Acta Veterinaria Brasilica June 12 (2018) 49-54

Acta Veterinaria Brasilica

Journal homepage: https://periodicos.ufersa.edu.br/index.php/acta/index

Original Article

Detecting estrus in Canindé goats by two infrared thermography methods

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A R T I C L E I N F O

Article history
Received 25 July 2017
Received in revised form 25 February 2018
Accepted 04 March 2018

Keywords:
Electronics in agriculture
Locally adapted goats
Reproduction
Semiarid region

A B S T R A C T

The aim of this study was to evaluate the diagnosis of estrous in Canindé goats used infrared thermographic images. The Canindé goats used in experiment, belong to Production Center of Small Ruminants. The goats were subjected to estrus induction protocol and monitored with regard to their behavior and blood levels of hormones. Temperature measurements were continuous during estrous signs and even disappearance of behavioral characteristics. Were evaluated different areas in females: vulva, perivulvar, dorse and venter regions, delimited by a quadrant that allowed the specification of the assessed region. In addition to the specific behavior of estrus in ruminants were observed in the vulvar region perivulvar signals swelling and redness and parallel identified the temperature rise of that region by thermographic images. Were observed in the vulvar region perivulvar signals swelling and redness and parallel identified the temperature rise of that region by thermographic images in goats in estrus. There was a significant effect for all temperatures of the regions evaluated for the thermographic images and infrared thermometer, except for the temperature of the vulvar region. The temperatures measured using the thermographic camera submitted a difference in the data set, allowing measurements observed higher temperatures compared with the temperatures measured with an infrared thermometer. So, due the precision and speed of the method the thermographic images used for detecting estrus is applicable and important. In animal production, thermography has seen used in detection of metabolic disorders, diseases and infections. In addiction, is used in understanding thermoregulation due to chenges in temperature and the impact of environmental conditions.

INTRODUCTION

The application of assisted reproductive technologies allows the development of the breeding performance, with the main objective of maximizing the reproductive potential of the female, intense use its biological potential to extrapolate its natural possibilities (KARACA et al., 2016). Several techniques increase the differential selection, as also accelerate the progress shortening the interval between generations (SOURI; MIRMAHMOUDI, 2014). The assisted reproduction techniques commonly used in goat breeding, including artificial insemination, hormone induction of estrus and ultrasound (PIETROSKI et al., 2013). However, the estrus detection can be a challenge for technologies application, and consequently the ovulation prediction is affected, considered a challenge for goat industry, emerging as a significant component of profitability in artificially bred herds (SOUZA et al., 2011).

Traditional methods of estrus detection are routinely used in observation of female behavior that are affected

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http://dx.doi.org/10.21708/avb.2018.12.2.7243

by duration and frequency of observations (NAGAPPA et al., 2015). Furthermore, when associated with increased herd size and the need for improvements work, has increased dependency on estrus detection tools, with emphasis non-invasive diagnostic methods. In this context, an experimental level, the infrared thermography through visible colors, it has assumed an important role as a safe method, non-invasive and used in analysis of different areas of animal production, especially in the identification of thermal comfort in real time (SYKES et al., 2012). In the livestock industry has been used for applications in the diagnosis of lesion members, evaluating the scrotal temperature as measure of fertility in cattle and goats (WESCHENFELDER et al., 2013) and evaluation of heat stress in dairy cattle (MONTANHOLI et al., 2008). In addition, it has been revealed as auxiliary and efficient technique for estrous detection in dairy ruminants (TALUKDER et al., 2014), especially in identifying the increase in the vulva area of the surface temperature difference between the phases of estrous and diestrus (SIREGAR et al., 2016). This fact is a consequence of changes in body temperature during the estrous cycle, due to increased blood flow, which increases with the approach of ovulation (OLA et al., 2006).

The use of infrared thermography in goats is poorly described, most related to understanding of thermoregulation in changes in surface temperature and the impact of environmental conditions on animal welfare (NAAS et al., 2014). The applicability of this technique in reproductive diagnosis presents important expectations. However, there is no technical patterning, complicating reliability of the result.

