Animal Biotelemetry

METHODOLOGY

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Bio-loggers inserted in intravaginal sponges, or subcutaneously, as tools to measure body temperature

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Abstract

The body temperature of animals can be measured by thermistors, thermocouples, or radiotelemetry devices that are implanted surgically under the skin, although the suitability of subcutaneous temperature as an indicator of core temperature can be limited because of abnormal temperature readings, probably affected by ambient temperature and animal inactivity. This study compared the use of bio-loggers designed to monitor subcutaneous temperature (Tsub), with their use embedded in intravaginal sponges to measure vaginal temperature (Tvag). Three ewes were implanted with a subcutaneous temperature bio-logger that was configured to record Tsub every 30 min for a month. Ewes were given an intravaginal sponge for 12 days two days later. Inside the sponges were installed programmed bio-loggers that measured Tvag every 5 min. The ambient temperature (Tamb) and relative humidity were monitored using mini data-loggers. Mean Tsub was lower ($P < 0.001$) during the day (38.02 ± 0.02 °C) than at night (38.10 ± 0.02 °C), with maximum Tsub (38.57 °C) at 20:00 h and minimum temperature (37.36 °C) at 08:00 h; however, mean Tvag was higher ($P < 0.001$) during the day (38.71 ± 0.01 °C) than at night (38.62 ± 0.01 °C), with maximum Tsub (39.02 °C) at 20:55 h and minimum temperature (38.33 °C) occurred at 08:25 h. Mean Tsub (38.08 ± 0.02 °C) was lower ($P < 0.0001$) than was Tvag (38.65 ± 0.10 °C) in the daytime and at night ($P < 0.001$). Both temperatures had a 24-h rhythm ($P < 0.0001$), but differed ($P < 0.001$) in the mean midline estimating statistic of rhythm (MESOR) (Tvag: 38.67 ± 0.02 °C, Tsub: 38.09 ± 0.02 °C), amplitude (Tvag: 0.21° ± 0.01C; Tsub: 0.25 ± 0.01 °C), and acrophase (Tvag: 18:27 ± 0.38 h, Tsub: 20:48 ± 0.44 h). The coefficient of correlation between the two temperatures, measured simultaneously for 12 d was 0.644 ($P < 0.01$), and between Tamb and the two physiological temperatures, measured at the same time throughout the 12 d experiment, was 0.319 ($P < 0.01$) for Tsub and 0.287 ($P < 0.01$) for Tvag. The linear regression analysis of the 24 h circadian rhythm in Tsub and Tvag indicated a high coefficient of determination with Tvag (0.9255) and a lower coefficient of determination with Tsub (0.4292). In conclusion, the integration of a mini body temperature logger into a vaginal sponge, or their subcutaneous insertion, provided a continuous and accurate record of body temperature. Furthermore, the strong correlation between mean 24 h circadian Tvag and Tamb, demonstrated the usefulness of Tvag in biometeorological studies in sheep. As an alternative to employing these devices subcutaneously, they can also can be utilized as a biomarker of core body temperature inserted in vaginal sponges.

Keywords: Sheep, Bio-loggers, Temperature, Subcutaneous, Vaginal

Background

Body temperature is an essential health and diagnostic sign [1]. Temperature is generally higher in the active periods of an animal's daily cycle than it is at rest [2], and hyperthermia occurs as a short-term increase in body

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temperature in response to stress [3]. Thus, physiological factors like body temperature can provide valuable insight into how management practices affect livestock, or how environmental and climatic factors, or the interaction with the human species affect wild fauna. Temperature is the most commonly measured physiological variable in domestic animals, and it is sensitive to even the smallest changes. It is also most closely related to a wide range of diverse physiological functions, including nutrition, reproduction, activity, stress reactions, and, obviously, the maintenance of health [4]. In rhesus monkeys, skin or nasal temperature changes following emotional stimulations have been reported [5]; moreover, measurable indicators of stress, such as body temperature have been documented to determine the effects of capture and tagging on wild animals [6].

The body temperature of animals can be measured by thermistors, thermocouples, or radiotelemetry devices that are implanted surgically under the skin (subcutaneous temperature, Tsub), which has been reported as more variable than is tympanic or rectal temperature (Trec), although Tsub is strongly correlated ($r=0.738$) with Trec in sheep, goats, and horses [7]. However, it has been concluded that the suitability of Tsub in cattle in the field as an indicator of core temperature can be limited because of abnormal temperature readings, probably affected by ambient temperature (Tamb) and animal inactivity, which can occur frequently [8].

Rectal or vaginal temperatures (Tvag) have been the "gold standard" measures of core body temperature, which were taken by inserting a thermometer into the rectum or vagina for around 30 s [9]. Trec and Tvag are substantially ($r=0.78$–0.95) correlated in sheep [10, 11], suggesting that Tvag, like Trec, can be utilized as an indication of core body temperature. Furthermore, Tvag may be more predictive of body core temperature than Trec because the former has a higher blood flow than the latter [12].

Trec is an accurate and repeatable measure for evaluating core temperature and quantifying heat stress responses in livestock [13]; however, Trec measured by a digital thermometer provides a cross-sectional picture of a species' temperature, only, because the animal has to be handled and restrained. In addition, stress-induced hyperthermia and metabolic heat production from flight responses can occur if the animal is restrained for a manual temperature measurement. In fact, the ability to measure Trec in free-range livestock has been hampered by the availability of devices that automatically record and transmit or store data without raising body temperature [14].

