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Original research

Determining the optimal time of insemination of goats using a thermal imager

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Abstract. The choice of the time of goat female insemination is one of the most significant factors determining the fertilisation result. Successful insemination can only be performed when it is close to ovulation (a few hours before). If insemination is carried out at the very beginning of the sexual hunt, then in females with a long course of this phenomenon, sperm may die before their contact with the ovule; if the sperm enters the genital system too late, the ovule may lose the ability to fertilise. Today, there are several ways to determine the fertile period of females, which have both advantages and disadvantages. Therefore, there is a need to improve existing and develop new methods for determining the optimal time of goat insemination. This can be a thermography (infrared thermography, infrared thermal imaging, thermal image, or thermal video) providing images in infrared rays, which shows a picture of the distribution of temperature fields. The investigation was focused on determining the dependence of the temperature gradients of goats' external genitalia according to the manifestation of the stages of the sexual cycle. It was found that the external genitalia are characterized by a significant area of the palette of «hot» colours during the excitation stage of the sexual cycle. The «cold» colours (green and blue) dominate at the maturation stage. The results of studies reliably confirm the pattern of the temperature increase of the goats' external genitalia during the period of sexual arousal compared to the maturation stage by 1.70 °C (5.1%) – 33.17 ± 0.18 °C and 31.46 ± 0.40 °C accordingly. So, for this purpose, using thermal imagers as a part of thermography is a way of preventive diagnosis of the optimal insemination time. It can be used for females of different species of animals and has a priority in animal reproduction in general. Such qualities as exceptional safety, autonomy and contactlessness make thermovisors irreplaceable in veterinary medicine.

Keywords: fertile period; sexual cycle; diagnostics; thermography

Визначення оптимального часу осіменіння кіз із використанням тепловізорів

Анотація. Вибір терміну осіменіння самок один із найважливіших факторів, що визначає результат запліднення. Результативним осіменіння може бути лише у тому випадку, коли його проводять у час, близький до овуляції (за кілька годин до неї). Адже, якщо осіменіння проводиться на самому початку статевої охоти, то у самок із тривалим перебігом цього феномену спермії можуть загинути раніше, ніж відбудеться їх контакт з яйцеклітиною; за надто «пізнього» потрапляння сперми до статевих органів самки яйцеклітини можуть втратити здатність до запліднення ще до контакту зі сперміями. Нині існує низка способів визначення фертильного періоду самок, що мають як переваги, так і недоліки. Тому, виникає необхідність удосконалення існуючих і розробки нових методів визначення оптимального часу осіменіння кіз. Таким може бути термографія (інфрачервона термографія, інфрачервоне теплобачення, теплове зображення або теплове відео) – зображення в інфрачервоних променях, що показує картину розподілу температурних полів. Дослідження направлені на визначення залежності температурних градієнтів зовнішніх статевих органів кіз відповідно прояву стадій статевої циклічності. Встановлено, що у стадію збудження статевого циклу зовнішні статеві органи характеризуються значними за площею «гарячими» кольорами палітри, тоді як у стадію зрівноваження переважають «холодні» (зелений і синій). Результати досліджень достовірно підтверджують закономірність підвищення температури зовнішніх статевих органів у кіз під час стадії збудження статевого циклу порівняно зі стадією зрівноваження на 1,70 °C (5,1 %) – 33,17 ± 0,180 °C та 31,46 ± 0,40 °C, відповідно. Такими чином, використання з цією метою тепловізорів, як складової термографії, є способом превентивної діагностики оптимального часу осіменіння, може бути застосованим для самок різних видів тварин і загалом мати пріоритетне значення у репродукції тварин. А такі якості, як виняткова безпека, автономність і безконтактність, роблять тепловізори незамінними у ветеринарній медицині.

Ключові слова: фертильний період; статевий цикл; діагностика; термографія

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Introduction

The choice of the time of female insemination is one of the most imperative factors determining impregnation success. Based on theoretical considerations, the optimal time is the most favourable for the meeting of sperm with the ovule, which, in turn, is possible with the full manifestation of the stage of arousal of the sexual cycle (Foote, 1975; Koshevoj et al., 2011; Arrebola et al., 2012; Skliarov et al., 2021).

