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Infrared thermography technique for detecting estrus in Surti does

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Accurate and timely estrus detection in goats (*Capra hircus*) is crucial for maximising production and reproductive performances as well as effective herd management for economic sustainability. Current methods of estrus detection in goats, including visual observation, tail painting, teaser animals, mucus discharge observation, behavioural changes, hormone testing and ultrasonography, are time-consuming, labour-intensive and influenced by the duration and frequency of observations, as well as herd size. Therefore, there is a need for a simple, quick, accurate, non-contact, non-invasive diagnostic tool for optimal estrus detection.

Piccione *et al.* (2003) found that sexually mature cows have a body temperature rhythm during estrus. This suggests that changes in body temperature could be a viable method for detecting estrus. While rectal or vaginal temperature measurements are traditionally considered the gold standard for determining core body temperature using thermometers (Vickers *et al.* 2010), they are somewhat laborious and time-consuming. As an alternative, measuring surface temperature could offer a quick and easy solution.

Objects with temperatures above absolute zero (0 K or -273°C) emit infrared radiation that is correlated with body temperature. Therefore, the higher the temperature of an object, the more infrared radiation will be emitted (Olasehinde 2021). Thermal mapping or thermographic images of such surfaces will display the temperature distribution of that region, which can be analysed using specialised software. These thermograms represent differences in temperatures of an object's surface with different colours; for example, the hottest areas are recorded as white or red, and the coldest areas are blue or black (Colak *et al.* 2008).

This technique is called as infrared thermography, and it has been used in the livestock industry to measure heat transfer and blood flow patterns by detecting body temperature changes (Chacur *et al.* 2016). Infrared thermography has been used to measure skin temperatures of the orbital area, flank, and ears in relation to rectal temperature (Sevegnani *et al.* 2016), for early diagnosis of subclinical and clinical mastitis (Berry *et al.* 2013), to assess heat stress in dairy cattle (Montanholi *et al.* 2008), to evaluate scrotal temperature as a measure of fertility in cattle and goats (Weschenfelder *et al.* 2013), for estrous detection in dairy ruminants (Talukder *et al.* 2014), to measure vulvar surface temperature difference between the phases of estrus and diestrus in cattle (Siregar *et al.* 2016) and ewes (George *et al.* 2014), to predict ovulation (Talukder *et al.* 2014), to detect early signs of viral and systemic infections (Vadlejch *et al.* 2010), and to assess animal welfare (Stewart *et al.* 2007) and animal behaviour (Reichard *et al.* 2010). Therefore, considering the importance of this relatively new technology option and its utility for efficient, quick, and easy estrus detection as an aid to enhancing goats' reproductive performance, this work was done to study the skin thermal pattern and evaluate the potential of the infrared thermography technique for estrus detection in Surti goats.

The present study was conducted on 24 apparently healthy and cyclic Surti does maintained at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat, under standard housing and management practices. The selected animals were divided into two groups, i.e. does that were in the estrus phase (E) and does that were not in the estrus phase (NE). Confirmation of estrus was done by parading a sexually active apronized buck thrice during the day (morning, noon and evening hours), along with observations for the presence of behavioural signs such as standing to be mounted, wagging of the tail, frequent micturition, restlessness, mounting on other animals, clustering around a buck and frequent bleating. Physiological signs like vulvar oedema, vulvar hyperemia, cervico-vaginal mucus discharge and opening of cervical-os were also observed to confirm the estrus. Whole blood samples from the jugular vein were collected once from both E and NE groups on the day the E group exhibited estrus. The vacutainer tubes without anticoagulant were used to collect blood samples and kept undisturbed for clotting. Serum was harvested from the coagulated blood without disturbing the clot for assaying estrogen and progesterone