Behavioral responses to the insertion of a rectal thermometer likely indicate many animals find Trec measurement uncomfortable, and for the veterinarians it is a time-consuming procedure, and a possible source of cross-contamination and damage to the patient or veterinarian [15]. Remote sensing techniques have been utilized to continuously assess body temperature to alleviate the stress produced by handling and constraint. The focus on noninvasive and continuous measures of body core temperature in animals has expanded [16], in addition to the development and implementation of technical solutions.

Intravaginal sponges with a button temperature sensor have been used to monitor the thermophysiological behavioral stress responses of sheep transported by road [17], and intravaginal recording devices based on telemetry have been used in cows [18, 19] and mares [20]. Recently, an indwelling vaginal temperature sensor based on a bio-logger in ewes has been used to validate the relationship between manual Trec and automated Tvag in Merino ewes [11]. In addition, an intravaginal sensor that combines temperature and a drug release device has been used to detect heat stress in animals on pasture, with minimal disturbance to the animal [14]. The use of bio-loggers to measure the physiological status of livestock; e.g., body temperature, respiration, blood pressure, heart rate, and activity has become commonplace. Those devices can provide invaluable insights into movement, feeding behavior, energy expenditure, and physiological processes. Bio-sensors can monitor real-time animal responses to stressors such as managing, housing, and feeding, which are otherwise difficult to measure and more difficult to differentiate from changes to fluctuating temperature from manually collecting temperature data, and these external factors affect an animal's resilience to stress [21]. For example, bio-loggers were used to quantify the effects of density on cattle stress [22], and to monitor temperature, heart rate, and activity of sheep managed under intensive housing conditions [23]. However, because these bio-loggers have been designed to be inserted subcutaneously, it is necessary to quantify their capacity to predict core (vaginal) temperature, and to quantify the influence of ambient temperature on the devices' performance.

This experiment aimed to determine whether bio-loggers initially designed to measure Tsub are also useful to measure Tvag, when embedded in intravaginal sponges. The level of agreement between both measurements in the same animal at the same time has been determined, as well as their relationships with Tamb.

Material and methods

Animals

In mid-May, three non-pregnant adult Rasa Aragonesa ewes (mean live weight ± S.D. = 47 ± 4 kg), received a

surgically implanted subcutaneous temperature bio-logger (DST milli-HRT ACT, Star Oddi, Gardabaer, Iceland) (13 mm × 39.5 mm, 12 g) that had been programmed to record Tsub data every 30 min for one month. Two days after they were implanted, ewes received an intravaginal sponge that contained 30 mg fluogestone acetate (Sincropart, CEVA Salud Animal, Barcelona, Spain) for 12 d. Programmed bio-loggers (DST micro-ACT, Star Oddi, Gardabaer, Iceland) (8.3 mm × 25.4 mm, 3.3 g) were placed inside the sponges to measure Tvag every 5 min for 12 d. Those ewes were placed in a communal pen (5 m × 7 m, 4.37 m^2/ewe) with five other ewes, 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.

Surgical procedures

We followed the procedures described by Abecia et al. [23]. Briefly, devices were sterilized by a 24-h immersion in 0.55% ortho-phthalaldehyde (CIDEX- OPA solution, Johnson & Johnson, New Jersey, USA). Animals were placed in dorsal recumbence using a cradle. The skin was prepared with a povidone-iodine soap solution (Betadine Scrub 7.5%, Alcon Laboratories, Inc. Fort Worth, TX), and 1 ml local anesthetic s. c. (lidocaine hydrochloride, Anesvet, Ovejero, León, Spain) was injected. The bio-logger was placed subcutaneously on the left thorax, through an incision in the skin with a pocket to hold the bio-logger. To affix the bio-logger in the skin pocket, a 2/0 absorbable suture (Novosyn, B-Braun, Melsungen, Germany) was connected to the logger through a small hole at the top of the bio-logger. The incision was closed by 2–3 sutures, and the scar was sprayed with aluminum (Aluspray, Vetoquinol, Madrid, Spain). A similar surgical procedure was used to remove the bio-logger at the end of the experiment.

Sponge modification

A small incision was made in the top of the sponge, and the bio-logger was placed inside and was fixed by silk suture to the sponge itself, through the small hole at the top of the bio-logger (Fig. 1). Following standard veterinary practice, before being inserted, sponges were dusted with an antibiotic powder preparation (neomycin sulfate, Framicas, Ovejero, León, Spain) in a bag. The sponges were inserted in the vagina of the ewes with the aid of an applicator that had been disinfected. They were removed by a gentle pull on the cord.

After subcutaneous and vaginal bio-loggers were retrieved, the data were downloaded to a communication box and the Mercury software v5.83 (Star Oddi, Gardabaer, Iceland). Tamb (°C) and relative humidity (RH, %) were recorded by mini data-loggers (Testo 174H, Testo SE & Co. KGaA, Titisee-Neustadt, Germany).

Statistical analysis

For Tsub and Tvag, mean (± S.E.) values were calculated at hourly intervals, and mean overall and mean daytime (07:45–20:45 h) and nighttime (20:50–07:40 h) values were compared by analysis of variance. Pearson correlation coefficients between Tsub, Tvag, and Tamb, measured at the same time were calculated. Regression analyses were conducted among Tsub and Tvag, and Tamb.

The circadian rhythms in Tsub and Tvag were estimated by fitting the individual time-series measurements of each sheep to the cosine curve of a 24 h activity rhythm, which was derived by the Cosinor Method through the Cosinor online platform (https://cosinor.online) [24].