To our knowledge, there are no studies in goats using the verification of the skin or vulvar temperature as estrous or ovulation indicator. Is noteworthy that in goats, hyperemia and swelling vulvar occurs discreetly, becoming complex estrus detection. Therefore, this study aimed to evaluate the diagnosis of estrous in Canindé goats used infrared thermographic images.

MATERIALS AND METHODS

Animals and design experimental

Animal care procedures throughout the study followed protocols approved by the Ethics Committee for Animal Use (CEUA) at the Federal University of Semiarid (Universidade Federal Rural do Semi-Árido; UFERSA), number 34/2014 (23091.003895/2014-71). The animals belong to the Ruminant Management Center in UFERSA, located in the northeast of Brazil (Mossoró, RN, Brazil; 5°10′S, 37°10′W).

The climate is typically semi-arid with an annual average temperature of 26 – 27,5ºC and relative humidity 50%.

Experiment was conducted from March to April 2015, during the rainy season. A total of seven Canindé goats, aging 2.5 ± 1.0 years and weighing 32 kg were used. They were subjected to clinical reproductive examination, including ultrasound examination of the uterus and ovaries, and had no signs of reproductive and systemic diseases. The animals were sexually mature, with registration of at least one pregnancy per female before the beginning of the experiment. The animals were maintained under natural lightning conditions and divided into two groups with 3 and 4 animals, and stayed in collective corral. The animals were fed on a diet consisting of Tifton 85 (*Cynodon* ssp) and supplementation with commercial concentrate.

Estrous induction protocol

The females received two intramuscular injections of 0.8mL cloprostenol (Ciosin®, Schering-Plough-Coopers, Brazil) administered at a 9-day interval. The estrous cycles of the animals were monitored all days for reproductive hormone's dosage (Estrogen) in blood, vaginal cytology and alterations in the external genitalia. Additionally, females were monitored for behavior, considering the females in estrous that presented reflection of immobility in relation to mounts to the others females, frequency of urination and vocalization.

Hormonal measurements

The estrogenic phases were identified according to the blood estrogen values previously established for goats. Blood samples (3.0mL) were collected from the jugular venipuncture, into silicon tubes and centrifuged at 200 G for 15 min within 2 h. after this procedure, the plasma was separated and kept in a freezer (-18ºC). Estradiol levels were determined by immunoadsorption enzyme-linked assay (ELISA) (Elysis UNO®, Human®) containing microtiter wells coated with anti-antibodies estradiol rabbit. The procedures were performed according to the recommendations of the manufacturer of the ELISA kit. The results obtained for estrogen were in pg/ml.

Thermography and infrared data

The verifications of the temperatures were performed in all females, daily, always 07:00 am, using infrared thermometer (SCAN TEMP ST-600.00, Incoterm®, Porto Alegre-RS) and infrared thermographic images camera (FLUKE Ti200, Fluke Corporation®), to diagnose a particular temperature according to the methodology used. Temperature measurements were continuous during estrous signs and even disappearance of behavioral characteristics. The thermographic images were obtained while females were being fed, facilitating the process of obtaining images, monitored from a fixed distance of 1m of the female.

Were evaluated different areas in females: vulva, peri vulvar, dorse and venter regions, delimited by a quadrant that allowed the specification of the assessed region. These areas were cleaned with dry paper towels, without moisten or rub excessively, for not interfere in the skin real temperature.

Digital infrared thermal images were transferred to the computer and viewed using a SmartView analyzer software, version 1.7 (Fluke Thermography, Plymouth, Minnesota). This software allows the user to identify the temperature at any point (pixel) image. In addition, specific areas such as vulvar and peri vulvar region could be identified, and the mean, minimum and maximum values were calculated.

Statistical analysis

Spearman correlations were performed between the temperature variables measured by infrared thermometer and thermographic camera and the serum estrogen values.

