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Major differences between single or twin hair lambs in the immediate postpartum period: Metabolic and thermodynamic patterns detected by infrared thermography

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ABSTRACT

The objective was to evaluate the differences between hair lambs, born from single or twin births, regarding the latency periods for standing up and suckling, the vitality, glycemic, cortisol, and triiodothyronine concentrations, as well as the phenotypic characteristics related to the maintenance of homeothermy in the immediate postpartum. Single (n = 10) or twin (n = 12) Morada Nova lambs were evaluated after birth, during the first successful suckling (M0 = Timepoint 0), and at regular intervals of 20 min (M20, M40, M60). Lambs from single births had higher birth weight (3.09 vs 2.58 kg; $P \leq 0.05$) and higher serum triiodothyronine concentration (267 vs 209 ng/dL; $P \leq 0.05$) compared to twin lambs. There was a positive correlation between weight and blood glucose (0.57; $P \leq 0.05$) for both single and twin lambs. The type of birth did not affect vitality, which was negatively associated with cortisol concentration (−0.53; $P \leq 0.05$). Twin lambs had higher internal and ocular temperatures (39.29 vs 38.67 °C and 38.84 vs 38.13 °C; $P \leq 0.05$, respectively). Body surface temperatures increased over time in both groups ($P \leq 0.05$). An increase in the temperature of the hips region (ysingle = 27.88 + 0.019*time; R2 = 0.96; P = 0.019 and ytwin = 28.74 + 0.019*time; R2 = 0.94; P = 0.029) was observed for both single and twin lambs, which coincides with the region of brown adipose tissue deposition. The lowest absolute thermal variabilities between twin and single lambs in M0 and M60 were recorded in the midloin and integral dorsal area. The parturition type did not influence the latencies to stand up (P = 0.908) and for the first suckling (P = 0.888), and the vitality score (P = 0.353). Thus, single and twin lambs do not differ in neonatal behavior, but they presented specific metabolic strategies to regulate body temperature over time. Midloin and integral dorsal areas are anatomical regions suggested for use in serial thermographic monitoring. Infrared thermography may be an important complementary resource in neonatal care.

1. Introduction

Increasing the economic efficiency of sheep production may be achieved by reducing the mortality rate of lambs from birth to weaning (Hinch and Brien, 2014). It is estimated that worldwide lamb mortality rates can reach 20% of the total number of born lambs (Nowak and Poindron, 2006; Everett-Hincks and Dodds, 2008), mainly in the most prolific breeds (Binns et al., 2002). The high risk of postpartum mortality may be attributed to factors associated with the onset of labor and delivery of conceptus (Vannucchi et al., 2012), functional disorders or infectious diseases (Holmøy et al., 2017), and the inability to adapt to postnatal life (Dwyer, 2008; McKnight et al., 2020). The immediate postnatal period is critical for lamb survival (Horton et al., 2019), as more than half of lamb mortality cases occur in the first days after birth (Dwyer et al., 2016). Another relevant factor is neonatal behavior, especially the attitudes related to udder seeking, which contributes to

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ensuring colostrum and milk intake. These serve as primary exogenous energy sources and contribute to thermoregulation in newborn lambs. Therefore, usually, the starvation/hypothermia/hypoglycemia/exposure complex is a cause of neonatal mortality in sheep (Radostits et al., 2000).

It is possible that these factors are physiologically linked, making newborn lambs more susceptible to hypothermia, particularly from birth to 5 h postpartum, when a significant heat loss may occur (Plush et al., 2016b). Thermoregulatory pitfalls and low vigour lambs are included in a list of factors that account for roughly 50%–75% of all documented perinatal losses in sheep herds (Rook et al., 1990). High perinatal mortality is also associated with flocks that foster more lambs, and failure to provide appropriate nursing (Binns et al., 2002). These data demonstrate that veterinarians and producers need to work together to design technical and economic prevention programs that address these problems (Rook et al., 1990).

Sheep are homeothermic animals and, therefore, the ability to survive in the neonatal period is essentially dependent on the lamb's intrinsic responses to maintain its thermal balance after leaving the intrauterine environment (Plush et al., 2016a) and its relationship with the surrounding thermal environment after delivery (Dwyer and Morgan, 2006). Hence, inability or delay in the regulation of body temperature are factors that increase the risk of newborn lambs (Nóbrega et al., 2005). As multiparous animals, sheep have an incidence of multiple births that can vary according to the dam's breed, age, and nutritional status (Silva et al., 2017). Studies show that Morada Nova hair sheep presenting a body condition score from 2 to 3 (scale of 1–5; Kenyon et al., 2014) have a higher twinning rate than those presenting body condition score <2.0 (Sousa et al., 2019), which can reach up to 43% (Oliveira et al., 2016). However, multiple pregnancies are associated with intrauterine growth restriction, which potentially contributes to the reduction in placental size and low birth weight (Gootwine et al. 2007), as well to the lower lamb development in prenatal (Robinson et al., 1977) and postnatal life (Symonds et al., 2016).

In pregnancies with multiple conceptuses, the lamb's weight at birth decreases as the number of born lambs increases (Gootwine, 2005), reaching values up to 12% lower compared to those born from single births (Dickinson et al., 1962). Birth weight influences the postpartum survival rate of lambs (Dwyer, 2008), particularly in large litters. In general, lighter newborn lambs have a lower fat reserve (brown adipose tissue), are less vigorous, and require more time to stand (Dwyer and Morgan, 2006). Delay in first suckling and depletion of the neonate's brown adipose tissue reserves can lead to hypothermia in the newborn (Singer, 1998).

Thermal maps of sheep's body surface have been efficiently developed by infrared thermography (Pantoja et al., 2017; Seixas et al., 2017a; Barros de Freitas et al., 2018). This technique has been considered a safe and non-invasive method to monitor sensitive heat loss to the environment in wool sheep (Labeur et al., 2017; Menant et al., 2020) and under conditions of artificially induced cold stress (McCoard et al., 2014). However, no study in the scientific literature relates thermoregulatory responses associated with phenotypic characteristics in newborn lambs of tropicalized hair sheep breeds. Hair breed animals have short and silky hair coats and differ from exotic wool breeds, whose specimens present wool covering the neck and body, and no wool only on the head and lower parts of the limbs (Maia et al., 2011). Studies on the thermoregulatory ability of hair sheep breeds in natural environmental conditions are also important for the selection of genotypes and animals potentially more resilient and productive when raised in a tropical environment (Titto et al., 2016; Pantoja et al., 2017). This information may be essential to broaden the understanding of the thermal biology of hair sheep in an extremely sensitive phase of life and, consequently, provide information for the development of management strategies that help to reduce neonatal lamb mortality.

Therefore, the objective of the study was to evaluate the differences of hair lambs, from single or twin births, regarding the latency periods for standing up and for performing the first suckling, vitality, glycemic, cortisol, and triiodothyronine concentrations, as well as the phenotypic characteristics related to the maintenance of homeothermy in the immediate postpartum period.

2. Material and methods

2.1. Bioethics

The work was carried out in accordance with the Brazilian Guide for the Care and Use of Animals in Studies and Scientific Research (Brasil-Conselho Nacional de Controle de Experimentação Animal, 2016). Sanitary protocols for the species were regularly adopted, according to Costa (2002). The experimental procedures were previously approved by the Ethics Committee on the Use of Animals of Embrapa Southeast Livestock (Protocol 09/2017) and the results were reported according to ARRIVE Guidelines (Animal Research: Reporting of *In Vivo* Experiments) (Kilkenny et al., 2010).