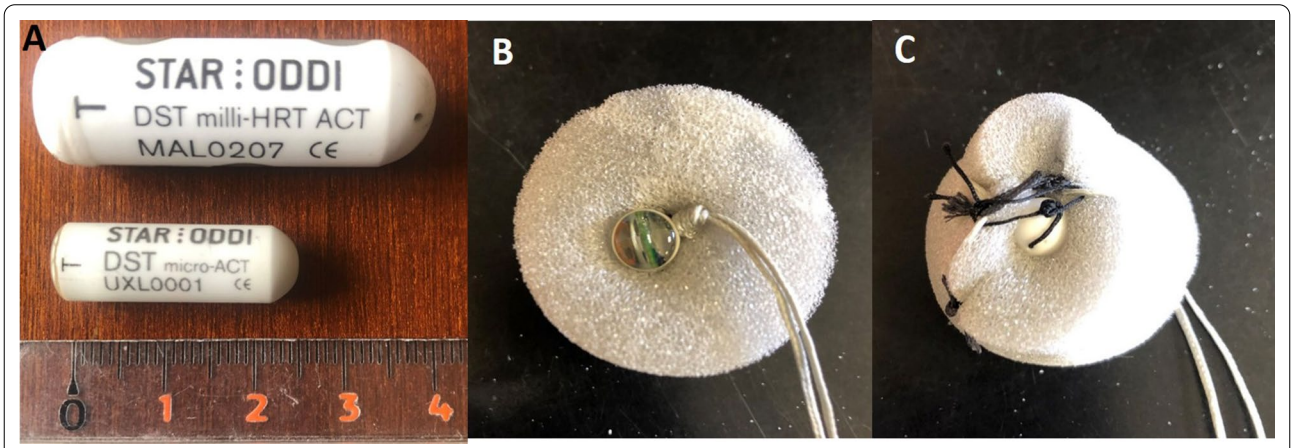

Fig. 1 Bio-loggers that were either surgically implanted (DST milli) (**A**) or inserted into a vaginal sponge (DST micro) (**B**–**C**), in three non-pregnant adult Rasa Aragonesa ewes

MESOR (Midline Estimating Statistic of Rhythm; i.e., the average value around which the variable fluctuates), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) were measured for each variable in each individual. A $P<0.05$ indicated that the time-series fit a 24-h rhythm.

The entirety of data collection period for Tvag and Tsub was analyzed separately to test circadian rhythm, and the overlapping times during the 12 days of the study between Tvag and Tsub were used to compare both temperatures. A Bland–Altman plot (difference plot) has been used to analyze the agreement between both temperatures.

Results

Tamb and RH in the 12 d of the experiment are shown in Fig. 2. Ewes were exposed to a mean Tamb = 19.76 ± 0.12 °C and a mean RH = 51.49 ± 0.31%. Ambient temperature was higher and RH was lower in the day than they were at night (21.32 ± 0.17 vs. 17.90 ± 0.12 °C, and 48.89 ± 0.41 vs. 54.57 ± 0.44%, respectively) ($P<0.0001$). The mean highest and mean lowest Tamb occurred at 18:30 h on the seventh day (29.00 °C) and at 06:45 h on the third day (12.80 °C), respectively, and the highest RH and the lowest RH occurred at 02:15 h on the third day (79.90%) and at 17:30 h on the tenth day (31.80%), respectively.

Figure 3 shows the Tsub and Tvag of an individual sheep in the 12 d of the experiment, and Fig. 4 shows a representative day for one of the three ewes. Mean (± SE) Tsub was significantly ($P<0.001$) lower in the day than it was at night (Table 1). Maximum Tsub occurred at 20:00 h and minimum temperature occurred at 08:00 h (Fig. 5, Table 1). Mean Tvag, however, was significantly ($P<0.001$) higher in the day than it was at night (Table 1). Maximum Tsub occurred at 20:55 h and minimum temperature occurred at 08:25 h (Fig. 6, Table 1). Mean Tsub was significantly ($P<0.0001$) lower than was Tvag in the day and at night ($P<0.001$) (Fig. 7, Table 1), with coefficients of variation of 15.2% and 9.8%, for Tsub and Tvag, respectively.

The analysis of the circadian rhythms of Tsub and Tvag revealed that both exhibited a 24-h rhythm ($P<0.0001$) (Fig. 7), but differed significantly ($P<0.001$) in mean MESOR, amplitude, and acrophase (Table 1).

The coefficient of correlation between Tsub and Tvag measured simultaneously on 12 d was 0.644 ($P<0.01$), the coefficient of determination was 0.4146 ($P<0.01$) (Fig. 8). The Bland–Altman method of comparison was calculated by using Tvag minus Tsub (Fig. 9). The mean (± S.D.) difference between Tvag and Tsub was 0.58 ± 0.44 °C, with a 95% confidence interval of − 0.298 to 1.456 °C. The coefficient of correlation between Tamb and the two physiological temperature, measured at the same time throughout the 12 d experiment was 0.319 ($P<0.01$) for Tsub and 0.287 ($P<0.01$) for Tvag (Fig. 10); however, the linear regression analysis between the 24 h circadian rhythm in Tsub and Tvag revealed a high coefficient of determination with Tvag (0.9255) and a lower coefficient of determination with Tsub (0.4292) (Fig. 11), which confirms the coincidence between the acrophase of Tvag and the time at which the highest (18:30 h) Tamb occurred (Fig. 7).