Goats are animals with seasonal reproduction, which occurs in August–February, depending on the geographic zone, and lasts for 45–60 days. At the beginning and the end of the mating period, the signs of hunting are weakly manifested. The bright manifestation of hunting is observed from September to February, and the best insemination is found from November to December. The sexual cycle of goats lasts 18–21 days on average, with fluctuations from 5 to 17 days. The average cycle duration in young goats is 17–18 days, although there are shortened (defective) cycles lasting even 5–7 days. According to W. Hip, the duration of proestrus is 2-3 days, estrus – 24–38 hours, metestrus – 2–3 days, and diestrus – 10–12 days. When classified by A.P. Studentsov stage of sexual arousal lasts 2–5 days, including heat – 24–36 hours. Sexual arousal coincides with the heat, which occurs in 1–1.5 days from the beginning of the heat and lasts from 27 to 44 hours, but can vary from 12 hours to 3 days; ovulation often occurs after 20–30 hours from the beginning of the heat (with fluctuations of 12–60 hours) and ends after 30–36 hours from the launch of the heat, i.e. at the end of its first day. Unimpregnated goats begin to come to the heat again from the 5th day after insemination (Fatet et al., 2011; Koshevoj et al., 2011; El-Tarabany et al., 2019).

Effective insemination can only be performed when it is close to ovulation (a few hours before). If insemination is carried out at the beginning of the sexual heat, sperm may die before their contact with the ovule in females with a long course of this phenomenon; if the sperm enters the genitals too late, the ovule may lose the inseminating ability even before contact with the sperm (Koshevoj et al., 2011; Skliarov et al., 2021).

Today, there are several ways to determine the fertile period of females, which are based on monitoring the behaviour of animals, assessing the condition of the vagina and cervix, studying the characteristics of oestrous mucus and hormone levels, etc. (Foote, 1975; Jiang et al., 2016; Widayati et al., 2018). To approach insemination before the beginning of ovulation, it is necessary to identify the beginning of the heat as precisely as possible. In practice, the heat in goats is detected with the help of test goats, supplemented by examination of the external genitalia, vagina, and cervix (Koshevoj et al., 2011).

However, there is a need to improve existing and develop new methods for determining the optimal time of goat insemination. One of the urgent problems arose after the introduction of oestrus stimulation and heat synchronization when signs of heat (e.g., erubescence of the external genitalia) are weak. (Ajbazov & Aksenova, 2012; Habeeb & Kutzler, 2021). One of the ways to diagnose this condition is thermography (infrared thermography, infrared thermal imaging, thermal image, or thermal video), which provides infrared pictures of the distribution of temperature areas (Koshevoj et al., 2013; Faust et al., 2014; Jasti et al., 2019). The use of infrared thermography in goats is poorly described. It is related to understanding the thermoregulation of surface temperature changes and the impact of environmental conditions on animal welfare (Nääs et al., 2014; McManus et al., 2016). The use of this technique in reproductive diagnostics raises important expectations (Montanholi et al., 2008; Scolari et al., 2009). However, there is no technical template, which complicates the reliability of the result. There aren't many studies on using goat skin or vulva temperature testing as an indicator of estrus or ovulation. (Façanha et al., 2018).

Our work aimed to evaluate the possibility of using thermal imagers for thermographic diagnostics to determine the optimal time of goats' insemination.

Materials and methods

We studied the optimal time of goat female insemination at the Department of Surgery and Obstetrics of Farm Animals of the Dnipro State Agrarian Economic University, the Department of Veterinary Surgery and Reproductology, and the Training and Production Center of the State Biotechnology University.

Methods of thermographic diagnostics include the determination of the temperature gradient (thermoscopy) and qualitative assessment of the colour palette (thermography). A thermoscopy of the goat female external genitalia was performed using a thermal imager TI-120. Analysis of thermograms was performed using standard IR Analysis Software.

During the study, the surrounding temperature was 19 ± 1 °C at a relative humidity of 61%.

The temperature gradient and the colour palette of the external genitalia were determined preventively. Then, detailed studies were performed following the diagnostic instructions about the optimal time of insemination.

Results

Thermography is a method of functional diagnostics based on the registration of infrared radiation from the body and body tissues in proportion to its temperature. The method can read and diagnose remotely without contact with the animal, which is its main function. The essence of the method is to visualize the colour palette, determine the local temperature gradient and assess colour indicators for computer monitoring (Faust et al., 2014; Jasti et al., 2019).

