# INFRARED THERMOGRAPHY IN REPRODUCTION

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***ABSTRACT***

*Thermography has been applied many times for studies carried out on "human" and "animal" reproduction. Numerous of these had as objective the identification of some specific pathological conditions through the use of a thermographic monitoring protocol.*

*Thermographic approach to the reproductive pathological condition influencing the body or local cutaneous areas can be routinely applied in veterinary medicine only after a specie-specific definition of the emission index of each cutaneous area interesting for diagnostic procedures. Some scientific works reported the possibility to identify cows during the estrous period or the pregnancy status. During the last decade the Authors had been performing different experiments to set up these specie-specific characteristics. At the start time, the objective was to create a strong relation among different imaging diagnostic tools usually utilized in veterinary medicine considering the also the thermographic approach. Among the studies carried out by our group, some reported specific thermographic monitoring sessions for the optimization of hormonal treatments during ovarian synchronization protocols in the mare and the ewe. In general, cutaneous temperature of vulvar and peri-vulvar areas may be useful for increase the awareness about the ovulation time. In these areas, the thermal radiation is related to the cutaneous or sub-cutaneous blood flux variations. During the estrous time an increase of blood efflux is usually depending by the level of secreted estrogens by the growing ovarian follicles. Because of its no-invasivity and the high sensitivity in measure temperature differences across the skin surface it appears clear that thermography could be applied to the monitoring of scrotal surface temperature. An extensive literature about thermography application in diagnosis, and follow-up monitoring in case of varicocele is available today. As a consequence of the use in human andrology, there was an increasing interest of animal andrologist on infrared thermography and the first studies had came out in the eighties. They showed like thermography could be used to assess scrotal/testicular thermoregulation in bull by providing an image on the base of the infrared emissions with an accuracy of 0.10°C.*

***Key words***

*Thermography, reproduction, veterinary medicine, cutaneous or sub-cutaneous blood flux variations, thermoregulation.*

Thermography has been applied many times for studies carried out on human and animal reproduction. Many of these studies had, as the main objective, the identification of some specific pathological conditions through the use of a thermographic monitoring protocol. Some studies, published for human medicine, described the use of this method for the early diagnosis of cases of uterine cervix lesions in women (Smaga et al. 2003). It has also been reported that thermal cameras have been used to increase research and application on reproduction in carnivores (Durrant et al., 2006). Unfortunately, at the present time, nobody uses this technique as principal diagnostic tool for reproductive diseases, where it is usually considered for supplementary or ancillary examination to increase the diagnostic precision. The main con-

straint to the common use of the thermographic monitoring for diagnostic purposes is the absence of specific protocols for its application. A number of image analysis software packages are available and can be modified depending on the needs of the operator. The operator has to understand fully how to operate the thermal camera and what type of measurements have to be considered in each environmental condition. Unfortunately, the potential measurement errors related to the individual animal variations block the set up of species-specific software. Individual variations are usually linked to the presence of some factor influencing the infrared emission from a cutaneous area (species-specific factors like hair length, cutaneous vascular bed amplitude and thickness) while the environmental factors can be considered by the thermal camera settings protocol. Continuing research activities on the definitive interspecies differences in terms of thermal characteristics (cutaneous thermal exchange equation) may help in resolving these problems in practical and diagnostic applications.

A thermographic approach to a reproductive pathological condition influencing the body or local cutaneous areas can be routinely applied in veterinary medicine only after a species-specific definition of the emission index of each cutaneous area of interest for diagnostic procedures. Some scientific research has reported the possibility of identifying cows during the oestrous period or their pregnancy status.

Hurnik et al. (1985) studied the relationship between differences in body surface temperatures and oestrus in Holstein-Friesian dairy cows, and the possibility of using this technique to determine the onset of oestrous. Because inaccuracies were encountered in determining the oestrous cycle during the experiment, the authors did not recommend thermographic for routine detection of oestrous, but it is nevertheless completely adequate in skin temperature studies, or, more precisely, in the studies of body surface temperature changes. Hellebrand et al (2003) concluded that the stage of pregnancy of heifers in their usual environment (pasture or barn) cannot be determined by simple monitoring with a thermal imager, but they did find that the external temperature of the genitalia follows the core body temperature, and thus thermography can be utilized for oestrous climax determination.

During the last decade the authors have been performing different experiments to set up these species-specific characteristics. Initially the objective was to create a strong relation between different imaging diagnostic tools often used in veterinary medicine considering also the thermographic approach. Among the studies carried out by our group, some reported specific thermographic monitoring sessions for the optimization of hormonal treatments during ovarian synchronization protocols in the mare and the ewe. In general, cutaneous temperature of vulvar and peri-vulvar areas may be useful for increasing the awareness of the ovulation time. In these areas, thermal radiation is related to the cutaneous or sub-cutaneous blood flux variations. During oestrous an increase of blood efflux is usually dependent upon the level of oestrogens secreted by the growing ovarian follicles.