Discussion

This study confirms the effectiveness of placing these types of bio-loggers in a vaginal sponge to monitor core temperature in sheep, and the strong correlation between circadian Tvag and Tamb. In an extensive literature review [13], benefits and disadvantages of vaginal

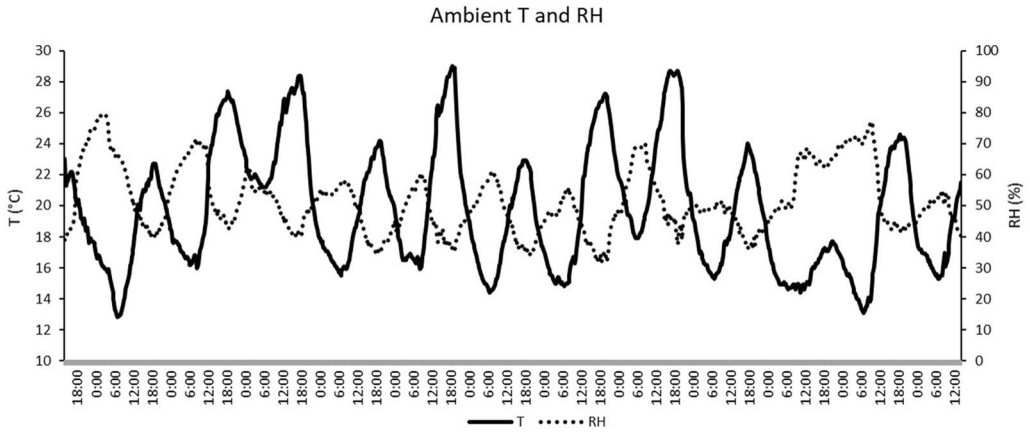

Fig. 2 Ambient temperature (T) and relative humidity (RH) in the 12 d experiment involving non-pregnant adult Rasa Aragonesa ewes

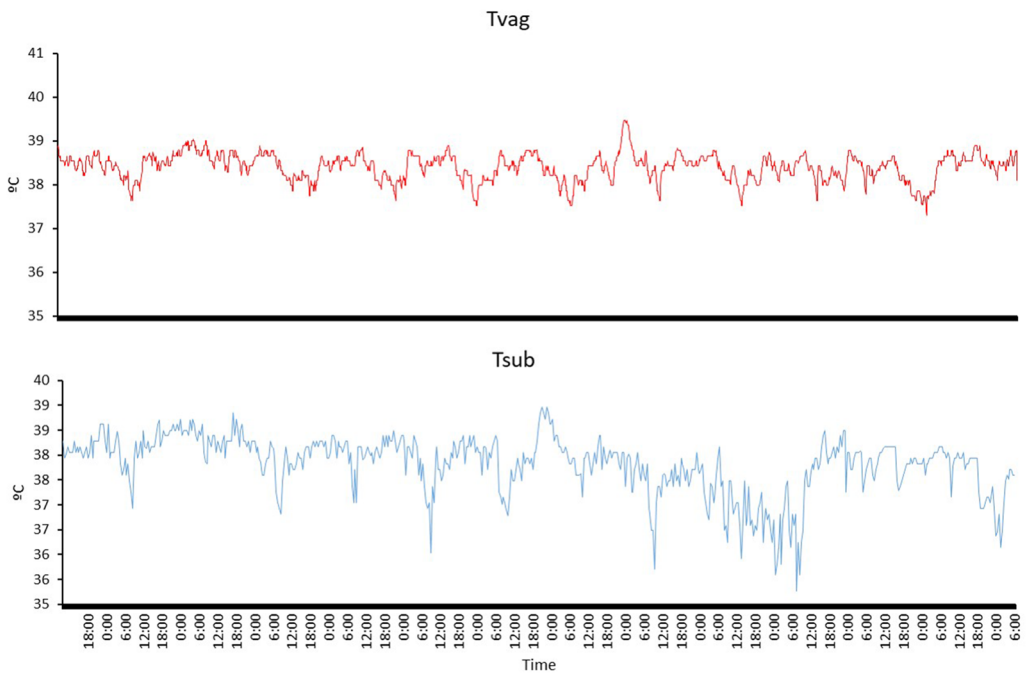

Fig. 3 Subcutaneous and vaginal temperatures for 12 d in one of three non-pregnant adult Rasa Aragonesa ewes that were surgically implanted with a subcutaneous bio-logger programmed to record subcutaneous temperature every 30 min and an intravaginal sponge with a programmed bio-logger to monitor vaginal temperature every 5 min