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hormones using commercially available ELISA kits (Diagnostic automation, USA), luteinising hormone (LH) and follicle-stimulating hormone (FSH) (Cloud-clone Corporation, USA). Comparisons of hormones were done to confirm the E and NE phases. The levels of these hormones were statistically compared between two groups, E and NE does, by t-test using SAS 9.3 software. Higher LH, FSH, estrogen and lower progesterone indicated the estrus phase. Ear temperature (or tympanic temperature) was recorded with the help of a non-contact infrared thermometer (BPL AccuDIGIT thermometer, India), while the rectal temperature was measured with the help of a clinical thermometer (Rossmax Swiss GmbH, Switzerland). Thermal images of the posterior body parts of the goats (Figs. 1 and 2) were captured using an infrared thermal camera (FLIR T420-Flir Systems Commercial Co. USA). While performing thermography, a constant distance of 1 m was maintained between the camera and the target surface on the animal body. Ambient temperature and relative humidity were determined during thermography using a real-time automatic data logger. Images captured using a thermal imaging camera were subjected to FLIR Image analysis software, and final observations for surface temperature were recorded after correcting for distance, ambient temperature, and relative humidity in the software itself. Pearson correlation analysis was performed using SAS 9.3 for surface temperatures of the vulva, pudendum, rectum, tympanic, as well as rectal temperature.

Most of the estrus-specific behavioural signs, viz. standing to be mounted, wagging of the tail, frequent micturition, restlessness, mounting on other animals, clustering around a buck, frequent bleating, and physiological signs like vulvar oedema, vulvar hyperemia, cervicovaginal mucus discharge and opening of cervical-os were observed in the does of the E group. Similar estrus-specific sexual behaviours in goats were also reported by Bedos *et al*. (2012), Kerketta *et al.* (2014) and Debora *et al*. (2018).

Serum concentrations (Mean±SEM) of estrogen, progesterone, LH, and FSH hormones of estrus and non-estrus Surti does are presented in Table 1. Perusal of data revealed that the serum concentration of estrogen, FSH, and LH was significantly higher ($P \leq 0.01$), and the serum concentration of progesterone was significantly lower ($P \leq 0.01$) in goats showing signs of estrus than non-estrus. The values of all these hormones were within normal physiological range, which corroborated well with the incidence of estrus. Regressing corpus luteum of the previous cycle results in lowering circulatory progesterone levels that pave the way for the next estrous cycle. Anterior pituitary gonadotropins such as LH and FSH are responsible for follicular growth (Amiridis and Cseh 2012). During the follicular phase, in proestrus, gonadotropin-dependent follicles begin to develop under the influence of FSH (Gama and Bressan 2011). FSH stimulates granulosa cells to produce estrogen in increasing levels (Hafez and Hafez 2000), thereby creating positive feedback for GnRH to rise. The spurt in GnRH levels results in a drastic LH rise before ovulation. Synergistic to rise in FSH, LH secretion from the anterior pituitary increases during estrus, results in its preovulatory surge. Thus, a period spanning the estrus phase is marked with higher levels of LH, FSH and estrogen and lowered progesterone, as observed in the present study.

Surface temperatures (Mean±SEM) of the vulva, pudendum, rectum, and tympanic and rectal temperature of estrus and non-estrus Surti does are presented in Table 2. A significant difference ($P<0.05$) in tympanic and rectal temperature between two groups of animals was observed. The tympanic temperature was higher by 1.73°C, whereas rectal temperature was higher by 1.15°C in group E than NE. The rectal temperature in goats varies from 38.8°C to 40.50°C (Singh and Islam 2014), and daily variation in rectal temperature ranges from 0.3°C to 1.9°C (Piccione and Refinetti 2003). The higher rectal temperature during estrus may be attributed to increased physical activity (Walton and King 1986), estradiol, LH surge, and the GnRH hypothalamic response in cyclic cows (Talukder *et al.* 2014).

The surface temperature of the vulva and rectum were significantly ($P<0.05$) higher in does in estrus than non-estrus does. Does in estrus had 1.02°C higher vulvar surface temperature and 0.78°C higher rectal surface temperature

Table 1. Serum concentrations (Mean±SEM) of estrogen, progesterone, LH, and FSH hormones of estrus and non-estrus Surti does

Group	Estrogen (pg/ml)	Progesterone (ng/ml)	LH (pg/ml)	FSH (pg/ml)
Does in estrus (E) (n=12)	38.50^{b}±2.22	1.23^{a}±0.18	4639.28^{b}±197.66	11.79^{b}±0.69
Non-estrus does (NE) (n=12)	17.93^{a}±0.40	2.92^{b}±0.20	2597.70^{a}±33.55	7.27^{a}±0.45
Overall (N=24)	28.21±2.41	2.08±0.22	3618.49±234.34	9.53±0.62

Means bearing different superscripts across columns differ significantly at $P \leq 0.01$.