In a recent study performed by our group (unpublished data) a monitoring protocol was designed (i) to determine the effect of two GnRH analogues (Ovsynch protocols) administered at two different times of a day for oestrous synchronization, and (ii) to study the relation of vaginal electrical impedance and vulvar and perivulvar temperature recorded with infrared thermography with pregnancy rates in Italian Mediterranean buffaloes inseminated with sexed frozen semen. Thirty-two healthy Mediterranean buffaloes with variable post-partum period (16 to 174 day) were used. Before starting the synchronization protocol, a trans-rectal ultrasound examination had been carried out to identify the follicular status in the ovaries. Follicles ≥3mm in diameter and the corpus luteum(CL) were measured and recorded. The Ovsynch protocol (Pursley et al., 1995; De Rensis & Lopez Gatius, 2007) was started regardless of the stage of the oestrus cycle in animals. All the female buffaloes were inseminated with sexed frozen semen (X-chromosome bearing spermatozoa), from a bull of known high fertility, 18-21 hours from administration of the second dose of GnRH (Figure 1.). There was a well de-

fined temperature drop between the 2nd administration of GnRH and the time of insemination only in buffaloes pregnant at 42 days after artificial insemination (AI). This type of thermal profile during an Ovsynch protocol may be easily explained. The 2nd administration of GnRH represents a hormonal input that simulates the natural pre-ovulatory surge and, 18-21 hours after, only the conceiving females were at the perfect time for AI. The decrease in vulvar temperature during that period may be related to a natural decrease of the circulating oestrogens in the immediately preovulatory period creating a good environment for the sperm oviductal reservoir and the subsequent fertilization.

Essential pre-requisites for successful artificial insemination are the accurate detection of oestrous and ovulation, which is classically performed using a "teaser", trans-rectal palpation and ultrasonographic examination of reproductive tract. An alternative method could be the measurement of electrical impedance of vaginal mucus and perivulvar and vulvar temperature. Reports on using infrared thermography in detection of oestrous in the mare are limited, so the aim of this study was to assess perivulvar and vulvar temperature using infrared thermography as a non-invasive method for the monitoring of the oestrous cycle in the mare. Nine trotter mares were used, five with foal and four without foal. The thermographic monitoring was considering a three stages protocol: T1 (follicle with 0 > 3cm diameter), T2 (follicular growth), T3 (ovulation). At each scanning, the thermography was performed first on perivulvar and vulvar regions by Thermacam P25, followed by trans-rectal palpation and ultrasonographic examination of the reproductive tract using an 8Mhz ultrasound probe. Finally, the measurement of electrical impedance was performed using an endo-vaginal Draminisky probe. Ten blood samples were collected from five mares to measure serum progesterone and oestrogen concentration in stages T2 (5) and T3 (5).

The analysis showed a positive correlation within the thermographic parameters, which were Perivulvar Maximum Temperature (PMaxT), Perivulvar Mean Temperature (PMeanT), Vulvar Maximum Temperature (VMaxT), Vulvar Mean Temperature (VMeanT). There was a simultaneous increase of these parameters (Table 1.). These parameters that increased were positively correlated with the Diameter of the Greater Follicle (DGF) and the Echotexture of the Follicular Wall (EFW), and negatively correlated with the presence of corpora lutea (CL). These data suggest an increase of maximum and mean perivulvular and vulvar temperature during follicular growth and a decrease in temperature during the establishment of CL. These changes may be because the mare, under the influence of oestrogen, has an increase of hyperemia of the vulvar region. A negative correlation was also found between PMT and the values of Electrical Impedance (EI). The increase of PMT is associated with lower values of EI, which occur during the follicular growth. Moreover, the PMT was positively correlated with serum oestrogen concentration and negatively correlated with serum progesterone concentration which occurs during the follicular growth and ovulation times respectively. The authors (Calabria et al. 2010) concluded that their preliminary results are encouraging the possible use of thermography as an auxiliary noninvasive method during oestrous cycle monitoring in mares.