2.2. Location, experimental period, and climate

The experiment was carried out in Embrapa Southeast Livestock, São Carlos, Brazil (21°58′30″S, 47°50′58″W, at 911 m alt), from March to April 2018, in autumn. The climatic subtype of the region is classified as Cwa, tropical altitude, according to Köppen's classification, characterized by dry winters and hot, rainy summers. The average annual air temperature is 21.3 °C, with an average maximum temperature of 27.8 °C, and an average minimum of 16.7 °C. The annual average relative humidity is 73.2% (Embrapa-Empresa Brasileira de Pesquisa Agropecuária/Embrapa Pecuária Sudeste, 2018). During the study, the pasture (outdoor) and barn (described in Section 2.4) microclimates were permanently monitored in order to compare indoor and outdoor environments. During the entire experimental period, the mean value recorded for the Temperature Humidity Index was 72.4, an indication of a thermal environment with normal conditions or absence of heat stress for the newborn animals.

2.3. Sheep reproductive protocol

For the production of lambs, twenty adult ewes (*Ovis aries*) of the Morada Nova breed (3.8 ± 1.0 years old and 39.8 ± 4.2 kg) with a body condition score of 2.8 ± 0.3 (scale of 1–5, according to Kenyon et al., 2014) were used. The ewes had their cycles synchronized and, when estrus was detected, they were mated through assisted natural mating (5–8 ewes/ram). Two matings were performed at 12-h intervals, with males of the same breed, of libido and seminal quality previously assessed in a breeding soundness evaluation. Pregnancy diagnosis was performed 30 days after breeding, by transrectal ultrasonography in B-mode (DP-3300 Vet, Mindray Bio-Medical Electronics Ltd, China) with a 5.0–7.5 MHz linear transducer. Sixteen ewes were diagnosed as pregnant and had their pregnancies monitored.

2.4. Management in the gestational phase and birth monitoring

The ewes were kept in a single batch, on a 0.8 ha pasture of Aruana grass (*Panicum maximum* cv. Aruana) during the day (6:00 a.m. to 6:00 p.m.), in a rotational grazing system. Daily, the ewes received a concentrated compound of corn, soybean meal, and vegetable oil. Average daily consumption of dry matter was 1.20 kg/day, being 0.77 kg via pasture (23.9% dry matter [DM]; 11.0% Crude protein [CP]; 2.2% Ethereal extract [EE]; 72.8% neutral detergent fiber [NDF]; 42, 1% acid detergent fiber [ADF]; 1.83 Mcal of metabolizable energy [ME]/kg DM) and 0.43 kg via concentrate (89.2% DM; 11% CP; 4.4% EE; 15.11% NDF; 3.9% ADF; 2.61 Mcal of ME). The diet was formulated following the nutritional requirements indicated by the NRC (2007) for pregnant ewes with a live weight of 50 kg, with one fetus during the last third of gestation. On the day of lambing, the ewes weighed 45.7 ± 5.6 kg and had a body condition score of 3.5 ± 0.3.

At the end of the day, ewes were taken to a covered maintenance semi-opened barn adjacent to the pasture, where they stayed at night (6:00 p.m. to 6:00 am). This system of semi-intensive farming with part-time outdoor (day) and part-time indoor (night) is traditionally adopted by the sheep owners in the region and takes place during the whole year in spite of weather conditions. The barn was a ground-level shed, 30 m long, 8 m wide, 4.5 m high on the central ridge, and 3.5 m high on the lowest point, surrounded by 1.20 m high wire mesh walls, covered with fiber cement tiles. The concrete floor was partially covered with soft straw bedding. The animals had *ad libitum* access to water, both in the barn and pasture.

From day 140 of gestation on, the ewes were continuously observed until parturition occurred. The method of uninterrupted visual monitoring was adopted, carried out by four trained observers. Once the prodromal stage of parturition was detected, when the ewes have a lack of appetite, seek isolation from the group, and show anxiety and agitation (Lickliter, 1985), the dams started to be observed more closely. Once the liquefaction of the cervical mucus plug was verified, the 60-min interval was adopted as the period for the normal progression of parturition. Otherwise, veterinary medical assistance would be called for obstetric assistance, which was not necessary for any of the experimental animals. Ewes lambed 22 newborns, either single (n = 10) or twins (n = 12). The outdoor birth rate was 17/22 (7 single and 10 twins) and it was 5/22 (3 single and 2 twins) outdoor.

2.5. Evaluation of neonatal behavior, vitality, and birth weight

None of the ewes abandoned their lambs and all of them exhibited maternal behavior and stimulated their offspring properly. After birth, the latency periods for standing up and for performing the first successful suckling (Dwyer and Morgan, 2006) were recorded with a digital stopwatch. Neonatal behavioral progress was assessed as described by Dwyer et al. (2005) (details in Supplementary Material, Tab. Sup 1). The standing behavior was characterized when the lamb stood on all four legs for more than 5 s. Successful first suckling was determined as the moment when the lamb was able to approach the udder, grab the teat with its mouth, and suckle continuously. Concomitantly, the animal should present appropriate movements of the mouth and head and wag its tail, demonstrating this behavior for at least 5 consecutive seconds. All lambs successfully performed the first suction, with effective teat suction occurring on average within 36.9 min after lambing.

The vitality of each lamb was visually evaluated and a score (1–5 points) was assigned based on the lambs' attitudes, as described by Holst (1987) and Hergenhan et al. (2014), and considering the birth as the timeframe for the vitality evaluation, as proposed by VannucchiRodrigues et al., 2012 (Table 1). Immediately after vitality evaluation, the lambs were led to the shed together with the ewes. The lambs were conducted/handled with care so that the operator did not touch the lamb, avoiding the iatrogenic occurrence of conductive thermal changes in the body surface. The lambs were kept with their mothers in an individual pen (1.5 m^2/animal) for 24 h, for weighing, thermographic scanning, and evaluation of physiological variables of interest. Lambs were weighed using a portable digital scale (10 g–50 kg).

Table 1
Values and definitions for attributing the postpartum lambs' vitality score.

Score	Description
1	Does not stand for at least 40 min; little or no teat-seeking drive; does not appear alert or active.
2	Attempts to stand after 30 min; low teat-seeking drive and tendency to follow ewe; shows some alertness but not very active; does not appear coordinated in attempts.
3	Shakes head within 30 s; attempts to stand within 15 min; seeking teat within 10 min of standing; follows ewe but distracted by other moving objects; generally alert and active; coordination may be lacking.
4	Attempts to stand within 10 min of birth; seeking teat within 5 min of standing; strong tendency to follow ewe; alert and active and movements well coordinated.
5	Attempts to stand within 5 min of birth; follows ewe closely; very alert and active.

*Scale defined by Holst (1987) and adapted by Hergenhan et al. (2014).

2.6. Thermographic evaluation of body surface temperatures

In the shed, the body surface temperature of lambs was evaluated by infrared thermography. The first recording of thermograms occurred immediately after confirmation of successful suckling (Timepoint Zero, M0), which occurred in 38.7 min for single lambs and 39.8 min for twin lambs, after lambing. Subsequently, three more consecutive evaluations were performed, at regular 20-min intervals (M20, M40, and M60). For the generation of thermograms, a portable thermographic camera (Testo 890–2, Testo AG, Lenzkirch, Germany) was used, equipped with a 640 × 480-pixel detector, 42° × 32° (15 mm) lens, thermal sensitivity <40 mK (<0.04 °C at an ambient temperature of 30 °C), and a temperature range from −20 to 350 °C, in the manual focus option (Pantoja et al., 2017).

The thermographic images were obtained from the anatomical areas of the right ocular globe and dorsal region (Fig. 1), with the thermographic camera positioned perpendicularly to the anatomical region of interest and at a prefixed distance of 0.5 m from the animal's eyeball and 1.0 m from the dorsal region. The adopted emissivity was 0.98 (Hoffmann et al., 2013). The generated thermograms were later analyzed with IRSoft Version 4.5 software (Testo AG, Lenzkirch, Germany). Ocular temperature (°C) was analyzed through circular tracing over the orbital region, comprising the eyeball and approximately 1 cm of the eye cavity, in order to include the lacrimal gland (Pantoja et al., 2017). The maximum values of ocular temperature (hot spots) were used in the statistical analysis, as proposed by Schaefer et al. (2007) and Hoffmann et al. (2013), since the sensitivity and specificity of infrared thermography for monitoring internal body temperature increase when maximum eye temperature is used (Johnson et al., 2011).