Thermal vision is a method of recording infrared radiation of a body whose temperature is above absolute zero. It uses special high-sensitivity devices – thermal imagers (Lahiri et al., 2012; Morozov et al., 2018).

It is necessary to consider the movement, artefacts, core temperature and energy of external heat sources for the reliability of results.

To reduce the effect of energy from external heat sources, thermography should be carried out under a shelter protected from sunlight. It is best to perform thermography in low light. The surrounding temperature should be within 10–20 °C, but any constant temperature is acceptable. The place where the thermography is performed must be with a continual flow of air so that there is no false decrease in temperature. The animal should be kept away from drafts for practical reasons.

The minimum size of a body whose temperature can be measured depends on the distance between the thermal imager and the object. The highest resolution of the thermogram equals 0.5 mm. It may be achieved if the device is placed 30 cm away from the studied entity. Yet, sufficiently reliable results can be obtained at a longer distance – 1–2 m or more.

Thermographic images were unified and analysed (maximum, minimum and average values), as well as their thermo- and histograms taking into account temperature, humidity, and distance to the object (Koshevoj et al., 2013).

The results of the investigations established the dependence of the temperature gradient of the external genitalia of goats on their physiological state (Fig. 1 and 2).

In the stage of estrus, the temperature of the external genitalia is 33.17 ± 0.18 °C, while in diestrus – 31.46 ± 0.40 °C (Fig. 1). In the stage of sexual arousal, the external genitalia are characterized by large «hot» (yellow and red) colours of the palette in contrast to the «cold» (green and blue) inherent in the equilibrium stage (Fig. 2). In the arousal stage, the temperature of the external genitalia increased by 1.70 °C (5.1%) compared to the equilibrium stage.

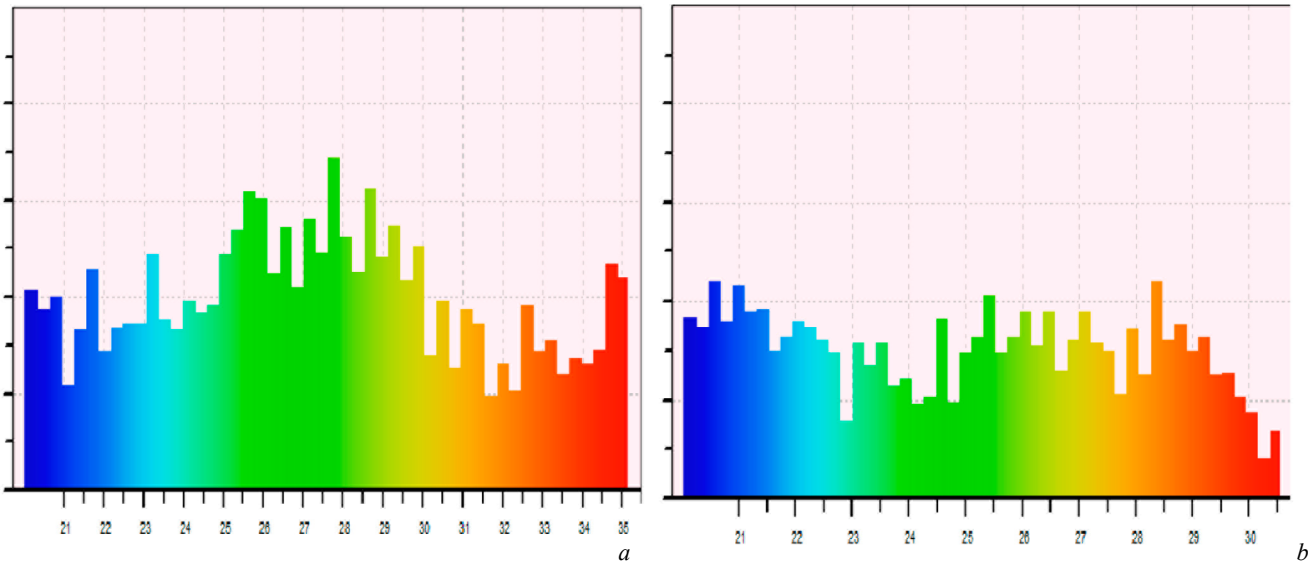

Fig. 1. Thermoscopy of the external genitalia of goats: *a* – stage of arousal of the sexual cycle (estrus); *b* – equilibrium stage (diestrus)