In other work (Stelletta et al., 2006) reported the use of thermographic monitoring in ewes during a classical hormonal treatment to synchronize the ovulation. The aim of that study was to detect skin temperature differences of the perivulvar area between ewes in oestral and anoestral phases. Twenty-four dairy ewes, 16 in oestral phase and 8 in anoestral phase, were investigated. Oestrus was synchronized using intravaginal progestagen-impregnated sponges (fluorogestone acetate, FGA) for 14 days and after the sponges were removed, ewes were treated with PMSG (pregnant mares serum gonadotrophin). Thermography sessions were carried out 50 hours after sponges were removed, from a distance of 1 m from the vulvar area. A skin emissivity of 0.95 was assumed. The control subjects with no synchronization treatment were in a seasonal anoestral period. The analyses performed on the resultant thermo-

grams were (i) a qualitative and quantitative analysis taking into account the mean perivulvar area temperature, (ii) a quantitive analysis of the temperature differences between unfleeced adjoining areas, and (iii) a quantitative frequency analysis of temperature using an interval of 0.2°C. A significant difference between the two groups was observed in values expressed as mean temperature of perivulvar area ($P < 0.05$). The subjects in oestrous and in anoestrus ranged in temperature from 35.9°C to 37.7°C with an average of 36.9 ± 0.5°C and 34.2°C to 36.5°C with an average of 35.42 ± 0.63°C respectively. The superficial temperatures detected in unfleeced areas of posterior anatomical regions may be taken into account in the study of circulatory and/or hormonal variations in ewes. The mammary skin receives only 2% of the regional blood flow and so this area may be used as a control for other adjoining areas because the emitted heat increase is proportional to the parenchymal blood flow. The animals in oestrous show an increase in blood flow to the genitals and hormonal changes. These variations are able to explain the increase of heat emitted by the unfleeced skin of posterior areas. Thermography is a technique useful to point out the different nature of heat transmission from underlying areas of the skin. The ewes with oestrous induced by synchronization have shown a different thermal behavior that can be detected by thermography sessions in adjoining areas receiving blood from common arteries (internal iliac –pudendal artery) (Figure 2.).

***Infrared thermography application in veterinary andrology***

The first applications of infrared thermography in human andrology date back to the 1970s. Scrotal thermography has been demonstrated, since that time, to be a useful diagnostic method, especially in varicocele (Comhaire et al., 1976; Comhaire, 1977) and testicular tumors (Lee and Gold, 1976). In particular, the relation between temperature elevation and seminal quality was known more than twenty years before (Hanley, 1956). Because it is non-invasive and has high sensitivity in measuring temperature differences across the skin surface, it appears clear that thermography could be applied to the monitoring of scrotal surface temperature (Kulis et al., 2012). Extensive literature is available about the application of thermography in the diagnosis and follow-up monitoring in cases of varicocele. Because of its use in human andrology, there has been an increasing interest from animal andrologists in using infrared thermography and the first studies were reported in the 1980s. Research showed that thermography could be used to assess scrotal/testicular thermoregulation in bull by providing an image based on the infrared emissions with an accuracy of 0.10°C (Coulter, 1988; Purohit et al., 1985). Researchers have shown that the surface temperature of the scrotum is highly correlated with deep testicular temperature (Coulter et al., 1988) and that infrared temperature thermograms provide accurate information about testicular thermoregulation (Coulter, 1988; Kastelic et al., 2001; Purohit et al 1985; Lunstra and Coulter, 1997).

In contrast with human colleagues, veterinarians are faced with more difficult conditions because of the “patients” and the working environment. Kastelic et al. (1996d) studied the effect of environmental factors affecting the measurement of bovine scrotal surface temperature with infrared thermography. Although it is established that many biological systems have diurnal rhythms, no significant changes were found on scrotal surface temperature, so scrotal thermography can be performed at any time of the day. Feeding had been shown to alter thermography. Within 30 minutes after the start of feeding an increase of scrotal temperature can be observed, with an effect lasting for several hours. Recumbency can have an effect depending on ambient temperature and floor material. In conditions of high environmental temperatures, cool floors, such as rubber mats and metal gratings, may absorb heat from the scrotum, resulting in scrotal warming after rising. Ambient temperature has a great effect on the surface temperature of the lower surface of the scrotum, a small effect on the top of the scrotum and an intermediate effect on the average scrotal temperature. In particular, a rapid change in am-

bient temperature leads to an apparent overcompensation response 3 hours later, followed by a slow return to normal temperature after 23 hours. Moisture on the scrotum decreases scrotal temperature and at least 30 minutes following drying is required for the temperature to return to normal. Once the limitations are fully understood the thermography has been demonstrated to be a sufficiently robust technique (Kastelic et al., 1996d).