To analyze the dorsal temperatures, this region was divided into four regions of interest: shoulder, midloin, hips, and rump, as described by Labeur et al. (2017). The temperatures of each region were analyzed through a rectangular tracing, with the maximum, minimum, and mean temperatures (°C) being recorded. Additionally, the mean temperature of the dorsal region was analyzed according to a polygonal tracing, which integrated the total area of the dorsal surface.

2.7. Internal temperature, hormonal dosages, and blood glucose

Immediately after recording the thermograms, the internal body temperature was assessed via rectal thermometry (Labeur et al., 2017), using a digital clinical thermometer (Digital TH186, Onbo Electronic, Shenzhen, China; measuring range 32.0–43.9 °C, max error ± 0,2 °C, self-checking system), in order to coincide with the same times adopted for the acquisition of thermograms (M0, M20, M40, and M60). After the generation of the first thermogram (M0), blood samples from lambs were collected by venipuncture, in vacuum tubes, without anticoagulant (Vacutainer, New Jersey, USA). The samples were centrifuged at 1350×*g* for 15 min for complete separation of serum, which was fractionated in polypropylene microtubes and stored at −20 °C until analysis.

In the laboratory, serum cortisol and triiodothyronine (T3) concentrations were evaluated by radioimmunoassay. Cortisol measurements were carried out with the Cortisol Immuchem Coated Tube kit (MP Biomedicals, LCC Diagnostics Division, Santa Ana, USA). T3 concentrations were measured using the T3 Antibody-Coated Tubes kit, T3 Tracer [125I], and T3 Standards Set (MP Biomedicals, Inc., Diagnostics Division, Santa Ana, USA). Sensitivity and intra-assay coefficient of variation (CV) were 0.17 μg/dL and 12% for cortisol and 6.7 ng/dL and 11% for T3, respectively. Glucose concentrations were determined in

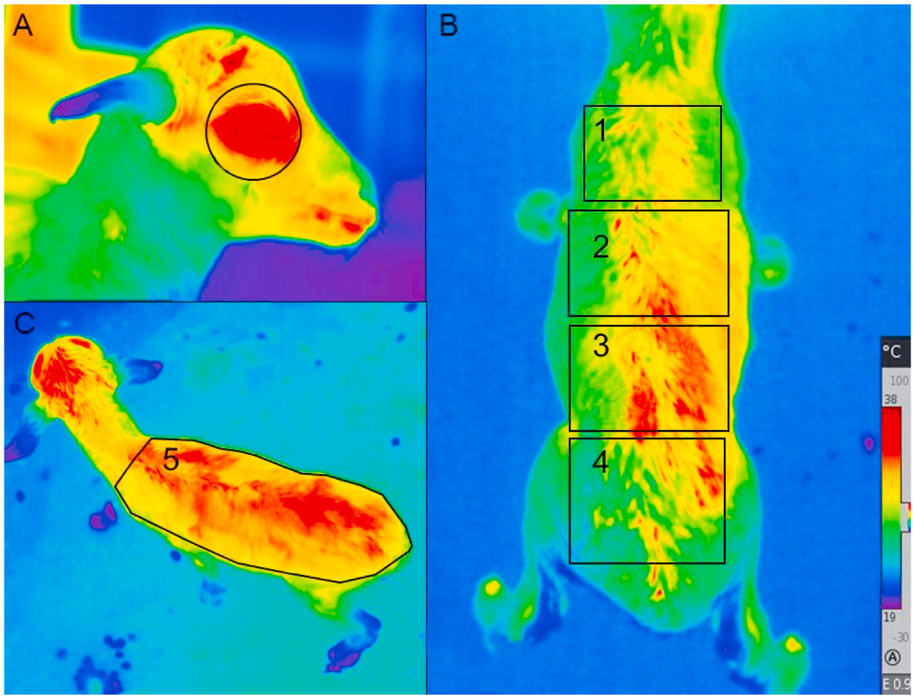

Fig. 1. Illustrative thermographic images of the evaluation of the ocular surface temperature analyzed through circular tracing over the orbital region (A), shoulder (B1), midloin (B2), hips (B3), rump (B4), and integral dorsal region (C5), in accordance with Pantoja et al. (2017), and Labeur et al. (2017).

whole blood (Katsoulos et al., 2011), using a digital device equipped with a biosensor and automatic calibration (On Call® EZ, Acon Biotech, Hangzhou, China).

2.8. Biometeorological variables and temperature Humidity Index (THI)

During the experiment, the air temperature (AT, °C) and the relative humidity (RH, %) were permanently monitored through an automatic meteorological station installed in the barn (indoor). The station was equipped with psychrometric sets protected in a micrometeorological shelter. The sensors were coupled to an automatic data acquisition system (CR1000 Datalogger®, Campbell Scientific, Logan, USA), programmed to take readings every 5 s and calculate means every 15 min. Also, the same variables were monitored in the pasture area (outdoor) using an automatic weather station (Fig. 2). In order to calculate the Temperature Humidity Index (THI), the microclimatic data corresponding to each timepoint of evaluation of the lamb's body surface were considered. The THI calculation adopted the following equation: $THI = [(0.8AT) + RH (AT - 14.3) + 46.3]$, in which: AT is the air temperature measured in a dry bulb thermometer (°C) and RH is the relative humidity (%), as proposed by Thom (1959). Its interpretation was based on the livestock weather safety index (LCI, 1970) which considers $\leq$74 as normal, 75–78 as alert, 79–83 as danger, and $\geq$84 as emergency (Seixas et al., 2017b). Indoor and outdoor AT, RH, and THI were tested and no significant contrasts (paired *t*-Test; $P > 0.05$) were observed during the experiment. The THI values used to correlate with all other variables refer to the time closest to each lambing, and to the subsequent timepoints in which thermograms were acquired.

2.9. Statistical analysis

Data analysis was carried out using the libraries from the nlme and stats packages from the R software, version 3.5.1 (R Core Team, 2019). The normality of variables and residuals in the different tested models was verified by the Shapiro-Wilk test. Variables were also tested for homoscedasticity. ANOVA was used to test the effect of parturition type on the concentrations of cortisol, triiodothyronine, blood glucose, latency for successful suckling, and birth weight. The animal effect (lamb) was included as a random factor, gender as a secondary effect, and birth weight as a covariate (except when the independent variable was birth

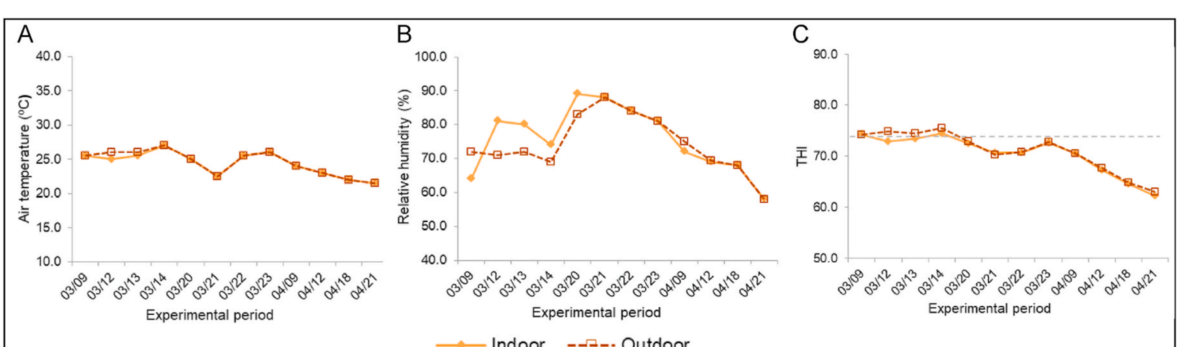

Fig. 2. Profiles of air temperature (A), relative humidity (B), and Temperature and Humidity Index-THI (C) recorded throughout the experimental period (March to April 2018). The dashed line indicates the THI reference value below which animals are in thermal comfort (LCI, 1970).

weight). In order to test the effect of parturition type on the vitality score, non-parametric ANOVA was used through the Kruskal-Wallis test.