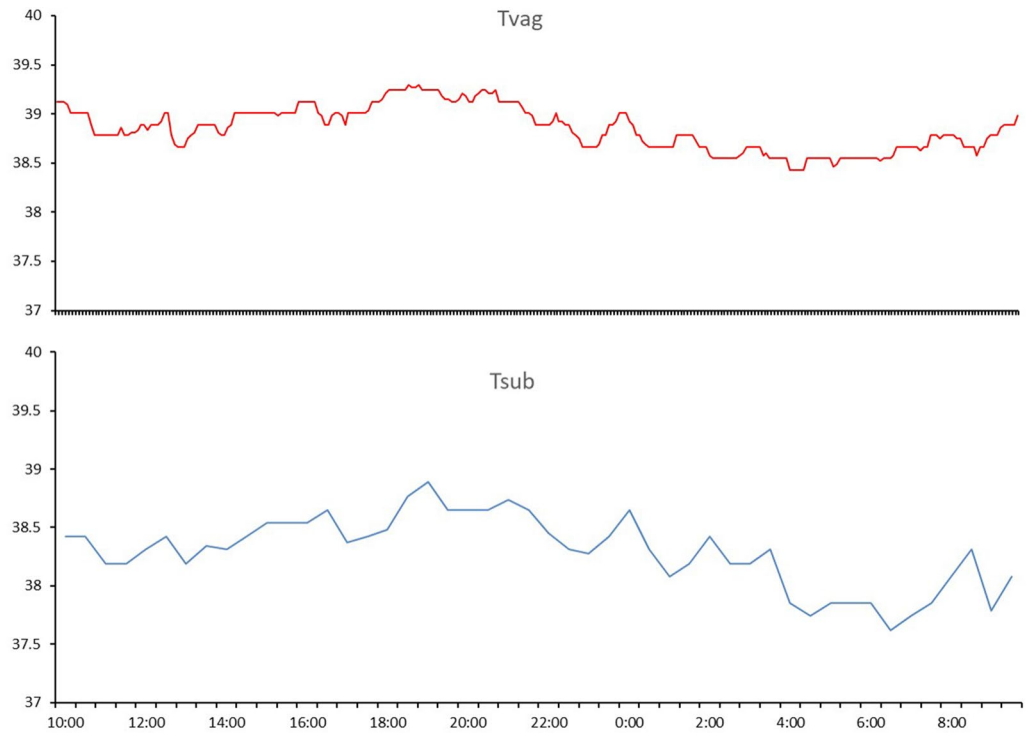

Fig. 4 Subcutaneous and vaginal temperatures on one day in one of three non-pregnant adult Rasa Aragonesa ewes that were surgically implanted with a subcutaneous bio-logger programmed to record subcutaneous temperature every 30 min and an intravaginal sponge with a programmed bio-loggers to monitor vaginal temperature every 5 min

Table 1 Mean (± SE), vaginal (Tvag) and subcutaneous (Tsub) temperatures (°C), during the day or night, and maximum, minimum and MESOR (midline estimating statistic of rhythm; i.e., the average value around which the variable fluctuates), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) of three non-pregnant adult Rasa Aragonesa ewes that were surgically implanted with a subcutaneous bio-logger programmed to record subcutaneous T every 30 min and an intravaginal sponge with a programmed bio-logger to monitor vaginal T every 5 min

	Mean	Day	Night	Max (time)	Min (time)	MESOR	Amplitude	Acrophase
Tvag	38.65 ± 0.10^{a}	$38.71 \pm 0.01^{a,x}$	$38.62 \pm 0.01^{a,y}$	39.02^{a} (20:55 h)	38.33^{a} (8:25 h)	38.67 ± 0.02^{a}	0.21 ± 0.01^{a}	$18{:}27 \pm 0.38^{a}$
Tsub	38.08 ± 0.02^{b}	$38.02 \pm 0.02^{b,x}$	$38.10 \pm 0.02^{b,y}$	38.57^{b} (20:00 h)	37.36^{b} (8:00H)	38.09 ± 0.02^{b}	0.25 ± 0.01^{b}	$20{:}48 \pm 0.44b^{b}$

[a,b] $P < 0.001$ between Tvag and Tsub

[x,y] $P < 0.001$ between day and night

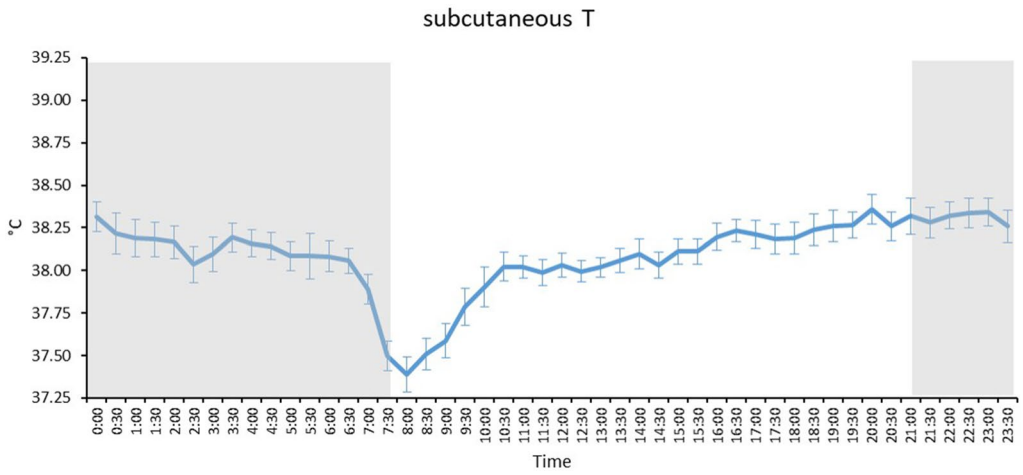

Fig. 5 Mean (± S.E.) 24 h subcutaneous temperature of three non-pregnant adult Rasa Aragonesa ewes that were surgically implanted with a subcutaneous bio-logger programmed to record subcutaneous temperature every 30 min for 12 d (grey areas indicate nighttime)