In normal conditions, scrotal surface temperature of a bull is 5-6°C lower than abdominal temperature (Arteaga et al., 2005; Brito et al., 2004; Brito et al., 2003; Kastelic et al., 1996a; Kastelic et al., 1997) and has a positive top-to-bottom gradient, with the top warmer than the bottom (Figure 3)(Kastelic et al., 1996a). This gradient is due to the scrotum being vascularized from the top to the bottom. Conversely, the testicular artery, once it reaches the ventral pole of the testis, diverges into several smaller arteries before entering the testicular parenchyma (Setchell, 1970). There should thus be a negative temperature gradient but the opposing temperature gradient of the scrotum complements that within the testis, resulting in a relatively uniform intratesticular temperature (Kastelic et al., 1995; Kastelic et al., 1996a). Scrotal temperature of the area overlying the cauda epididymis increased following ejaculation and electroejaculation due to the contraction of the cauda epididymis during ejaculation (Kastelic et al., 1996b). Most reported studies have been conducted in bulls, working in two main areas: the study of thermoregulation and its relation to testicular activity and the use of thermography in monitoring scrotal surface temperature to predict fertility.To record a good scrotal thermogram it is necessary to hold the infrared scanner approximately 1 m behind the bull and to orient the scanner perpendicularly to the paired testes in the scrotum (Brito et al., 2012; Lunstra and Coulter, 1997).

***Environmental factors affecting bull thermoregulation***

Techniques for studying the effects of heat on the testis include a whole body heating or a local heating of the testis. The first technique consists of exposing the whole animal to a hot environment (Setchell, 1998), however there are two complicating factors with this approach. Firstly, the body reacts to heat stress in a variety of ways that involves physiological, metabolic and endocrinological changes that can affect indirectly the testis. Secondly, the sweat glands of the scrotum are not controlled independently of those of the general body surface (Robertshaw and Vercoe, 1980), so the ability to produce sweat can be influenced by the prior heat exposure. Kastelic et al. (1997) used this technique to study testicular thermoregulation in two different ambient temperatures. Scrotal surface temperature was shown to be influenced by external temperature with an increasing of about 2.5°C when passing from 15°C to 25°C either in the top, bottom or average temperature. The temperature gradient was not affected. The same experiment was than performed after castration resulting in the evidence that the testis has a limited influence on scrotal surface temperature (Kastelic et al., 1997).

Local heating of the testes has usually been achieved in one of three ways, induced cryptorchidism, scrotal insulation or short-term heating, usually by immersion in a water bath (Setchell, 1998). Of these techniques, the most useful in thermography studies is scrotal neck insulation (Brito et al., 2003; Kastelic et al., 1996c). Kastelic et al. (1996c) used an infrared thermal camera to assess the insulation effect on scrotal surface temperature. Images were taken prior to insulation and then at 24 and 48 hours after insulation. At the same time they monitored scrotal subcutaneous temperature, intratesticular temperature and semen quality. Insulation resulted in a decreased scrotal surface temperature of the top of the scrotum after 24 hours, similar to the neck, and an increased temperature of the bottom scrotal area. After 48 hours, temperature returned to the same level as pre-insulating, suggesting some compensatory mechanism. Despite the return to a normal scrotal temperature an increased intratesticular temperature was observed at 48 hours, presumably because heat radiation from the

testicular vascular cone and possibly countercurrent heat exchange were impaired (Kastelic et al., 1996c). Insulation was also correlated with a decreased of seminal parameters, particularly an increase in abnormal sperm (Barth and Bowman, 1994; Brito et al., 2003; Fernandes et al., 2008; Kastelic et al., 1996c). Insulation of the neck of the scrotum may be also a suitable model for simulating over conditioned bulls with a large amount of fat in the scrotal neck (Coulter and Kastelic, 1994). Dietary energy is another factor that affects scrotal thermoregulation and seminal quality (Coulter et al., 1987). Thermography was used to assess the dietary energy's effect on male reproduction (Coulter et al., 1997). After a period of 168 days after weaning, scrotal surface temperature was measured in different lines of beef bulls fed with moderate or high-energy diets. The authors did not find a significant effect of diet on the top, bottom or average scrotal surface temperature, but there was an effect on the gradient, with the bulls on the high-energy diet having a smaller gradient than those on the moderate-energy diet (3.4 vs 3.9°C) (Coulter et al., 1997).