A mixed linear model with ANOVA was used in repeated measures over time. Model parameters were estimated by restricted maximum likelihood, with the animal effect (lamb) included as a random factor. Internal, ocular surface, and body surface temperatures were tested for the effects of parturition type, timepoints (M0, M20, M40, M60), and their interactions. All models were adjusted with birth weight as a covariate in order to account for variations in the dimension of the dorsal region. Different covariance structures were tested until the best fit was found for each variable according to the criteria of the lowest AIC values. Unstructured covariance and compound symmetry structure were the best to fit the models. The comparison between means of the tested effects was performed using the Tukey test. The significance level previously adopted for all analyses was less than or equal to 5%. A linear regression analysis was carried out, considering internal, ocular surface, and body surface temperatures as dependent variables and as independent variables at different times (M0, M20, M40, M60).

The relationships between body surface temperatures, behavior, vitality score, blood glucose, and serum cortisol and triiodothyronine concentrations, birth weight, and THI at timepoints M0 and M60 were analyzed using Pearson correlations. Correlation coefficients (r) were considered significant when $P \leq 0.05$ and classified as being high ($r > 0.68$), moderate ($0.36 < r < 0.67$), or low ($r < 0.35$), according to the estimated values (Taylor, 1990). Principal Component Analysis was applied to identify the components that explain the observed variation. Variables were standardized in order to remove the effect of unit differences. To select the number of principal components, the screen plot visual evaluation and the Kaiser criterion (Kaiser, 1958), with eigenvalues≥1, were chosen, explaining at least 70% of the total variance of the original data.

3. Results

The results for the characteristics of behavior, vitality, birth weight, body temperatures, and THI recorded at different evaluation times are shown in Table 2. At M0, there was no difference in the means of internal body temperature and ocular temperature between lambs born from single or twin births ($P > 0.05$). The parturition type did not influence the neonatal behavior regarding latencies to stand up ($P = 0.908$) and for the first suckling ($P = 0.888$), vitality score ($P = 0.353$), nor serum glucose ($P = 0.356$), and cortisol concentrations ($P = 0.931$) (Table 3). However, the parturition type determined differences in birth weight, in which lambs born from single births were, on average, 0.51 kg heavier than their twin counterparts. Gender also had a significant effect on birth weight, as males were heavier than females. Animals born from

Table 3
Means and standard error for the effect of parturition type on behavior, vitality, weight, blood glucose, and serum hormonal concentrations of newborn hair lambs.

Variable	Parturition Type		Main Effects (P-value)		
	Single	Twin	PT	Cov	Gender
Latency – standing up (min)	11.2 ± 1.75^{a}	11.5 ± 1.40^{a}	0.908	0.033	0.484
Latency – first suckling (min)	38.7 ± 6.15^{a}	39.8 ± 4.48^{a}	0.888	0.24	0.071
Vitality (1–5)*	3.20 ± 0.63^{a}	3.38 ± 0.74^{a}	0.353		
Birth weight (kg)	3.09 ± 0.187^{a}	2.58 ± 0.146^{b}	0.048		0.038
Blood glucose (mg/dL)	50.1 ± 5.49^{a}	38.8 ± 5.40^{a}	0.356	0.055	0.847
Cortisol (ng/mL)	44.3 ± 13.6^{a}	45.8 ± 10.2^{a}	0.931	0.612	0.646
Triiodothyronine (ng/dL)	267 ± 19.5^{a}	209 ± 14.7^{b}	0.029	0.931	0.356

PT = parturition type; Cov = covariate (birth weight); Gender = secondary effect; F = F-test F; P = P-value. * Kruskal-Wallis test.
a, b different lowercase letters indicate significant difference in columns ($P \leq$ 0,05).

Table 2
Descriptive statistics for variables of behavior, vitality, weight, blood glucose, serum hormonal concentrations, and body temperatures of newborn hair lambs.

Variable	Unit	n	M0			M20			M40			M60		
			Mean	SD	Amplitude	Mean	SD	Amplitude	Mean	SD	Amplitude	Mean	SD	Amplitude
Latency – standing up	min	18	11.03	4.82	18.8									
Latency – first suckling	min	20	36.91	15.93	48.2									
Vitality score	*	18	3.28	0.67	3									
Birth weight	kg	22	2.68	0.55	1.8									
Blood glucose	mg/dL	17	43.29	15.38	51.0									
Cortisol	ng/mL	15	47.38	25.49	72.6									
Triiodothyronine - T3	ng/dL	15	229.2	46.29	172.0									
Internal temperature	°C	22	39.10	0.61	1.9	39.07	0.55	2.1	38.85	0.66	3.0	39.00	0.50	1.8
Ocular IRT	°C	22	38.21	0.62	2.7	38.54	0.79	2.7	38.63	0.79	2.7	38.45	0.90	3.1
Maximum shoulder IRT	°C	22	32.72	2.17	7.4	33.11	2.20	7.9	33.18	2.66	10.5	33.32	2.26	7.3
Minimum shoulder IRT	°C	22	26.64	3.05	11.3	27.12	3.37	12.3	27.15	3.45	12.5	27.61	3.38	13.1
Mean shoulder IRT	°C	22	29.21	2.84	9.8	29.62	2.91	10.2	29.85	2.97	10.8	30.05	3.07	10.8
Maximum midloin IRT	°C	22	32.26	2.44	9.9	32.14	2.05	6.9	32.17	2.59	9.2	32.22	2.04	6.5
Minimum midloin IRT	°C	22	25.58	4.75	22.6	26.67	3.08	10.3	26.82	2.94	11.7	27.20	3.29	13.4
Mean midloin IRT	°C	22	28.62	2.97	9.5	28.74	2.99	10.1	29.10	2.96	11.1	29.40	2.94	11.7
Maximum hips IRT	°C	22	31.73	2.09	7.5	31.49	2.27	7.8	32.29	2.25	8.5	32.58	2.24	8.0
Minimum hips IRT	°C	22	26.05	2.96	10.2	26.39	2.92	10.5	26.87	3.23	11.7	27.19	3.37	13.3
Mean hips IRT	°C	22	28.33	2.90	10.2	28.49	2.88	9.7	29.06	3.02	11.2	29.51	2.98	11.4
Maximum rump IRT	°C	22	31.49	2.20	8.4	31.50	1.87	8.3	32.01	2.36	8.4	32.53	2.00	6.2
Minimum rump IRT	°C	22	25.71	2.79	10.4	26.36	3.16	10.2	26.52	3.15	11.8	26.76	3.36	12.7
Mean rump IRT	°C	22	28.12	2.88	9.5	28.54	2.93	10.3	29.04	3.07	11.6	29.39	2.92	11.1
Maximum dorsal area IRT	°C	22	33.45	1.86	7.1	33.36	1.87	7.2	33.50	2.62	9.8	33.50	2.20	7.6
Minimum dorsal area IRT	°C	22	25.05	2.90	10.1	25.58	3.15	10.5	25.82	3.29	12.7	26.33	3.54	13.8
Mean dorsal area IRT	°C	22	28.39	2.85	10.3	28.70	2.91	9.6	28.98	3.10	11.2	29.45	2.94	11.4
THI	*	22	73.0	5.2	16.6	72.3	5.4	18.3	72.16	5.4	19.1	72.3	5.3	19.6

M0 (timepoint zero) = immediate moment to first successful suckling; M20 = 20 min after M0; M40 = 40 min after M0; M60 = 60 min after M0.
IRT = Surface temperatures measured by infrared thermography. SD = Standard deviation. * dimensionless variable.

single births also had higher serum concentrations of triiodothyronine than twins after birth.