Data analyses were performed using the methodology of linear mixed models (SAMPAIO, 2002), exemplified below:

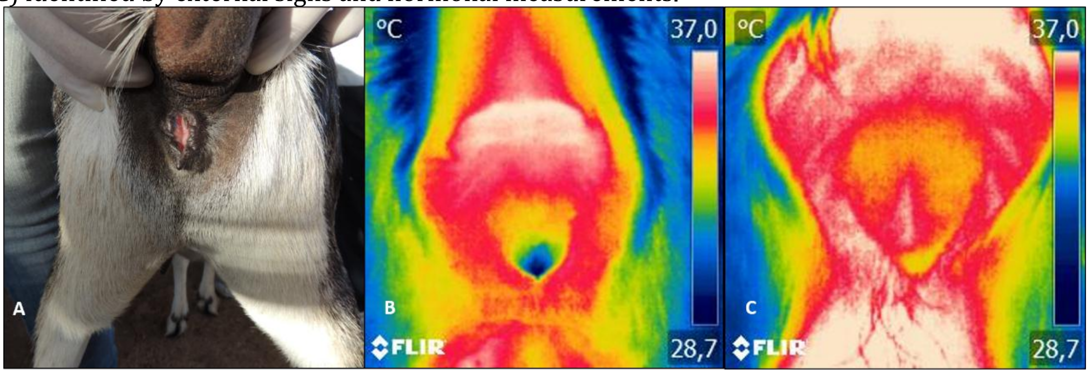
$$Y_{ijke} = \mu + T_j + D_k + e_i$$

Y_{ij} Set of dependent variables;
μ Overall average of each variable;
T_i Diagnostic method (infrared thermometer and thermographic images);
D_k Collection days (1, 2, 3, 4...);
e_i Random error associated with each observation.

RESULTS

Induction of luteolysis resulted in estrous synchronization in all animals, but the ovulation not was valued. In addition, to the specific behavior of estrus in ruminants (vocalization, restlessness and stilling how touched the back) were observed, and in the region perivulvar and vulvar, signals discreet swelling and redness was observed (Figure 1.A). Parallel to these characteristics, was identified the temperature rise of that region by thermographic images (Figure 1.C).

Figure 1 – Photography (A) and thermographic image of vulvar and perivulvar regions of Canindé goats in proestrous (B) and estrous (C) identified by external signs and hormonal measurements.



In thermographic images of the regions of the dorse and venter, a region focal showed higher temperatures, still when compared to the vulva ($p = 0.1558$) and perivulvar ($p = 0.0015$) regions that are naturally vascularized and receive more blood supply, this situation.

There was a significant effect ($p < 0.05$) for all temperatures of the regions evaluated by thermographic images and infrared thermometer, except for the temperature of the vulvar region (Table 1). In this case, for vulvar evaluation, no difference in the method used ($p = 0.1558$), so any method can be used. However, the temperatures measured using the thermographic camera submitted a difference in the data set, allowing measurements observed lower temperatures compared with the temperatures measured with an infrared thermometer (Figure 2, 3).

The temperature measured by the infrared thermometer showed a higher correlation with estrogen, which was more evident for the temperature of the vulva (R = 0.46718) and perivulvar temperature (Table 2).

Table 1 – Temperatures regions of the vulva, perivulvar, dorse, ventral using thermographic images and infrared thermometer in Canindé goats estrous detection Canindé breed.

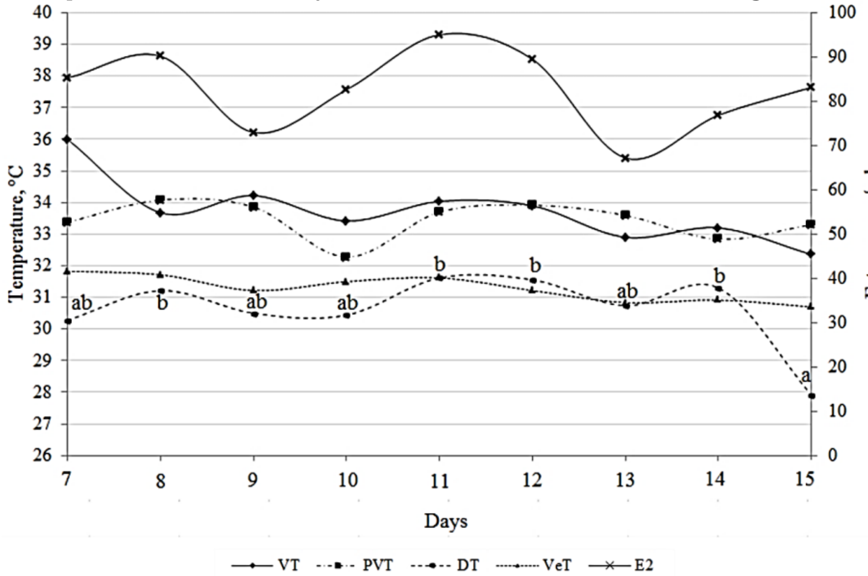
Regions of the body	Thermographic images	Infrared thermometer	p < 0,05
Vulva	33.81 ± 0.18	34.17 ± 0.18	0,1558
Perivulvar	33.43 ± 0.15[b]	34.05 ± 0.14[a]	0,0015
Dorse	30.74 ± 0.15[b]	32.96 ± 0.15[a]	<0,001
Venter	31.34 ± 0.15[b]	33.64 ± 0.15[a]	<0,001