Table 2. Surface temperatures (Mean±SEM) of the vulva, pudendum, rectum, and tympanic & rectal temperature of estrus and non-estrus Surti does

Group	Tympanic temperature (°C)	Rectal temperature (°C)	Vulvar surface temperature (°C)	Rectal surface temperature (°C)	Pudendal surface temperature (°C)
Non-estrus does (12)	35.28^{a}±0.69	37.62^{a}±0.13	36.60^{a}±0.35	38.02^{a}±0.28	36.86±0.33
Does in estrus (12)	37.01^{b}±0.46	38.77^{b}±0.15	37.62^{b}±0.15	38.80^{b}±0.13	37.51±0.18
Overall (24)	36.14±0.44	38.20±0.15	37.11±0.21	38.41±0.17	37.19±0.20

Means bearing different superscripts across columns differ significantly at $P<0.05$.

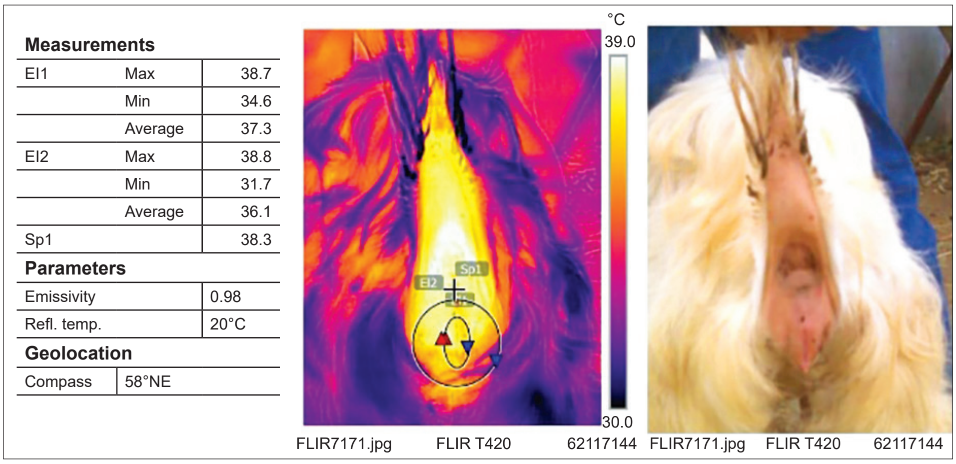

Fig. 1. Thermogram of non-estrus doe.

than the does that were not in estrus. A non-significant difference in pudendal temperature was observed between the two groups of animals, although it was higher for does in estrus. The thermogram of both the groups of does is presented in Figs. 1 and 2. Marquez *et al.* (2019) concluded that the difference in radiated temperature between control and estrus synchronised groups is attributed to increased radiated temperature in cyclic cows during the estrus period. Talukder *et al.* (2014) postulated that estradiol, LH surge and GnRH hypothalamic response in cyclic cows may increase the radiated temperature, which could be corroborated by the serum levels of different hormones observed in this study. In mammals, the skin plays a vital role in transferring heat between the body surface and the environment and the heat loss efficiency depends on the temperature gradient between the two. The insensible mode of heat loss is activated when the superficial temperature increase results from redirecting the blood flow to the body's surface by vasodilation (Habeeb *et al.* 1992). Stelletta *et al.* (2017) reported an increase in vulva temperature during the estrus period until approaching ovulation and then the vulva temperature decreased at the end of the estrus period. Similar to the findings of the present study, the higher vulvar surface temperature was also reported by Talukder *et al*. (2014), Debora *et al.* (2018) and De Ruediger *et al.* (2018). Osawa *et al.* (2004) studied the variations of vulva temperatures in cows and observed an increase in vulvar surface temperature in estrus compared with the diestrus periods. Further, in the present study, the rectal temperature was higher than the surface temperature of the vulva in both groups of animals, which is in agreement with the finding of Pamungkas *et al.* (2020).