When all timepoints were considered together, animals born from single and twin births showed significant differences in internal body temperature ($F_{1;19} = 20.00$; $P = 0.0003$) and ocular temperature ($F_{1;19} = 6.17$; $P = 0.0225$) (Fig. 3). Lambs born from twin births had an internal temperature 0.62 °C higher, as well as an ocular temperature 0.71 °C higher than lambs from single gestations.

Regression analysis showed that there was a subtle decrease in the internal temperature of animals over time ($P \leq 0.05$), with a more significant reduction (0.4 °C) for twin animals (Fig. 4A). There was no significant effect of time on ocular surface temperature (Fig. 4B). In general, the mean temperatures recorded on dorsal region thermograms increased over time, regardless of parturition type (Fig. 4G).

From M0 to M60, single birth animals exhibited increases in temperatures ($P \leq 0.05$) for the shoulder, hips, and rump regions (Fig. 4C, E, Fig. 4F), while twin birth animals showed a positive linear effect for the hips and dorsal region (Fig. 4E and G). The regression equations show that the increase recorded in the animals' dorsal region was approximately 0.02 °C per minute from M0 to M60, regardless of parturition type. At M60, the numerical differences in the temperatures of lambs born from twin or single births, in decreasing order, were recorded in hips and ocular globe (0.7 °C), rump (0.6 °C), internal temperature and integral dorsal area (0.5 °C), midloin (0.3 °C), and shoulder (−0.1 °C).

At M0, the times required to stand up and perform the first suckling were negatively correlated with birth weight, vitality, ocular temperature, and surface temperature in different dorsal regions (Fig. 5).

Body weight was positively correlated with blood glucose ($r = 0.57$) for both single and twin lambs. Cortisol was negatively associated with vitality ($r = -0.55$). At M60, the times required to stand up and perform the first suckling were negatively correlated with the mean surface temperatures of the different dorsal segments and integral dorsal area. Cortisol was positively associated with mean midloin surface temperature ($r = 0.58$). There were high and positive correlations between the surface temperatures of the different anatomical regions and of these with THI, both at M0 and M60.

Principal component (PC) analysis was applied to allow visualization of the relationships between the variables in each of the principal components, at M0 and M60 (Table 4). Twenty-two PCs explained 100% of the total variance for M0, while 15 PCs explained this variance for M60. The three main components (PC1, PC2, and PC3) selected at each timepoint explained 77.20% of the total variance observed at M0 and 78.38% of the variance observed at M60.

At M0, PC1 showed similar coefficients (eigenvectors) for the maximum (midloin and hips), minimum (shoulder, hips, rump, and dorsal area), and mean (midloin, hips, rump, and dorsal area) surface temperatures and THI. PC2 was composed of weight, blood glucose, and vitality, with positive coefficients, as well as of the ocular surface, maximum (midloin, rump, and dorsal area), and minimum (midloin) temperatures, while the latency to stand up had a negative coefficient. PC3 was composed of the variables blood glucose, cortisol and triiodothyronine, ocular surface temperatures, and latency to stand up, which presented a negative coefficient.

At M60, PC1 was composed of surface temperatures and THI, with similar coefficients, while latency for the first suckling had a negative coefficient. PC2 was composed of weight, vitality, latency to stand and first suckling, cortisol, and internal and ocular surface temperatures. PC3 presented high positive values for weight, vitality, blood glucose, and triiodothyronine and a negative coefficient for latency to stand up. The contribution of the variables to the formation of the main components at the moments of the first successful suckling and 60 min later is shown in Fig. 6.

The variables with the greatest contribution were projected in a two-dimensional space to highlight the relationships with the different PCs. At both M0 and M60, PC1 was mainly represented by the surface temperature and THI parameters, which are biophysical elements related to thermoregulation. PC2 showed higher vectors for cortisol, blood glucose, and standing up at different timepoints, that is, the metabolic variables made the greatest contributions to PC2. On the other hand, PC3 had higher vectors for cortisol and blood glucose at M0, while at M60 the most relevant vectors were blood glucose, body weight, and latency to stand up, with the latter variable in the opposite direction to weight.

4. Discussion

The present study demonstrated differences in the thermal biology of lambs born from single or twin births. Using behavioral evaluations and serial infrared thermography, it was possible to monitor the thermodynamics of different body segments in the first hours of life, associating these characteristics with birth weight and endocrine and vitality conditions. This knowledge allows for a greater understanding of the physiology of newborn hair lambs and may allow the monitoring of thermal balance to be carried out using infrared thermography technology as a complementary resource, aiming at improving the health condition of newborn lambs.

Our results indicate that parturition type did not affect the time required for the lambs to stand up and initiate the act of suckling. This likely occurred because the animals did not differ in terms of vitality, a characteristic that indicates the level of awareness and motor activity of the newborn in the first moments of life (Vanden Hole et al., 2018). However, regardless of parturition type, vitality was negatively associated with cortisol concentration, indicating higher secretion of this hormone in less active animals and with some degree of stress. Data from individual registers indicated that 87% of lambs born from single births and 50% of those born from twin births performed the first suckling attempt within less than 40 min after birth. The similarity in the latency to perform the first suckling between twin or single birth animals, which varied by a maximum of 1 min, was also a direct consequence of the equality in the glycemic levels of these animals.

Around parturition time, metabolic changes are stimulated by cortisol, which promotes fetal maturation and results in increased

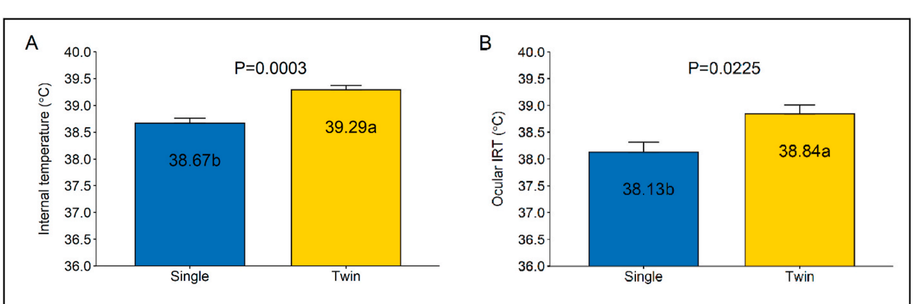

Fig. 3. Mean values (± standard error of the mean) of internal temperature (A) and ocular surface temperature (B) of hair lambs born from single or twin births. Lowercase letters (a, b) indicate a significant difference ($P \leq 0,05$).