Means followed by different letters in the same linea differ by the Ducan test ($p < 0.05$).

Table 2 – Correlation between temperatures of different regions and the estrogen of Canindé breed.

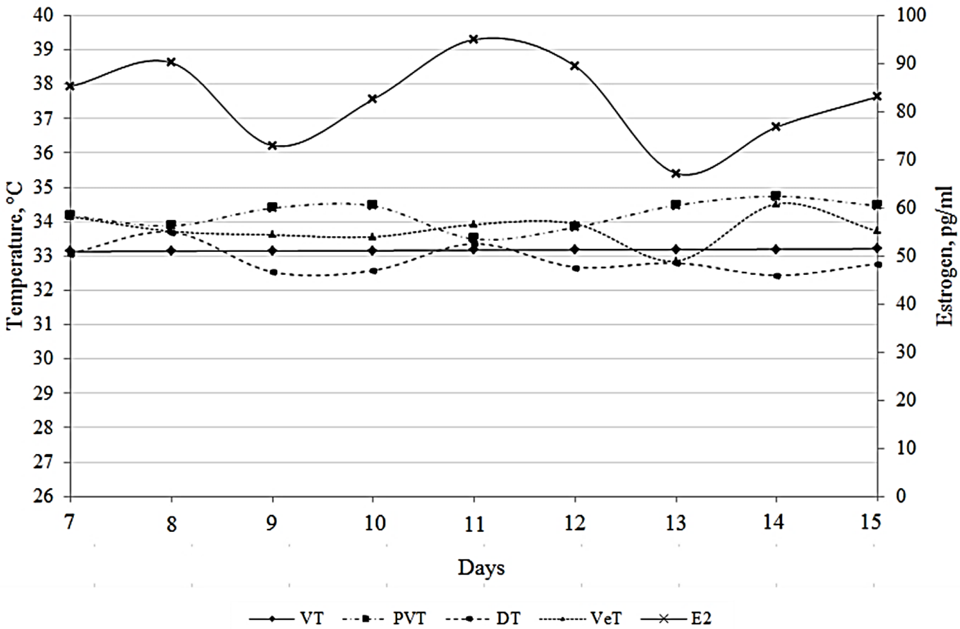
	Variables	E2	
		R	P value
Infrared Thermometer	Vulvar	0.46718	0.0012
	Peri Vulvar	0.52242	0.0002
	Dorse	0.41098	0.0050
	Venter	0.26183	0.0823
Thermographic camera	Vulvar	0.38273	0.0095
	Peri vulvar	0.07162	0.6401
	Dorse	0.15655	0.3044
	Venter	0.40548	0.0057

Figure 2 – Plasma estrogen concentration in Canindé goats treated with Cloprostenol on four day before until four days after estrogen peak and temperatures measured by infrared thermometer in different regions of the body.



VT = vulvar temperature; VeT= venter temperature; PVT = perivulvar temperature; DT = dorse temperature; E2 = estrogen.

Figure 3 – Plasma estrogen concentration in Canindé goat treated with Cloprostenol on four day before until four days after estrogen peak and temperatures measured by thermographic camera in different regions of the body.



VT = vulvar temperature; VeT= venter temperature; PVT = perivulvar temperature; DT = dorse temperature; E2 = estrogen.