### *Bull Breeding Soundness Evaluation*

Starting from the middle of 1990s, infrared thermography had been proposed as a support tool in the evaluation of breeding soundness in bulls. In some studies scrotal surface temperature was evaluated in basal conditions (Kastelic et al., 2001; Lunstra and Coulter, 1997) and in others its use was related to the testicular response to an exogenous GnRH administration (Gabor et al., 1998a; Gabor et al., 1998b; Vencato et al., 2012). In the first case, thermograms were taken prior to semen collection and then data were correlated with other factors including seminal parameters, testicular echotexture, testicular tone and scrotal circumference. Lunstra and Coulter (1997) described three possible classes on the base of scrotal thermogram. Numerous horizontal bands, with each band representing a narrow temperature range and reflecting progressively cooler temperatures as distance away from the body increased, characterize normal pattern. Scrotal thermograms that displayed some non-uniformity of band width or some asymmetry of bands can be classified as questionable. Scrotal thermograms that displayed very few bands, more marked disruption of band uniformity, or pronounced band asymmetry (hot spots) classified as abnormal (Lunstra and Coulter, 1997). Bulls exhibiting abnormal scrotal temperature patterns had a lower percentage of sperm with normal head morphology, tail morphology, and acrosome morphology, and had a higher percentage of sperm with proximal droplets when compared with bulls with normal or questionable thermograms patterns (Lunstra and Coulter, 1997). The authors concluded that bulls with abnormal scrotal temperature patterns achieved significantly lower pregnancy rates when use for natural mating, and that infrared thermography can be used to predict reduced fertility (Lunstra and Coulter, 1997).

The purpose of using thermography during a GnRH stimulation test is to determine the change in scrotal surface temperature and to relate this change to male fertility (Gabor et al., 1998a; Gabor et al., 1998b; Vencato et al., 2012). In those studies a thermogram was taken immediately before the GnRH administration and after 45 minutes (Gabor et al., 1998a; Gabor et al., 1998b) or every 15 minutes until 1 hour after GnRH administration (Vencato et al., 2012) (Figure 4). Gabor et al. (Which reference year), working with sexual mature bulls, found that 45 minutes after GnRH administration there was an increase in scrotal surface temperature. They also described an increasing in LH and Testosterone concentration after GnRH administration. There were also significant correlations between the number of spermatozoa and the percentage of live spermatozoa and other end points including thermographic measures. The authors concluded that infrared thermography is useful for predicting the number and percentage of live spermatozoa (in association with testicular size and echotexture) (Gabor et al., 1998a). Vencato et al. (2012) performed GnRH testing in young bulls with poor semen

production. In their study bulls could be divided in two groups in relation to the variations of scrotal surface temperature at 60 minutes after GnRH administration. A group that had a significant decrease of scrotal temperature and a group that had a significant increase in temperature compared to scrotal surface temperature before GnRH administration. Although in both groups there was an increase in serum testosterone level, the amount of the increment was significantly higher in the group with a decreased scrotal temperature after GnRH administration. In the weeks after GnRH administration there was an increase in quality parameters of semen, and again the increment was higher in the group with the decreased temperature after GnRH administration (Graphic 1). Analysis of Pearson correlation indexes revealed a significant negative correlation between testosteronemia and scrotal surface temperature variation pre- to post-GnRH administration (Vencato et al., 2012).

***Other species***

There are few reported studies from other species.

Thermography was recently used (Ramires-Neto et al., 2012) to study, in stallions, the efficiency in testicular thermoregulation. Stallions of different ages were conditioned in an environment with an ambient temperature of 30-32°C. The results of the research demonstrate that normal stallions of different ages can maintain a constant testicular temperature even under heat stress conditions, since no difference in scrotal surface temperature was detected. The authors concluded that thermography has potential for use as a complementary examination technique in andrological evaluations.

Thermography was used to monitor scrotal surface temperature changes in response to GnRH administration in male alpacas (Stelletta et al., 2009). The authors performed GnRH on male animals in different conditions: after female isolation (T1), after female exposure (T2) and after female exposure with mounting (T3). The measured scrotal temperatures are reported (Graph 2) and shown that in all groups a decreased temperature was observed after GnRH administration. Moreover, a significant negative correlation was found between scrotal temperature variation and testosterone variation after GnRH administration suggesting that thermography can be use to assess the effect of GnRH administration (Stelletta et al., 2012).

Thermography had also been used to evaluate the safety of testicular biopsies in llamas (Heath et al., 2002). Thermographic images were obtained prior to biopsy, immediately after the biopsy and then once a week for 6 weeks. The biopsy process had no effect on scrotal temperature measured with thermography, although 3 animals of 9 showed a transient alteration in the thermogram patterns 1 week after biopsy (Heath et al., 2002).