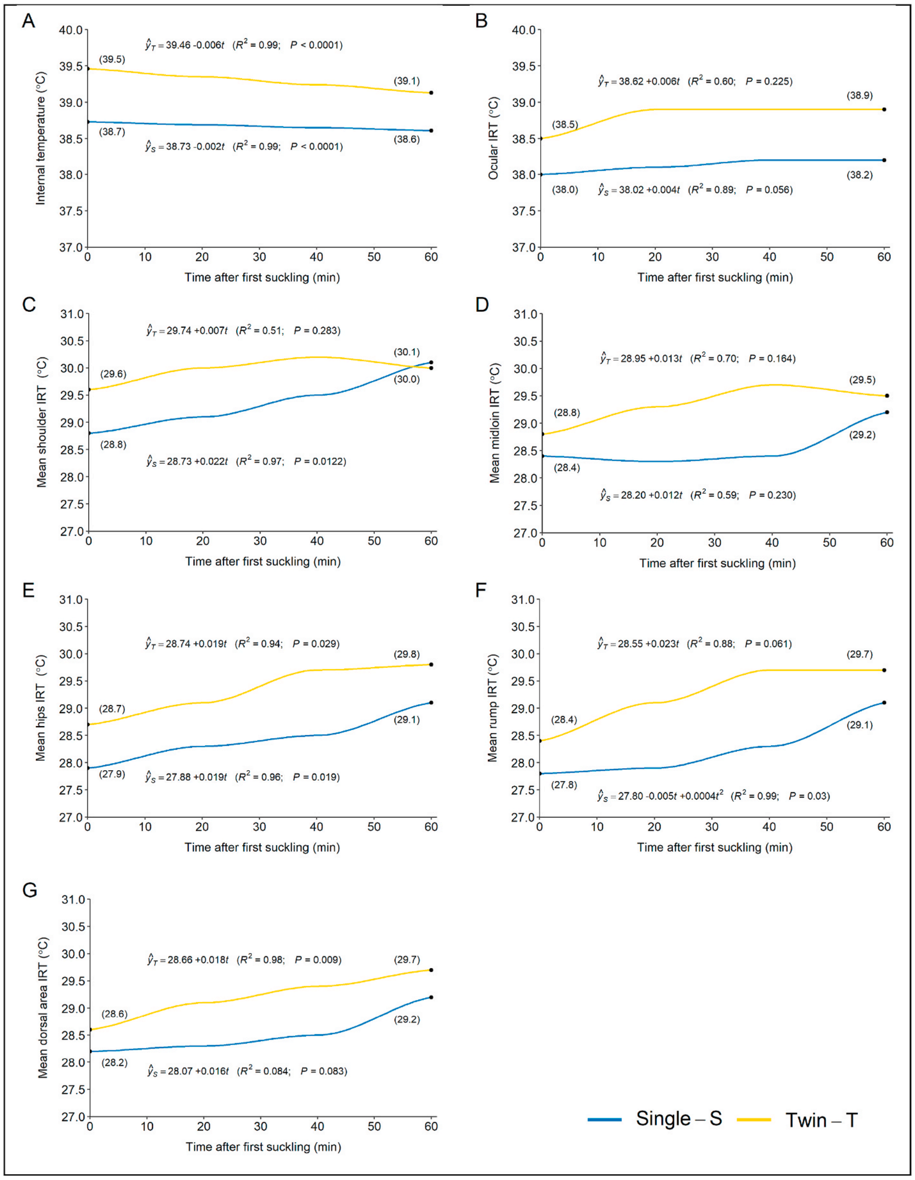

Fig. 4. Internal temperature (A), ocular temperature (hotspot) (B), and mean surface temperatures profiles of shoulder (C), midloin (D), hips (E), rump (F), and dorsal area (G) surfaces, observed in newborn hair lambs, born from single or twin births, at different times after first suckling.

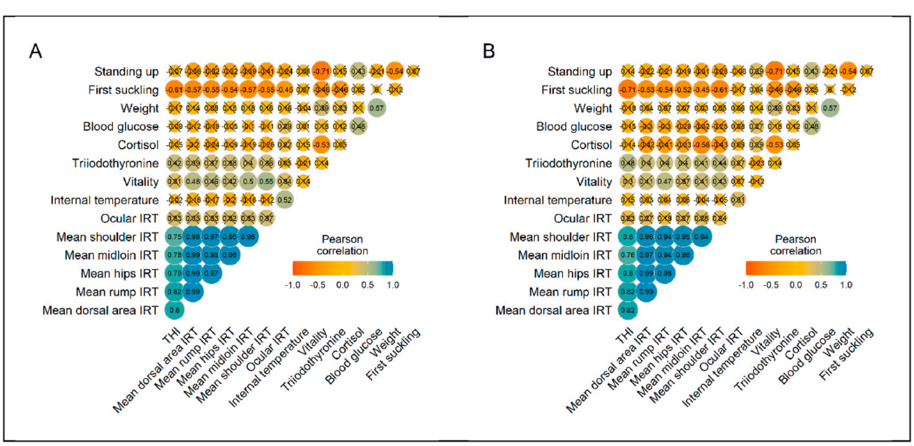

Fig. 5. Linear correlation matrices between behavior, vitality, and temperature variables, evaluated at the timepoints of the first successful suction-M0 (A) and 60 min later-M60 (B) in newborn hair lambs. IRT = surface temperatures evaluated by infrared thermography. Significant correlation values are presented in the non-marked circles (P ≤ 0.05).

Table 4
Principal component analysis showing eigenvalues, percentage of variance, and the share of total variance along with weighting coefficients for behavior, vitality, and temperature variables of different body regions of newborn hair lambs after successful suckling.

Variable	Timepoint Zero			Timepoint 60		
	PC1	PC2	PC3	PC1	PC2	PC3
Standing up	-0.12	**-0.34**	**0.35**	-0.12	**0.52**	**-0.23**
First suckling	-0.18	-0.03	0.01	**-0.26**	**-0.24**	-0.21
Weight	0.03	**0.37**	-0.05	0.03	**-0.26**	**0.56**
Blood glucose	-0.06	**0.44**	**0.24**	-0.14	0.09	**0.59**
Cortisol	-0.01	0.12	**0.55**	-0.22	**0.41**	0.21
Triiodothyronine	0.18	0.06	**0.29**	0.18	0.22	**0.23**
Vitality	0.16	**0.26**	**-0.36**	**0.23**	**-0.36**	**0.23**
Internal temperature	-0.04	0.08	-0.15	-0.01	**0.25**	0.17
Ocular IRT	0.10	**0.23**	0.01	0.09	**0.34**	**0.24**
Maximum shoulder IRT	0.21	-0.18	-0.19			
Minimum shoulder IRT	**0.25**	0.10	0.09			
Mean shoulder IRT	0.26	0.04	-0.02	**0.36**	0.02	-0.02
Maximum midloin IRT	**0.19**	**-0.30**	-0.10			
Minimum midloin IRT	0.12	**0.31**	**-0.32**			
Mean midloin IRT	**0.27**	0.02	0.00	**0.36**	0.00	-0.07
Maximum hips IRT	**0.23**	-0.13	0.04			
Minimum hips IRT	**0.25**	0.07	0.15			
Mean hips IRT	**0.26**	0.01	0.07	**0.36**	0.05	-0.03
Maximum rump IRT	0.21	**-0.26**	-0.03			
Minimal rump IRT	**0.25**	0.10	0.14			
Mean rump IRT	**0.27**	-0.02	0.03	**0.36**	0.03	-0.05
Maximum dorsal area IRT	0.20	**-0.28**	-0.16			
Minimum dorsal area IRT	**0.25**	0.10	0.14			
Mean dorsal area IRT	**0.26**	0.01	0.03	**0.37**	0.04	-0.05
THI	**0.22**	-0.05	0.16	**0.31**	**0.26**	-0.04
Eigenvalues	**13.88**	**3.25**	**2.17**	**7.14**	**2.62**	**1.99**
Accumulated proportion	**55.51**	**68.51**	**77.20**	**47.62**	**65.09**	**78.38**

PC1 = first principal component; PC2 = second principal component; PC3 = third principal component. IRT = surface temperatures evaluated by infrared thermography; THI = Temperature Humidity Index. The highlighted values indicate the variables with the highest contribution to each principal component, at different moments of the first successful suckling (Timepoint Zero) and 60 min later (Timepoint 60).

gluconeogenic activity and endogenous glucose production by the lamb (Hammon et al., 2012). Indeed, the blood glucose recorded even before the first suckling was higher (50.1 ± 5.49 and 38.8 ± 5.40 for single and twin lambs, respectively) than that reported in the literature for hair lambs, at 30 mg/dL (VannucchiRodrigues et al., 2012). This condition may be favorable to the lambs, as the availability of glucose increases the metabolic rate, through the activation of the glycolytic pathway and the production of pyruvate, with a positive balance in the production of energy in the form of ATP and heat generation (Randall et al., 2000). Considering colostrum as the first source of carbohydrates for the lamb, the quick manifestation of behavior that culminated in the first suckling was also essential for thermoregulation, as a possible delay in standing up may lead to the depletion of energy reserves before the lamb reaches the teat and effectively carry out the first feed (Nowak and Poindron, 2006). Thus, a slow behavioral progression can have negative consequences for the newborn, such as decreased survival due to late neonatal adaptation (Dwyer and Morgan, 2006; Macias-Cruz et al., 2018), especially if the lamb presents progressive hypothermia and needs to trigger active thermogenesis mechanisms, which demand energy expenditure. This argument is corroborated by the negative correlations recorded at M0 between the latency for the first suckling and the surface temperatures of most regions evaluated, which means that the higher the surface temperatures of the lamb are, the shorter the time to carry out the suckling will be. A similar effect, with regard to an inverse relationship between body surface temperature and behavior, was observed in hair lambs born from single births by Menent et al. (2020).