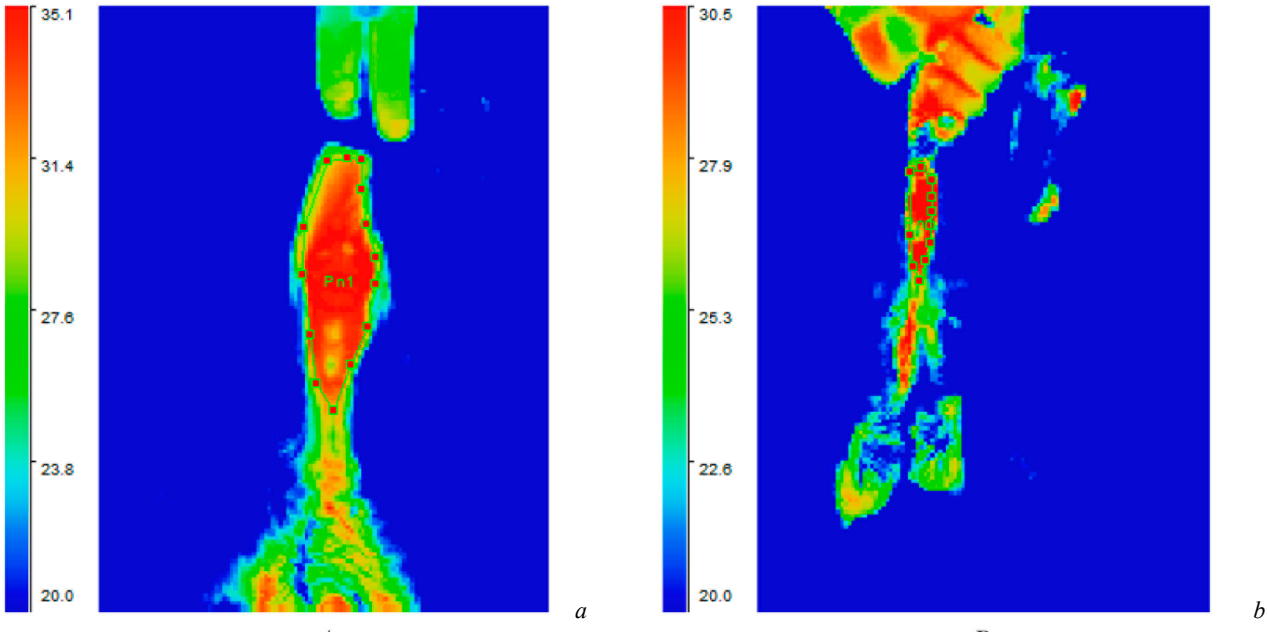

Fig. 2. Thermogram of the external genitalia of goats: *a* – stage of arousal of the sexual cycle (estrus); *b* – equilibrium stage (diestrus)

Discussion

The most favourable conditions for fertilization are the interrelation of insemination and ovulation over time and the necessary changes in the germ cells (sperm capacitation and detachment of the radiant crown of the ovum). The process of capacitation is a complex of biochemical and structural changes in sperm that occur in the female genital tract. This activates the enzyme systems in the sperm acrosome with a release of hyaluronidase, which separates the cumulus cells of the ovule and allows sperm to reach the transparent shell. After reaching the shell, a cortical reaction occurs. It facilitates the penetration of sperm through the transparent shell. The final preparation of ovules for fertilization is its release from cumulus cells (which is facilitated by sperm), as well as the activation of the ovule.

The sperm from the cervix is accumulated in the fallopian tubes 8 hours after insemination in sufficient quantities and completes its capacity. The release of the ovule increases the activity of sperm. When the ovule passes through the fallopian tube there is some detachment of cumulus cells, which facilitates the penetration of sperm. The most favourable period for fertilization is 8–10 hours after the beginning of the heat and during the next 12–15 hours.

The ability to fertilize ovules remains for less than 12 hours, and therefore insemination is often effective even after the end of the 6-hour heat (i.e., 4–6 hours before ovulation) (Kemp & Soede, 1997; Jablons'kyj & Homyn, 2006; Medvedev et al., 2019).