DISCUSSION

The arrival of a technological innovation generates a large impact on elements of an industry (HANNACHI; TICHIT, 2016). The use of thermographic images have long been used in production systems as a technological tool in the diagnosis of diseases (MCMANUS et al., 2016), as in studies of parasitic tick infestation in cattle (CORTIVO et al., 2016), in bioclimatology (CARDOSO et al., 2016; GEORGE, 2014) on animal welfare (STEWART et al., 2005) and animal reproduction (SCOLARI et al., 2009; RAMIRES NETO et al., 2011; SIMÕES et al., 2014; CRUZ JÚNIOR et al., 2015; ALVES et al., 2016). In present study, the identification of estrous in goats showed that the infrared thermography can be a promising technique, and to a highly repeatable method for evaluation of skin temperature in goats.

In present study, the estrus condition was verified by maybe higher circulating amount of the hormone estrogen than others, found between the 8th and 15th experimental day, and in mean, in the 11th day it occurred estrogen peak. At the same day (on the 11^{th} after the first injection) in the animals submitted to the analysis, higher values of vulvar and perivulvar temperature were observed. Studies on estrus detection goats are found and others ruminant species, as dairy cattle, the thermography to detect estrous have met with mixed results (VASCONCELOS et al., 2015). However, these results were also observed in cattle by TALUKDER et al. (2014), that descriptioned an increase in the temperature (increased to 1.5ºC) 24 h before ovulation corresponding the moment of estrus, while a reduction in the temperature of the vulva and muzzle 48 h prior to ovulation can be related to the corpus luteum regression. In this study, during the day, although the highest temperatures have been observed in the two methods used in same day.

In general, the infrared thermography present higher values as compared with the use of the infrared thermometer. Second MCMANUS et al. (2016), although it is sensitive in detecting changes in thermal patterns of animals, this methodology of thermographic images may not be accurate. This occurs because the animal is influenced by the thermal environment, due to the energy exchange between the animal and the environment (SYKES et al., 2012). Despite this, a positive correlation ($R = 0.38$) was observed between estrogen values and vulvar temperatures when the thermographic camera was used. However, this correlation was higher when the infrared thermometer was used ($R = 0.46$ and 0.52, for vulvar and perivulvar temperature, respectively). Note that changes in the vulvar and perivulvar regions, are closely related to sexual characteristic in estrous (SCOLARI et al., 2011). In gilts, vulva temperature thermal images were positively correlated with ambient temperature ($p < 0.01$), always with the highest vulva temperature throughout the day in the estrus period (SYKES et al., 2012).

The temperature measurements were only recorded for one cycle in all animals. In this fact, we evaluated the use of thermal imaging to differentiate first estrous from diestrus after synchronization, but not between estrous and diestrous of subsequent estrous cycles. So, further studies should be carried out to evaluate the use of thermography in goat. Since, estrous detection efficiency is fundamental to provide viability in animal production, with effect on the calving interval (IEP), fertility and conception rate in herds submitted to artificial insemination. This condition must be sought, because the activities related to reproduction are much more intense and failures would lead to serious problems in management, with major economic repercussions (SOLEILHAVOUP et al., 2016).

Surprisingly, a significant effect ($p < 0.05$) for all temperatures of the regions evaluated for the thermographic images and infrared thermometer was observed, except for the temperature of the vulvar region. In a similar study with sows, the thermography was used for identify the estrus and the vulvar region was the one that presented hight relation with this phase of the cycle (SIMÕES et al., 2014). In fact, this occurs due to the vulvar swelling and reddening, normally observed in mammalian species under the influence of estrogen.

The precision and speed of the method used for detection estrus is applicable and important, however, needs further studies. Therefore, the thermographic images combined with a visual detection of estrus and hormone levels of estrogen in Canindé goats is an option for breeders, because it is a technique faster and more accurate, allowing the smallest errors in estrus detection.

Acknowledgement

The authors acknowledge the support by the Laboratory of Adaptive Physiology and Genetic Resources (Laboratório de Fisiologia Adaptativa e Recursos Genéticos) and Bioclimatology Laboratory and Animal Welfare (Laboratório de Bioclimatologia e Bem-Estar Animal).

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