The observed birth weight was higher than values for Morada Nova lambs born from single (2.4 kg) and twin (1.9–2.1 kg) births previously reported in the literature (Fernandes et al., 2001; Quesada et al., 2002). Weight was positively correlated with blood glucose concentration. This indicates that heavier lambs at birth are less dependent on having to perform the first suckling immediately, as they have ensured, at least in the immediate postpartum period, sufficient substrate for their energy metabolism and maintenance of the first vital activities. Also, lighter lambs are usually less vigorous, take longer to stand up (Dwyer et al., 2003; Dwyer and Morgan, 2006; Pedernera et al., 2018), and have lower brown adipose tissue reserves (Symonds and Clarke, 1998). However, even having been born lighter than their counterparts, twin lambs presented a prenatal body development above the breed average (1.9–2.1 kg) (Fernandes et al., 2001; Quesada et al., 2002), possibly due to the proper nutritional management of the ewes.

Greater nutritional input to ewes during the gestational period favors the production and accumulation of fetal brown adipose tissue (Budge et al., 2000). In this tissue, electron transfer is uncoupled from ATP synthesis and the fatty acid oxidation energy is dissipated in the form of heat. The presence of uncoupling protein UCP1 (Unique Uncoupling Protein-UCP1), exclusive to brown adipose tissue, allows rapid heat generation at a maximum rate of 300 W/kg, much higher than the 1 W/kg production recorded in other tissues non-specialized in the production of metabolic heat (Symonds et al., 2012). In lambs, UCP1 expression is maximum on the first postnatal day, with brown adipose tissue being concentrated in the perirenal region (Symonds, 2013; Fuller-Jackson and Henry, 2018). This coincides with the thermographic region of hips adopted in the present study, with the thermogenic effect of brown adipose tissue being evidenced by infrared thermography technology, due to the progressive increase in the local surface temperature. This phenomenon has been described in rats (Al-Noori et al., 2016), mice (Redaelli et al., 2019), and humans (Law et al., 2018) under controlled ambient temperature conditions, and suggested in sheep (Hergenhan, 2012). However, as far as we know, this is the first time that a change in the baseline temperature has been described at the sites of brown adipose tissue deposits in the hair sheep breed, under natural conditions in a raising environment. Similar findings were previously described by McCoard et al. (2014) in wool sheep.

The absence of a significant difference in neonatal cortisol concentrations may be explained by the fact that all parturitions were eutocic and had a normal duration, which was 22.1 min (data not shown). This may have favored thermoregulation in twin lambs, given that there is evidence that cortisol is a positive regulatory factor for the UCP1 protein (Mostyn et al., 2003) and is known to increase the production of triiodothyronine in newborn lambs (Dwyer and Morgan, 2006), which stimulates metabolism and induces endogenous heat production. As the highest triiodothyronine was found in lambs from single birth, these animals were expected to produce more heat and be energetically favored. However, higher values of internal and body surface temperature were observed for animals from twin births. Thus, there is evidence that twin lambs are more sensitive to activating complementary thermogenic mechanisms. It is known that low concentrations of triiodothyronine may trigger thermogenesis by shivering, which contributes to increased endogenous heat production (Dwyer et al., 2016). In addition to chemical thermogenesis, which depends, to some extent, on the availability of circulating glucose and the mobilization of brown adipose tissue, possible participation of mechanical thermogenesis, which was not monitored in the present study, may have favored thermogenesis in twin lambs.

The temperature of an animal depends on the amount of heat contained per unit of tissue mass. Therefore, it is a variable that indicates an animal's heat storage condition and, ultimately, its physiological adaptability and tolerance to variations in ambient temperature. In the present study, both internal and ocular temperatures were higher in twin lambs. The internal temperature was in the range from 38.0 to 39.0 °C, considered normal for newborn sheep (Bal et al., 2012), and was similar to previously reported values of 39.2 °C for newborn lambs

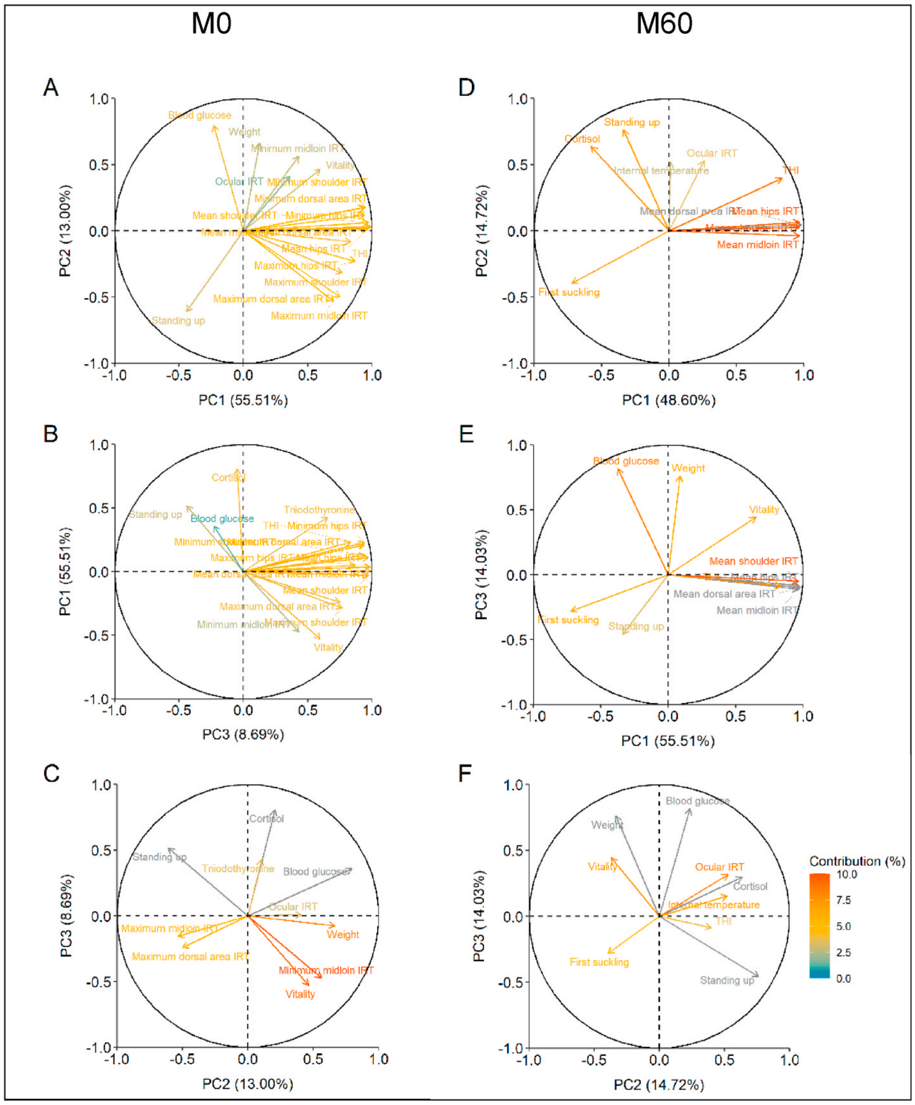

Fig. 6. Correlation circle indicating the contribution of the variables to the principal components at the moments of first successful suckling-M0 (A-B-C) and 60 min later-M60 (D-E-F): The x-axis indicates the first or second components and the y-axis indicates the second or third components. The arrows represent the variables and the length of the arrow indicates the magnitude of the correlation with the principal component.