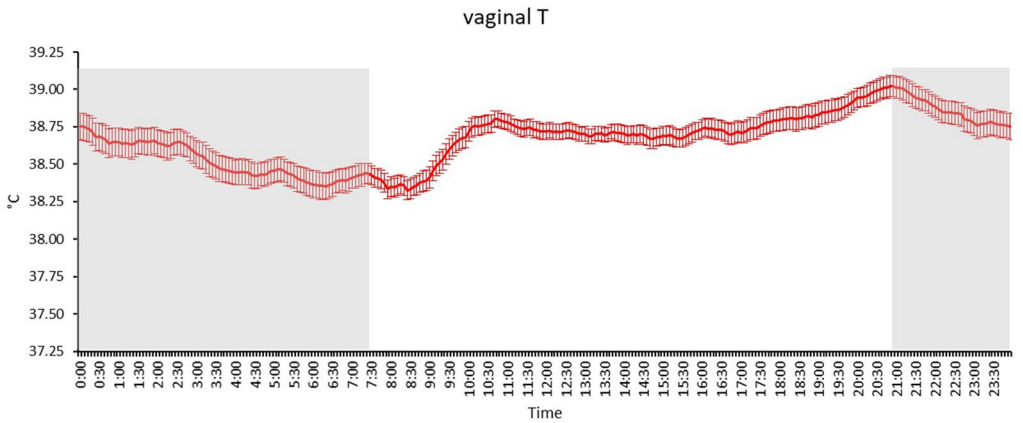

Fig. 6 Mean (± S.E.) 24 h vaginal temperature of three non-pregnant adult Rasa Aragonesa ewes that received an intravaginal sponge with a programmed bio-loggers to monitor vaginal temperature every 5 min for 12 d (grey areas indicate nighttime)

bio-loggers have been enumerated. They include robust correlation with core temperature, high collection frequency, accurate and detailed temperature distribution, easy operation, convenient to implant, catheterization without surgery, and no constraints on biological functions as benefits. Among disadvantages, risk of infection,

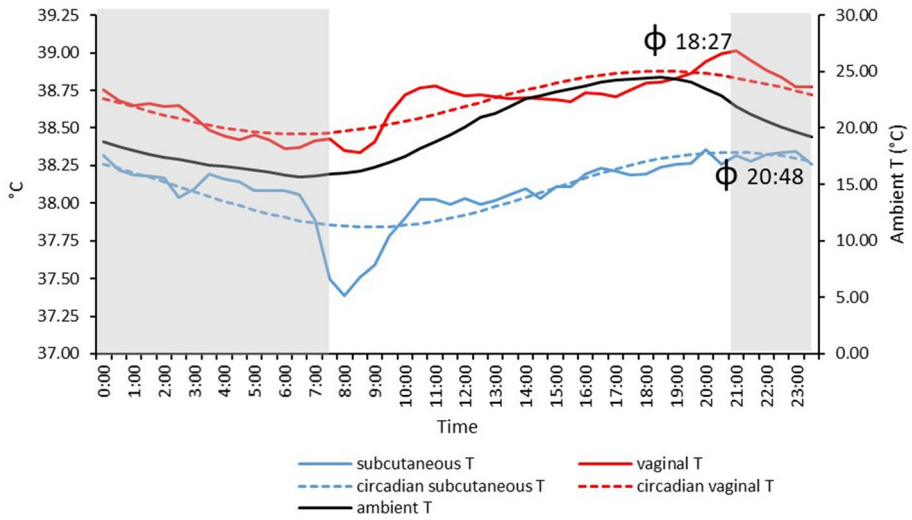

Fig. 7 Mean hourly subcutaneous and vaginal temperatures, and their respective cosinor curves, of three non-pregnant adult Rasa Aragonesa ewes that were surgically implanted with a subcutaneous bio-logger programmed to monitor subcutaneous temperature every 30 min and an intravaginal sponge with a programmed bio-loggers to monitor vaginal temperature every 5 min (ϕ acrophase) (grey areas indicate nighttime)

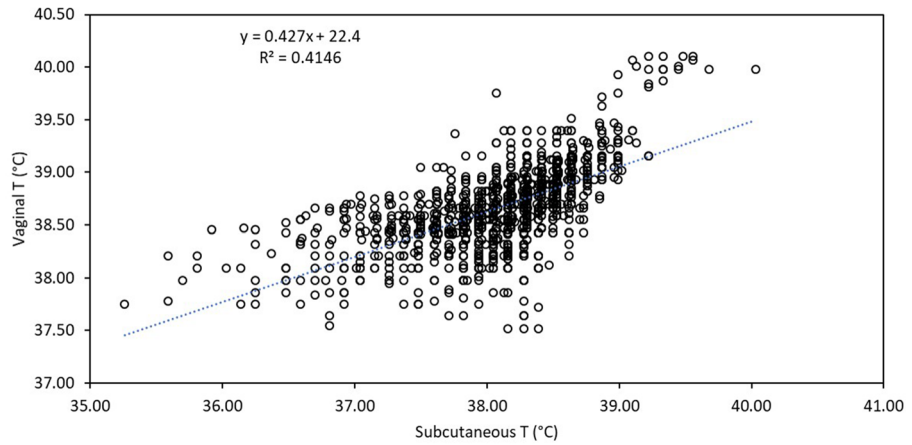

Fig. 8 Linear regression between subcutaneous temperature and vaginal temperature recorded at the same time in 12 d of three non-pregnant adult Rasa Aragonesa ewes that were implanted with a subcutaneous bio-logger programmed to monitor subcutaneous temperature every 30 min and an intravaginal sponge with a programmed bio-logger to monitor vaginal temperature every 5 min

influenced by increasing blood flow in estrus, female individuals only, data logger size limitations, duration restricted by battery power, and not real-time.

