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Thermographic Applications in Veterinary Medicine

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1. Introduction

Thermography is a non-contact, non-invasive technique that detects surface heat emitted as infrared radiation. Because skin temperature reflects the status of tissue metabolism and blood circulation, abnormal thermal patterns can signify areas of superficial inflammation or circulatory impairments.

Veterinary infrared thermography is a term indicating *in vivo* digitally imaging an animal with an infrared camera using computer interpretation of thermal maps. Various trials were performed with different species (horse, pig and cows)[1,2,3,4,5] to assess the validity of thermographic instrument. Infrared thermographic systems are capable of seeing energy emitted by most objects at a temperature above -35°C. Therefore colour or visible light does not interfere with the possible images seen by thermographic system. The maximum heat emitter is considered a black body which have an emissivity of 1 because it adsorbs all radiated heat. The emission factor of skin is approximately 0.93-0.98 depending on coat quantity and length. Heat is the primary sign of inflammation process and different disease processes affect the microcirculation of the skin. Therefore variations of the skin temperature become interesting indicator of such conditions that can range from specific vascular alterations to referred conditions also physiologically. Since skin temperature may be used in order to estimate tissue integrity because it reflects the underlying circulation and tissue metabolism.

Infrared thermography (IRT) uses thermal radiation emitted by objects to visualize and measure their surface temperature. The temperature is detected over wide areas, at a distance and measuring time is fast. It does not require physical contact and, therefore, it is entirely non-invasive. The colours of the images represent different temperatures, highlighting hot and cold spots and showing the map of the thermal distribution of an object or a body surface. Thermal imaging cameras can produce very sharp images of the distribution of body surface temperatures to a precision of 0.08°C. These characteristics allow the IRT to be applied where the temperature of live animals or carcases is difficult to measure under housing conditions or in the situation occurring during commercial slaughter.

Since the 2001 it has been carried out numerous efforts to introduce the thermography in the veterinary clinical practice and below are reported some clinical applications and experimental approaches.

2. Veterinary clinical applications of the thermography

The thermographic applications in veterinary medicine are very numerous considering the difficulties that in some cases due to the characteristics of the patients. In the past time the major application was on the equine diagnostic procedures above all for the lameness. More recently different applications were on bovine medicine and particularly for the mastitis and the welfare evaluation. In animals, body surface temperature is a function of blood flow and metabolic rate of underlying tissues. Thus, the physiological state of underlying cells could potentially be assessed by measuring skin temperatureusing IRT[6]. Infrared thermography and potential veterinary applications for this imaging technique have been described[6,7,8]. These reports mostly described thermographic imaging of spontaneous disease and attempts to correlate images to disease or injury diagnosed by other means. A study in cattle revealed successful utilization of thermography in the detection of localized sepsis in the pinna after contaminated growth stimulant pellets had been administered[7]. Infrared thermography has been used to predict changes in udder temperature[9] and to detect inflammation associated with hot- iron and freeze branding in cattle[10] and bovine viral diarrhea infection in calves[11]. Soles of hooves affected by subclinical laminitis commonly appear soft and warm long before the appearance of yellowish discoloration, lesions, and ulcers[12]. Our experiences were based on different species (bovine, ovine, south american camelids, horse, dog) and with the main objective of diagnostic procedures standardization[13,14,15,16,17,18,19,20]. The most important problem that have to be consider is the specie-specific heat transfer equation which is influenced by numerous factors. The following paragraphs report our experimental approaches for the different species.

3. Experimental approaches to the use of the thermography in veterinary medicine

3.1 Effect of GnRH test on scrotal surface temperature in Alpaca

Treatment with gonatropin realising hormone (GnRH) increases blood concentrations of LH and FSH. LH and GnRH directly act on Leydig cells stimulating them to release testosterone from the testis. Testis have a local regulation due to its structural organization (avascular seminiferous tubules compartment and vascularised interstitial compartment) and to its organization and hormonal control of spermatogenesis. The regulation of the testis functionality is based on general and local information through hyphophyseal LH and GnRH. Increments of interstitial testosterone level following the local action of GnRH is generally faster then hypotalamic-hyphophyseal-gonadal way. High interstitial testosterone level might activate a mechanism of secretion based on the neural local regulation of the blood flow and muscular contraction.

