the second half of the night (Sammito et al., 2016). An increase in HRV at night is a product of parasympathetic activity, particularly, because the HRV variables evaluated in the studies are, in most cases, associated with the parasympathetic nervous system (RMSSD), or reflect the interaction between the sympathetic nervous system and the parasympathetic nervous system. HRV is reduced in the day because of increased sympathetic nervous system activity, and increases again in the evening and in the first few hours after dark. The cosinor curve of HRV of the ewes in our study was similar to that reported in healthy infants and children, aged 2 mo to 15 yr (Massin et al., 2000). The acrophases for SDNN (0350 h) and RMSSD (0327 h) in the ewes in our study were very similar to those in children (0351 h and 0245 h).

In our study, there was an inverse relationship between BT and HR with HRV, which has been demonstrated using linear or nonlinear HRV methods (Kazmi et al., 2016). As reviewed by Sacha (2014), the correlation between HRV and HR is both a physiological and a mathematical phenomenon, such that the physiological dependence of HRV on HR is dictated by autonomic nervous system activity; i.e., the higher the parasympathetic nervous system activity, the slower HR and higher HRV. Eye temperature tended to be higher and HRV numerically lower in sheep given epinephrine during an infusion period compared with sheep that received saline (Sutherland et al., 2020), in agreement with the negative correlation observed in our study between BT and HRV.

Studies on the daily patterns in HRV in adult sheep are limited, although several studies used an ovine fetus as animal models for physiological studies because the sheep heart is similar to the human heart (Jensen et al., 2009; Lear et al., 2014, 2020, 2023). In adult sheep, Oishi et al. (2018) reported results similar to those in our study, with HRV lower in the day than at night; however, the physical activity evaluated in those animals had significant effects on all of the HRV variables evaluated and on HR. They concluded that a correction in the activity-specific component of HRV is indispensable for evaluating other effects on HRV. In a study of the effect of heat stress on HRV, Kitajima et al. (2021) drew the same conclusion. They reported that HRV, as measured by Polar HR monitors, decreased significantly with an increase in THI and locomotor activity of sheep and goats, although they suggested that there would be a threshold of THI around 80 that strongly affected HRV, such that high heat stress can affect the autonomic balance of animals non-linearly by inducing the sympathetic nervous system. However, the confounding effects of physical activity on HRV should be considered. In our study, we did not estimate locomotor activity; therefore, it remains to be confirmed whether an increase in HRV at night is associated with a reduction in locomotor activity that we have previously demonstrated in the same type of sheep and in the same housing (Abecia et al., 2024).

5. Conclusion

In conclusion, subcutaneous biologgers detected a circadian rhythm in BT and HRV in lactating adult sheep, variables that should be taken into account when assessing animal welfare and health status.

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CRediT authorship contribution statement

Carlos Palacios: Writing – review & editing, Methodology, Data curation. **Jaime Nieto:** Writing – review & editing, Methodology, Data curation. **Javier Plaza:** Writing – review & editing, Methodology, Data curation. **Francisco Canto:** Writing – review & editing, Methodology, Data curation. **José A. Abecia:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

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

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