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**Table 1. Values of thermographic, ultrasonographic and electrical parameters during the periovulatory period in mare**

| | T1 | T2 | T3 |
|---|---|---|---|
| TMinP | 28.6±0.80 | 28.84±0.32 | 27.72±0.54 |
| TMaxP | 34.2±0.38 $^{ab}$ | 34.41±0.22 $^{a}$ | 33.43±0.13 $^{b}$ |
| TMP | 31,98±0.51 $^{a}$ | 31.54±0,15 $^{ab}$ | 30.91±0.21 $^{b}$ |
| TMinV | 27.95±0.22 | 27.17±0.61 | 26.85±0.28 |
| TMaxV | 33.72±0.46 $^{ab}$ | 34.12±0.18 $^{a}$ | 33.14±0.14 $^{b}$ |
| TMV | 31.40±0.30 $^{a}$ | 31.31±0.19 $^{a}$ | 30.31±0.20 $^{b}$ |
| TMDt | 0.58±0.23 | 0.23±0.16 | 0.60±0.19 |
| θFM | 4.31±0.28 $^{a}$ | 4.72±0.21 $^{a}$ | 1.10±0.13 $^{b}$ |
| EPF | 2.30±0.12 $^{a}$ | 2.45±0.11 $^{a}$ | 1.0±0.00 $^{b}$ |
| CF | 1.58±0.07 $^{a}$ | 1.79±0.04 $^{b}$ | 1.0±0.00 $^{c}$ |
| EU | 1.50±0.29 $^{a}$ | 1.39±0.14 $^{a}$ | 0.92±0.05 $^{b}$ |
| ER | 320.00±21.99 | 391.11±23.12 | 366.67±22.11 |
| P4 | 0.2±0.00 | 0.23±0.01 | 0.31±0.03 |

Different letters among groups indicate a significant difference (P<0,05) Perivulvar Minimum temperature (PMinT); Perivulvar Maximum Temperature (PMaxT); Perivulvar Mean Temperature (PMT); Vulvar Minimum Temperature (VMinT); Vulvar Maximum Temperature (VMaxT); Vulavr mean Temperature (VMT); Delta mean temperature Perivulvar-Vulvar (ΔPVMT); Diameter of Greater Follicle (DGF); Follicle concistency (CF) grade 1= firm, grade 2=soft, Echotexture Follicular Wall(EFW)grado 1= anecogenic, grade 2= medium ecogenicity, grade 3=ecogenic; Uterine edema (UE) grade 1=mild, 2=moderate, 3=heavy; Electrical impedance (EI); Progesterone (PG); T1(DGF>3cm); T2 (follicle growing); T3 (ovulation).

*Figure 1: Thermographic images of vulva and perivulvar regions taken during administration of the second dose of GnRH (a) and AI (b) in a Ovsynch protocol. The measuring scale indicates the color relating to temperature (°C).*