(Macías-Cruz et al., 2016; Labeur et al., 2017). Although the internal temperature was higher in twin lambs, the transfer of thermal energy to the environment was more accentuated in these animals over time. This may be explained due to the fact that animals with smaller body mass have higher heat conductance and face greater heat loss due to their relatively smaller surface areas (Randall et al., 2000). Ocular temperature records (38.21–38.63 °C) were higher than the value of 32.7 °C reported by Vicente-Pérez et al. (2019). This difference may be attributed to the use of the mean temperature by these authors, instead of the maximum temperature point (hotspot), as described by Hoffmann et al. (2013) and adopted in this study. Regardless of parturition type, the ocular surface temperature was lower than the internal temperature, with a moderate positive correlation between these variables, as already observed in adult sheep (Kahwage et al., 2017).

Considering the surface temperatures of the dorsal anatomical regions, the recorded values were higher than those previously reported for newborn lambs (Labeur et al., 2017; Vicente-Pérez et al., 2019). This variation may be attributed to distinctions between genotypes and/or to the environmental conditions in each experiment. The linear correlation values show that variations in body surface temperatures were positively associated with THI, corroborating results from previous studies (Paim et al., 2012; Vicente-Pérez et al., 2019), and that these are more sensitive to the thermal environment than the internal temperature. It is known that air temperature and relative humidity influence the heat exchange of animals, both in hot and cold environments (Salles et al., 2016). In these situations, as THI cannot be manipulated in the open-pasture environment, lambs should be gathered in a shelter that provides higher air temperature and prevents continuous wind. Also, lambs can be warmed in a holding area, such as a warming box, to rest with thermostatically-controlled heat. Nevertheless, THI has been used as an index to monitor climatic conditions and potential environmental impacts on the productivity and animal health, as well as to guide management practices that help prevent the thermal stress (Lallo et al., 2018. Theusme et al., 2021; Wijffels et al., 2021). Therefore, for the correct acquisition and interpretation of the thermograms, the temperature and humidity input device in the thermograph must be activated, a resource available in the high-performance equipment recommended for biomedical applications. This reinforces the importance of monitoring THI in the parturition environment to safeguard the health of newborns, especially in colder climates or at times of the year when air temperatures tend to be lower. Therefore, for the correct acquisition and interpretation of the images, the temperature and humidity input device in the thermograph must be activated, a resource available in the high-performance equipment recommended for biomedical applications.

Nevertheless, body surface temperature has been used as a complementary and applicable indicator for field evaluation of the thermal balance of sheep (Pantoja et al., 2017; Seixas et al., 2017b). At the time of the first effective suckling (M0), the lower thermal variabilities between twin and single birth animals, in absolute value, occurred in midloin (Δ = 0.4 °C), in the integral dorsal area (Δ = 0.4 °C), and rump (Δ = 0.6 °C), with variations in internal temperature (Δ = 0.8 °C) and ocular globe (Δ = 0.5 °C). One hour after the first suckling (M60), the lowest thermal variabilities between twin and single birth animals, in absolute value, were recorded in shoulder (Δ = 0.1 °C), midloin (Δ = 0.3 °C), and integral dorsal area (Δ = 0.5 °C), being less than or equal to the variability of the internal temperature (Δ = 0.5 °C) or of the ocular globe (Δ = 0.7 °C). Based on the intersection of the regions with the lowest thermal variabilities between single and twin birth animals recorded at M0 and M60, it can be suggested that midloin and dorsal area are considered the anatomical regions of choice for serial thermographic monitoring of newborn hair lambs, regardless of parturition type.

The results obtained in the analysis of principal components helped to understand the relationships between the evaluated characteristics. In the analysis of the first principal component, regardless of the timepoints, the surface temperature vectors were close, with the same direction, and correlated with the THI. Thus, it is possible to infer that this component was influenced by thermal and environmental variables. The second principal component indicated that individuals with increased weight were more active and needed less time to stand up and reach the udder. On the other hand, the third main component discriminated, immediately after the suckling, the differences between individuals with lower and more reactive cortisol and blood glucose, in contrast to individuals that were lighter, less active, and had lower maximum temperatures. Meanwhile, 60 min after the first suckling, the third principal component exhibited higher vectors for blood glucose and body weight, and opposite directions on the latency to stand up, reaffirming that heavier animals were more active and required less time to get up and initiate the active agonistic interaction in search of the mother.

In practical terms, the results indicate that newborn hair lambs, regardless of whether they come from single or twin births, may have their surface temperature monitored individually, as an important complementary resource in the care adopted in the postpartum period. The midloin and dorsal regions behave differently but are interestingly useful. The average midloin temperature, regardless of parturition type, shows little difference between twin or single birth animals, either after birth or after the first suckling, which may favor its interpretation and use in clinical monitoring. However, adopting the first suckling as an easily visible event, the integral dorsal region may become the region of choice for monitoring. Despite the average dorsal temperature having a thermal variability of 0.5 °C between twin and single birth lambs, its use after the first suckling may be considered the most convenient one among the evaluated surface temperatures. The dorsal region is easy to view, which, in newborn lambs, has an estimated area of 318 cm^2 (Sousa, 2021), detectable at an orthogonal angle, ideal for the acquisition of thermographic images, regardless of whether the animals are standing up or lying down, static or moving. Its delimitation does not require in-depth anatomical knowledge for the segmentation of the thermographic image, which could dispense with the image post-processing step and give the producer greater autonomy in the use of thermography as a screening tool before triggering veterinary medical assistance, in case of problems.

In addition to the use for individual monitoring, with the purpose of clinical control of animals, it is possible to imagine other implications for the future use of thermographic monitoring of the surface of lambs, due to advances in knowledge. For use in herd management situations, on a larger scale, infrared thermography would allow scanning dozens of animals in a few minutes, as it does not require contact and is non-invasive (Luzi et al., 2013). The thermographic equipment to be used could be a stationary camera, fixed, for instance, inside a management barn (Hoffmann et al., 2013), or a portable camera, which can be attached to an unmanned aerial vehicle, such as a drone (Karp, 2020). In the latter case, its use can be designed for the detection and evaluation of newborn animals in pastures, as already reported for deer (Cukor et al., 2019), allowing collection and adequate neonatal care. Being carried out early in the morning, in order to avoid less interference from THI and direct radiation on the surface temperature of the animals, the adoption of daily overflights in maternity paddocks would be a management strategy to identify and evaluate newborn lambs in an open field.

5. Conclusions

Both single and twin animals showed similar neonatal behaviors, but their vigour score was associated with the postpartum cortisol concentration at birth. Twin lambs have a higher internal and ocular surface temperature than single lambs, and there is a correlation between internal and ocular surface temperature, regardless of the type of birth. The body surface temperatures of newborn lambs increase over time, and the midloin and dorsal regions proved to be interesting for thermographic monitoring, due to the ease of observation and small thermal variability among lambs from single or twin births. Finally, infrared thermography proved to be a useful tool to evaluate the instantaneous thermal condition of hair lambs in the immediate postpartum period, even capable of detecting the thermogenic effect in the region of brown adipose tissue accumulation in hair sheep newborns. New applications of the technique may be incorporated into individual or flock evaluation routines, as the frontier of knowledge on lamb thermal biology expands.

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Ethics

The experimental procedures were previously approved by the Ethics Committee on the Use of Animals of the Brazilian Agricultural Research Corporation-Embrapa Pecuária Sudeste (Protocol 09/2017).

Author statement

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Declaration of 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2022.103258.

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