Each of the currently proposed methods for determining the optimal timing of insemination of females has its advantages and disadvantages (Foote, 1975; Bugrov et al., 2005; Tsuma et al., 2015; Ljubec'kyj et al., 2020; Palomares, 2021; Thomas et al., 2021).

The visual method is watching the behaviour of the animals. Females in heat show anxiety, make sounds, shuffle from foot to foot, look around, wag their tails, have reduced appetite, and decreased productivity. They become more mobile, there is a search reaction focused on the male with a characteristic feature – a manifestation of the embracing reflex.

The visual method is the simplest and most accessible, common in practice, but not the most accurate (Heres et al., 2000; Alhamada et al., 2016; Mičiaková et al., 2018; Reith & Hoy, 2018). To clarify, the external genitalia, vagina, and cervix are examined using a visual vaginal method. At the same time, there are signs of swelling and redness of the mucous membrane of these organs, relaxation of the cervical canal, and leakage of mucus that accumulates at the bottom of the vagina, and then flows by gravity from the genital slit (Foote, 1975).

Twenty eight non-pregnant West African dwarf goat females under 24 months old were used to study the signs of heat. During estrus, most of them (94.7%) wagged their tails. Swelling of the vulva was observed in 68.4% of females. The frequency of urination was higher in the presence of males. Cervicovaginal mucus was much more abundant and elastic. The duration of heat was 1.91 ± 0.07 days and increased with the age of females. Signs of heat were less noticeable in younger females in comparison with the older ones (Kenfack et al., 2013).

The most problematic aspect of this method is that 20% of females' sexual cycles are anovulatory, and another 10% of ovulation occurs without visible signs of heat (Fricke et al., 2014).

New technologies for solving the estrus misdiagnosis problem need to be more effective than the visual inspection and tools that are currently used for estrus detection. Ideally, technologies that provide solutions to detection problems should provide the following: continuous (24 hours a day) monitoring of the female, accurate and automatic identification of estrus animals, and work during the female productive life. The working requirements should be minimized and a high degree of accuracy in identifying the appropriate physiological or behavioural conditions that strongly correlate with ovulation should be ensured. New approaches are focused on automating the detection of estrus using electronic technologies. These are pedometry, implantable impedance sensors, as well as pressure sensors, which are at different stages of development and use currently. (Stevenson & Britt, 1977; Senger, 1994; Chanvallon et al., 2014).

An additional tool for detecting heat in female dairy goats is activity monitoring by the use of a pedometer. So, a significant increase in estrus-related activity was observed during the day, night and 24-hour period. The largest increase was observed in the dark (Doherty et al., 1987).

The reflexological method, which is a probe with a tied apron, is used for this purpose. The male can show all his sexual behaviour such as vocalization, foreleg striking, mount, and close contact except copulation (Tsuma et al., 2015; Romano et al., 2016).

Vaginal cytology and vulvar biometrics can be used as a predictor of heat in goats (Leigh et al., 2010). Vaginal cytology is a simple technique that can be used to characterize the stages of the reproductive cycle, and diseases of the genital tract and is a useful tool for determining the optimal time of insemination. Vaginal smears were used to study variations in the vaginal mucosa at different phases of the heat cycle in dogs, sheep, goats, pigs, cows, and so on. Vaginal epithelial cells are classified into three cell types. It depends on the differences in their sizes: surface flat cells (diameter 40–65 μm) with light cytoplasm, intermediate squamous cells (diameter 20–40 μm) and parabasal cells (diameter 12–15 μm). They can be classified into four types: parabasal, intermediate, surface intermediate and surface cells. The relative proportion of different types of vaginal epithelial cells can be used as a marker of the endocrine medium because epithelial cells accumulate a large amount of glycogen and undergo rapid cell proliferation in the basal and parabasal layers under the influence of estrogen. They form more surface flat cells (Sharma, & Sharma, 2016).

The study of vaginal cell detachment during the oestrous cycle in West African dwarf goats showed that squamous epithelial cells were classified into parabasal, intermediate, and superficial. Their relative location was used to determine the reproductive status of cyclic and undescribed cells. The average number of epithelial cells and leukocytes was also compared between days of the cycle. Leukocytes and epithelial cells were present in vaginal smears throughout the cycle. The number of cells increased sharply at 1 and 2 days after oestrus. Starting on day 4 of the cycle, the rates dropped to levels during the oestrus, and the daily fluctuations after that were not impressive enough to distinguish between different stages of the cycle. Most smears were dominated by intermediate cells. It is a typical pattern of cell detachment in the anestrus and puberty periods. However, surface cells were more common in cycling smears, and were probably associated with the proestrus, estrus, and early metestrus phases of the sexual cycle. These results indicate that the model of vaginal cell detachment can be used to determine the reproductive status of goats (Ola et al., 2006).