The bio-loggers used in our experiment that were inserted into vaginal sponges previously had been inserted in another way to measure vaginal temperature. A vaginal ring made of silicone elastomer with an integrated, miniature temperature logger that was similar to ours, using Cynomolgus macaques (Macaca fascicularis) to design and test the product [25], was compared with subcutaneous loggers in the same animals. As in our experiment, Tsub and Tvag clearly exhibited a diurnal/nocturnal pattern, and the Tvag of the macaques were consistently higher than were those measured subcutaneously, which suggested that internal Tvag is more representative of core body temperature that are those measured close to the external skin surface. Pent et al. [14] used controlled internal drug release (CIDR) devices to record Tvag in ewe lambs, replacing a segment of the CIDR with a Star Oddi bio-logger, as in our experiment, and reported the usefulness of these methods for detecting monthly differences in vaginal temperature,

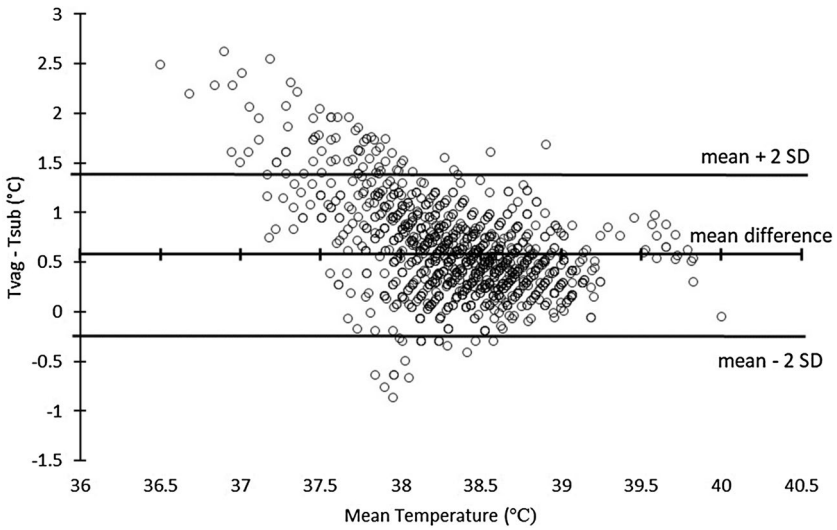

Fig. 9 Bland–Altman plot between vaginal (Tvag, °C) and subcutaneous temperatures (Tsub, °C) recorded at the same time of three non-pregnant adult Rasa Aragonesa ewes that were implanted with a subcutaneous bio-logger programmed to monitor subcutaneous temperature every 30 min, and an intravaginal sponge with a programmed bio-logger to monitor vaginal temperature every 5 min

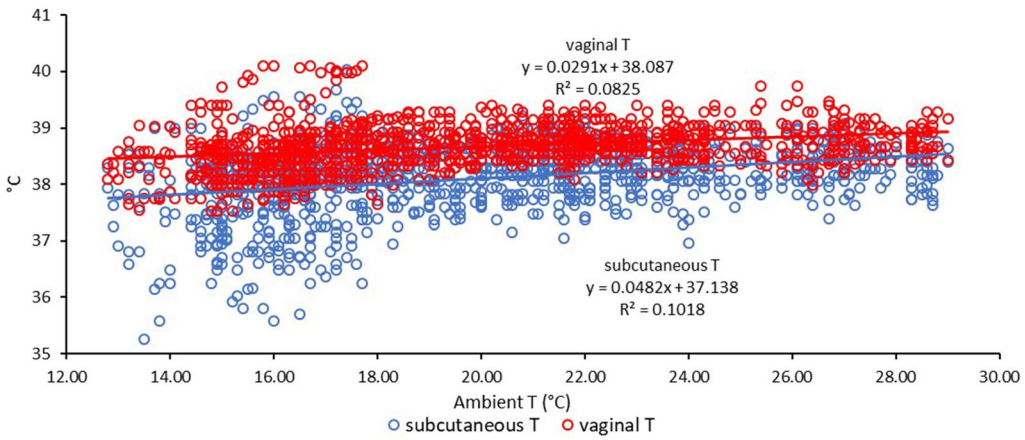

Fig. 10 Linear regression between subcutaneous temperature and vaginal temperature with ambient temperature (recorded every 30 min in 12 d of three non-pregnant adult Rasa Aragonesa ewes that received an implanted subcutaneous bio-logger programmed to record subcutaneous temperature every 30 min and an intravaginal sponge with a programmed bio-logger to monitor vaginal temperature every 5 min

considering the impact of tree species on animal physiology, as well as varying shade usage.

Although there are many published comparisons of surface temperature and Tvag in sheep, few have compared Tsub and Tvag. Abecia et al. [26] compared Tsub and Tvag in sheep as measured by radio-frequency temperature-sensitive cylindrical transponders and button data-loggers that had been inserted in intravaginal sponges, respectively, and ocular (by thermography) and Trec (by a thermometer). After inducing an acute stress, the vaginal data-loggers and the rectal thermometer detected differences between the pre- and post-stress temperature, but Tsub and ocular temperature were similar before and after the stress [26].