Correlation coefficients among surface temperature of vulva, pudendal, rectum, and rectal and tympanic temperature are presented in Table 3. A strong positive and significant correlation was observed for the surface temperature of the vulva with pudendum (0.779), rectal surface (0.781), rectal (0.683), and tympanic (0.409) temperatures. Significant positive correlation was also found for pudendal surface temperature with rectal surface temperature (0.695) and rectal temperature (0.523); rectal surface temperature with rectal temperature (0.594) and tympanic temperature (0.405); rectal temperature with tympanic temperature (0.636). De Ruediger *et al.* (2018) reported moderate to strong correlations between rectal temperature and vulva, muzzle and orbital area surface temperatures.

The findings of the present study concluded that the

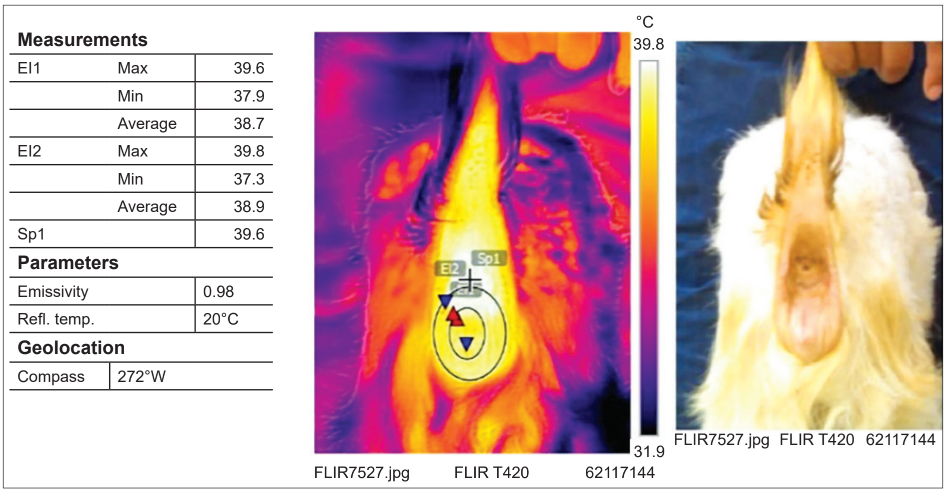

Fig. 2. Thermogram of doe in estrus.

Table 3. Correlation coefficients among surface temperature of vulva, pudendal, rectum and rectal and tympanic temperature

Temperature (°C)	Vulvar surface temperature	Pudendal surface temperature	Rectal surface temperature	Rectal temperature	Tympanic temperature
Vulvar surface temperature	1	0.779**	0.781**	0.683**	0.409*
Pudendal surface temperature	0.779**	1	0.695**	0.523**	0.203
Rectal surface	0.781**	0.695**	1	0.594**	0.405*
Rectal temperature	0.683**	0.523**	0.594**	1	0.636**
Tympanic temperature	0.409*	0.203	0.405*	0.636**	1

*Correlation is significant at the 0.05 level, **Correlation significant at the 0.01 level.

vulvar and rectal surfaces, along with rectal and tympanic temperatures, were significantly higher in the E group does. A strong, significant positive correlation exists between vulvar surface temperature and pudendal surface temperature, rectal surface temperature, rectal temperature as well as tympanic temperature. Thus, the infrared thermography technique holds the potential for detecting estrus in Surti does.

SUMMARY

The present study evaluated the potential of the infrared thermography technique for estrus detection in Surti does. Based on the presence of estrus-specific behaviour, physiological signs as well as higher LH, FSH, estrogen and lower progesterone hormone concentration, 24 Surti does were divided into two groups, viz. estrus and non-estrus does. Recording of surface temperatures, rectal and tympanic temperatures was done using an Infrared thermal camera (FLIR T420), digital thermometer and infrared non-contact tympanic thermometer, respectively. It was concluded that temperatures of the vulva, rectal surface, rectal and tympanic temperatures are significantly higher in estrus does. Further, a strong, significant positive correlation was observed between vulvar and pudendal, rectal surface temperatures, rectal temperature as well as tympanic temperature. Thus, it may be concluded that the infrared thermography technique holds the potential for detecting estrus in Surti does.

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