The increase in somatic cell count in goat's milk during the heat is associated with high plasma estradiol and low progesterone levels and suggests that their increase may be due to estrogen-induced proliferation and epithelial cell desquamation (McDougall & Voermans, 2002; Moroni et al., 2007).

An electrometric method can be used to determine the optimal time of insemination in goats. It consists in measuring the electrical resistance of the mucous membrane of the dorsum and vagina with special devices. The time of maximum secretion of mucus is accompanied by a decrease in the electrical resistance of the mucous membrane of the dorsum and vagina and often coincides with the optimal time of insemination (Leidl & Stolla, 1976).

Behavioural signs of heat are caused by hormonal changes. Endocrine hormones regulate ovarian functions: follicle development, ovulation, luteinization, and luteolysis. They are secreted by the hypothalamus (gonadotropin-releasing hormone), anterior pituitary gland (follicle-stimulating and luteinizing), ovaries (progesterone, estradiol, and inhibin) and uterus (prostaglandin F2 α–PGF2 α) (Aungier et al., 2015). Elevated concentrations of estradiol are secreted by the preovulatory follicle and, in turn, promote a surge in gonadotropin-releasing hormone. When progesterone levels are low, they allow the expression of behavioural estrus and the release of luteinizing hormone to induce ovulation. (Martínez-Álvarez et al., 2007).

Determining progesterone, estradiol, and prolactin is also practised in determining the optimal time of insemination (Foote, 1975; Roelofs et al., 2006; Sergeev & Malova, 2012; Malahova et al., 2015). Ovulation diagnostics using transrectal ultrasonography is promising for the practice of determining the optimal time of ovulation (Romano et al., 2016).

Today, several fully automated technologies are available including pressure-sensing systems, activity meters, video cameras, and vocalization recordings. These systems differ in many aspects regarding their sustainability and effectiveness as keys to their choise for practice. High priority is given to monitoring activity using sensors, especially accelerometer systems. There is a growing interest in exploring the potential of combining activity monitoring data and information from several other methods. It may lead to the best results regarding the sensitivity and specificity of detection. Future improvements are likely to require multivariate detection using data and systems that already exist. (Aungier et al., 2015; Banuvalli et al., 2015; Reith & Hoy, 2018).

One of the promising methods for determining the optimal time of goats' insemination is thermography. Perivulvar signals of oedema and redness are observed in the vulva in goats during the

heat. At the same time, an increase in the temperature of this area is identified using thermographic images. There was a significant effect on the temperatures of all areas evaluated by thermographic images and infrared thermometer, except for the temperature of the vulva. Temperatures measured with a thermographic camera showed a difference in the data set. They allowed us to observe higher temperatures compared to temperatures measured with an infrared thermometer. So, the thermographic images used to detect leaks are applicable and important due to the accuracy and speed of the method (Façanha et al., 2018).

Conclusions

The choice of the time of female insemination is one of the most important factors determining the success of fertilization. Today's requirements make it necessary to improve the existing and develop new methods of determining the optimal time of insemination of goats. A promising trend is thermal imaging diagnostics for determining the temperature gradient and the colour palette data of the female external genitalia infrared radiation.

It was found that in the stage of arousal of the sexual cycle, intensive "hot" colours of the palette characterize the external genitalia, while the stages of balance are dominated by «cold» (green and blue). The results of studies reliably confirm the regularity of the temperature increase of the external genitalia in goats during the stage of sexual arousal compared to the stage of equilibrium by 1.70 °C (5.1%) – 33.17 ± 0.18 °C, and 31.46 ± 0.40 °C accordingly.

The use of thermography by making thermal imagers provides preventive diagnosis of the optimal time of insemination. It can be used for females of different species and, in general, have priority in animal reproduction. Such qualities as exceptional safety, autonomy and contactlessness make thermal imagers irreplaceable in veterinary medicine.

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