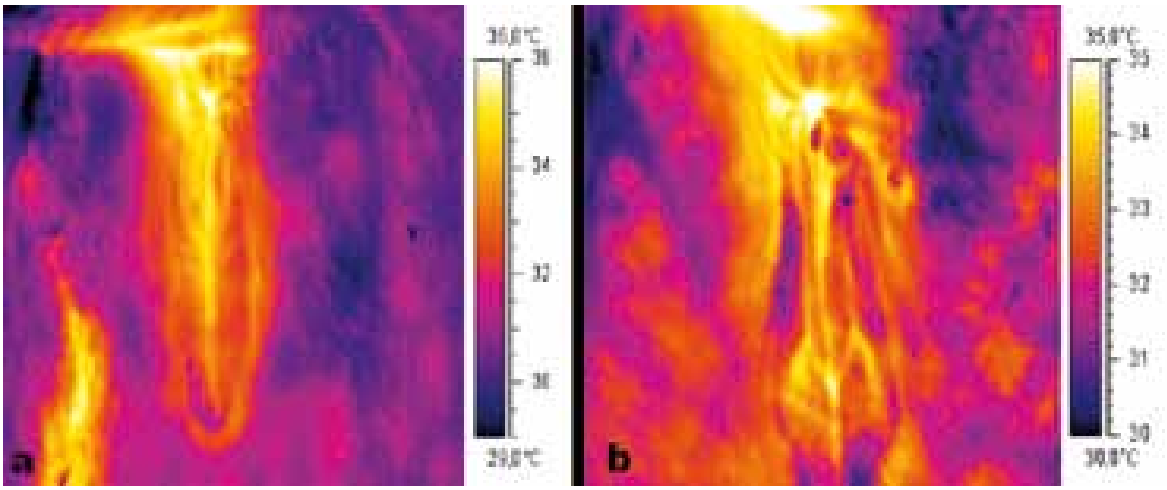



*Figure 2. Ewes vulvar thermograms during a synchronization protocol.*

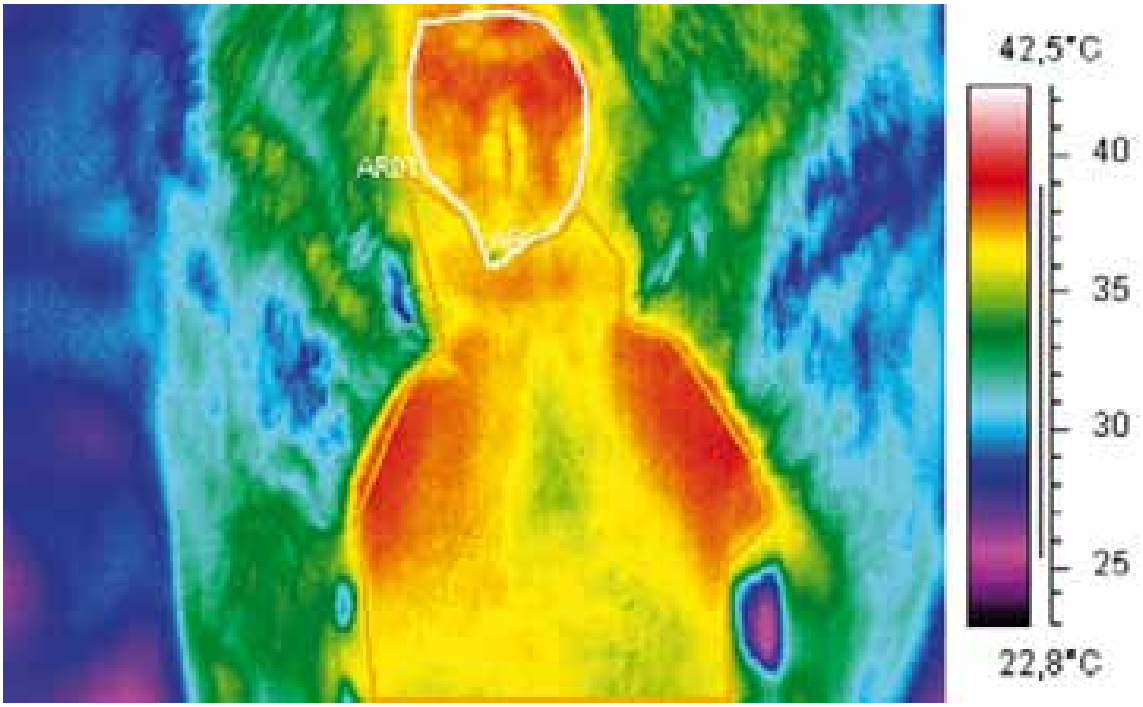


*Figure 3. Normal scrotal thermogram in bull.*

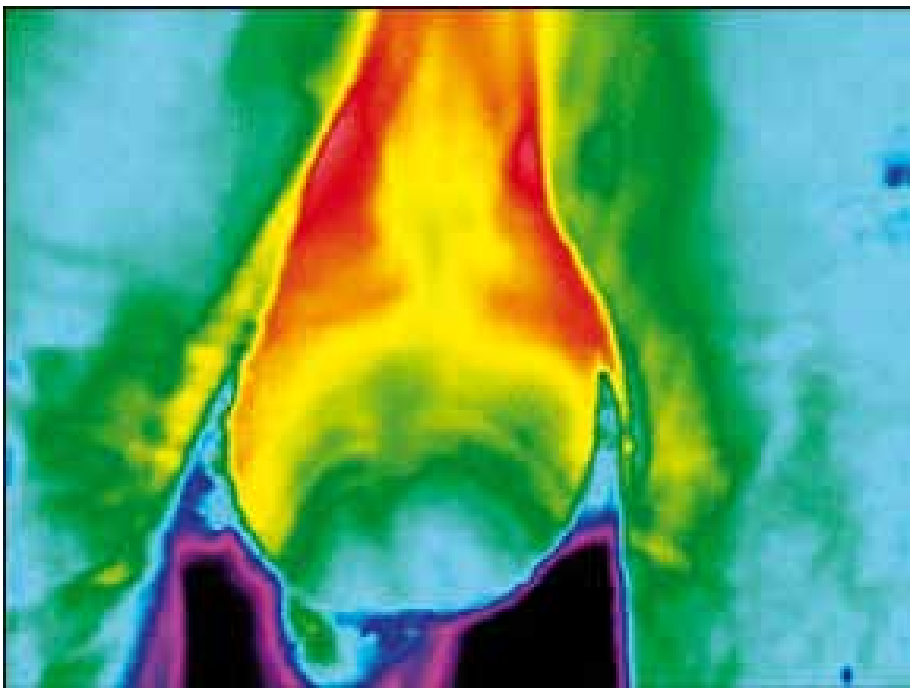
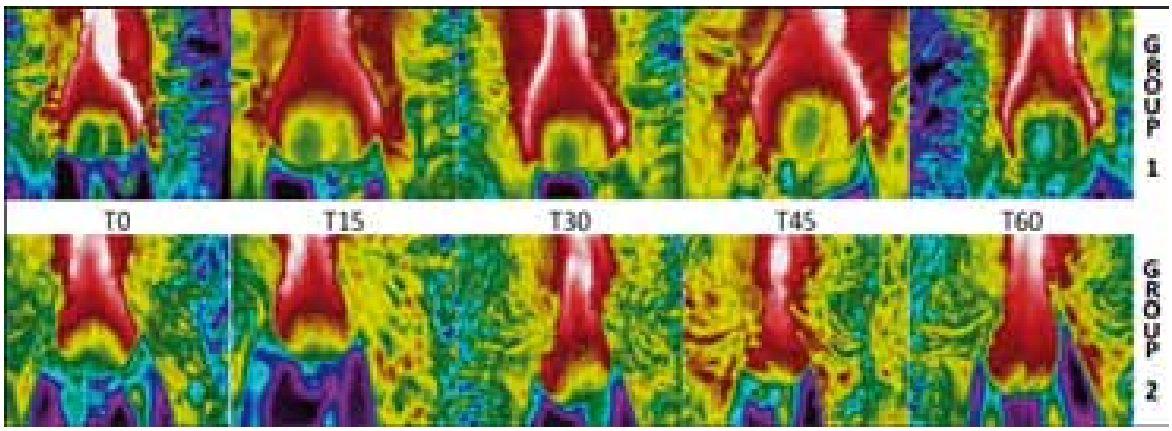
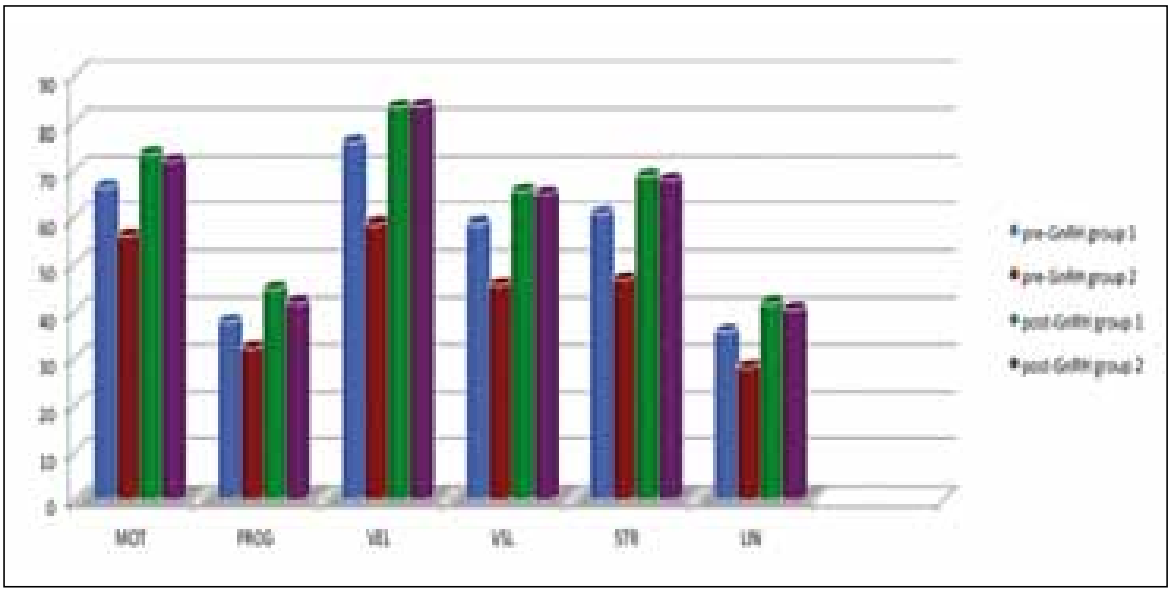


*Figure 4. Scrotal surface temperature monitoring after GnRH administration in bull (Vencato et al., 2012). Group 1=decreased temperature after 60; Group 2=increased temperature after 60 minutes.*



*Graphic 1: Sperm quality in groups with different thermal response to GnRH administration (Vencato et al., 2012) Group 1= decreased temperature after 60 minutes; Group 2= increased temperature after 60 minutes.*




*Graphic 2: Scrotal surface temperature in alpaca before and after GnRH administration (Stelletta et al., 2009).*