In our experiment, ewes exhibited a circadian rhythm in Tsub and Tvag. Piccione et al. [27] observed a distinct daily rhythm in core temperature in sheep as reflected by Trec, and, based on infrared thermography of the eye, forehead, and foot, a distinct daily rhythm in surface temperature. As in our experiment, the peak of temperature at the four sites was highest in the late afternoon or early evening, although the peak in Trec was highest later than was the peak at the other body sites, which was because of the difference in photoperiod between their

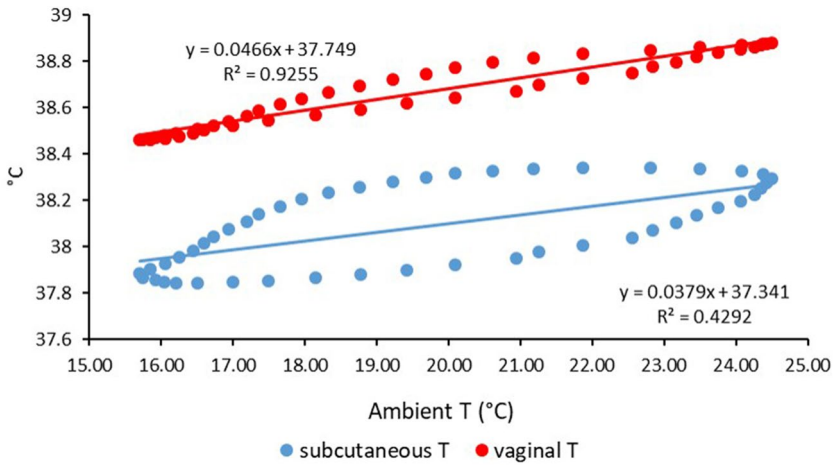

Fig. 11 Linear regression between subcutaneous temperature and vaginal temperature with ambient temperature, recorded at the same time in 12 d of three non-pregnant adult Rasa Aragonesa ewes, that received an implanted subcutaneous bio-logger and an intravaginal sponge with a programmed bio-logger to monitor vaginal temperature every 5 min

experiments [27]. In our experiment, Tsub and Tvag were measured simultaneously and under the same photoperiod, and Tvag had an earlier acrophase than did Tsub. Kearton [28] compared the circadian rhythms of Tvag and Tsub measured in the neck and the tail, reporting that the acrophase of Tvag was almost 2 h before that of peripheral temperature.

Changes in Tvag in sheep are caused by changes in vaginal and uterine blood, which probably influence Tvag at other stages in the estrous cycle [13]. Through a negative feedback mechanism, the progestagen in the vaginal sponges mimics the progestational activity of the corpus luteum and prevents ovarian activity in sheep by limiting the discharge of pituitary gonadotropic hormones and, thus, ovulation [29]. As a result, the ewes in our study were in a similar hormonal condition at the time of the Tvag measurements, and variations in temperature could not be attributed to sheep hormone levels.

In our experiment, Tvag was more strongly correlated with Tamb than Tsub was, which suggests that Tvag is a good variable for measuring changes in core temperature caused by changes in Tamb. A strong correlation between Tamb and Tvag, especially, in lambs in honeylocust silvopastures and open pastures has been reported [14]. Lowe et al. [30] reported a strong correlation between temperature humidity index (THI) and Tvag and ear canal temperature in sheep, although rainfall markedly effected core temperature, which underscores the effects of environment on daily temperature patterns in free-range animals. Tian et al. [31] measured Tvag and THI simultaneously in cows to quantify heat stress, and showed a high correlation between Tamb and Tvag. They came to the conclusion that heat stress had a significant impact on Tvag and the activity of dairy cows, which had an impact on production, by introducing a novel approach to monitoring heat stress that combined in vivo measurements and environmental index calculations.

In our experiment, Tsub and Tvag increased after ewes received concentrate. It has been reported that Tvag increased at the time feed was provided, reaching a peak at an hour before decreasing [32]. In sheep fed high roughage diets, the increase was greater, especially at low intake, and the rate of decrease was lower, especially after the afternoon meal. We previously observed [23] that ewes were at their lowest temperature right before feeding, when body temperature rose, and heart rate and activity peaks occurred right after feeding. Moreover, it has been found that body temperature and heart rate had notable peaks at the moment of food presentation, which worked as a zeitgeber for these variables [33], much as we did.

In the view of the results obtained in this work, the choice of one location or another will depend on factors such as the sex of the animals under study—the sponges could only be placed in females—the possibility or not of performing surgery for implantation and removal of the subcutaneous devices, or the animal's own size. Moreover, the choice of the vaginal route should take into account the possible temperature variations produced by the time of the female's estrous cycle, due to blood circulation, a fact that has been avoided in this work by having the ewes used their cycle synchronized.

Conclusion

The integration of a mini body temperature logger into a vaginal sponge, or their subcutaneous insertion, provided a continuous and accurate record of body temperature. Furthermore, the strong correlation between mean 24-h circadian Tvag and Tamb, demonstrated the usefulness of Tvag in biometeorological studies in sheep. As an alternative to employing these devices subcutaneously, they can also can be utilized as a biomarker of core body temperature inserted in vaginal sponges.

Abbreviations

Tsub: Subcutaneous temperature; Tvag: Vaginal temperature; Tamb: Ambient temperature; Trec: Rectal temperature; RH: Relative humidity.

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Author contributions

JAAb: conceptualization, funding acquisition, investigation, methodology, project administration, resources, writing—original draft; writing—review and editing; SL: investigation, methodology, writing—review; FC: investigation, methodology, writing—review; CP: conceptualization, writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted at the experimental farm of the University of Zaragoza, Spain (41° 63′ N), following procedures approved by the Ethics Committee for Animal Experiments at the University of Zaragoza. The care and use of animals were by the Spanish Policy for Animal Protection (RD 53/2013), which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Consent for publication

Not applicable.

Competing interests

The authors report no declarations of interest.

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