Aim of this work was to evaluate variations in terms of testosteronemia and scrotal surface temperature (ST) during GnRH test. Five adult males (4 Huacaya, 1 Suri) were tested to evaluate their testicular functionality through the GnRH test (administration I.M. of 6-9 mcg of GnRH analogue buserelin/male). Trial 1 (T1): males were completely isolated from

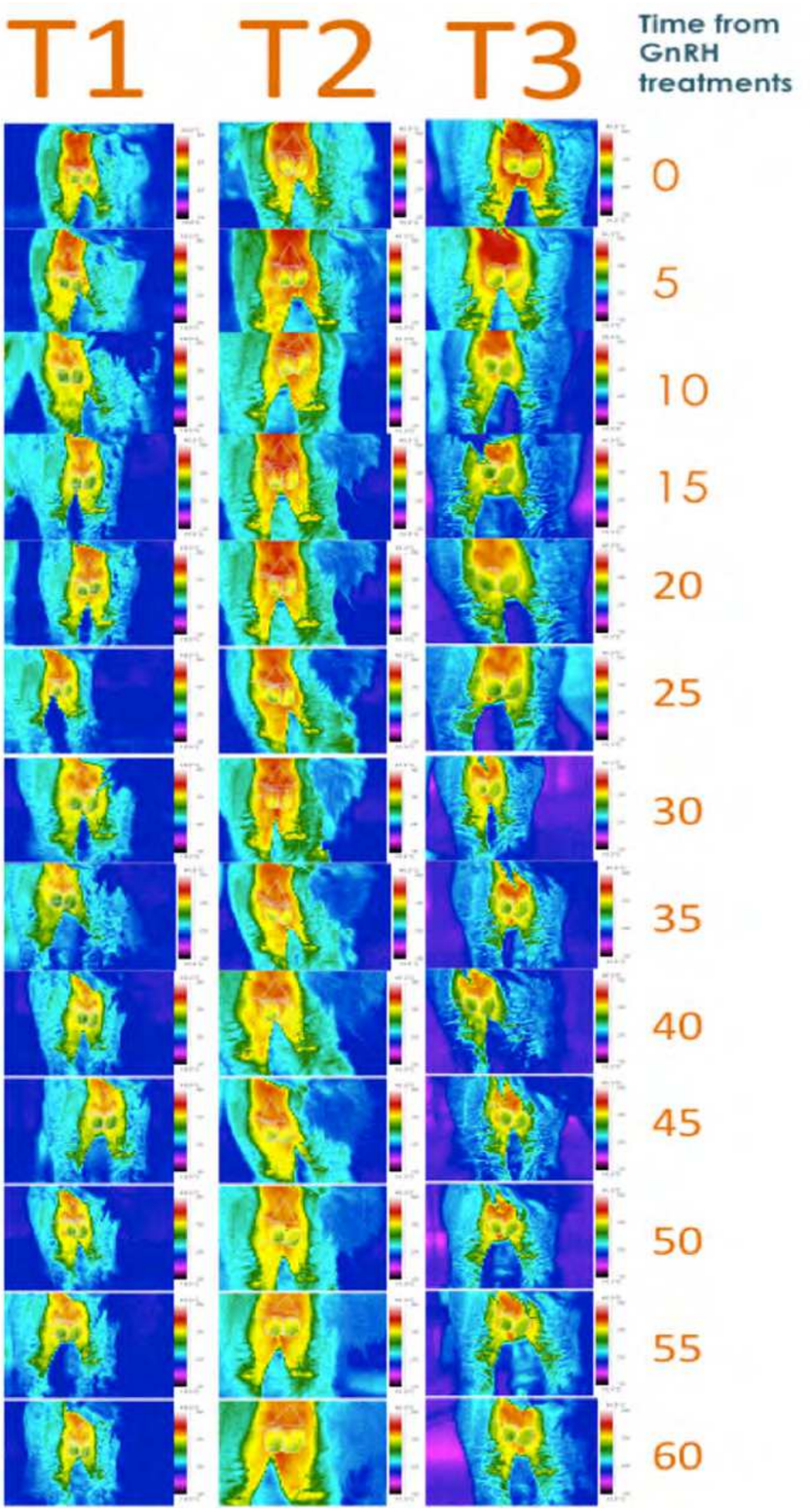


Fig. 1. Example of Alpaca's scrotum thermographic images during three trials. (T1: isolation from female; T2: exposure without mounts; T3: exposure with mounts)

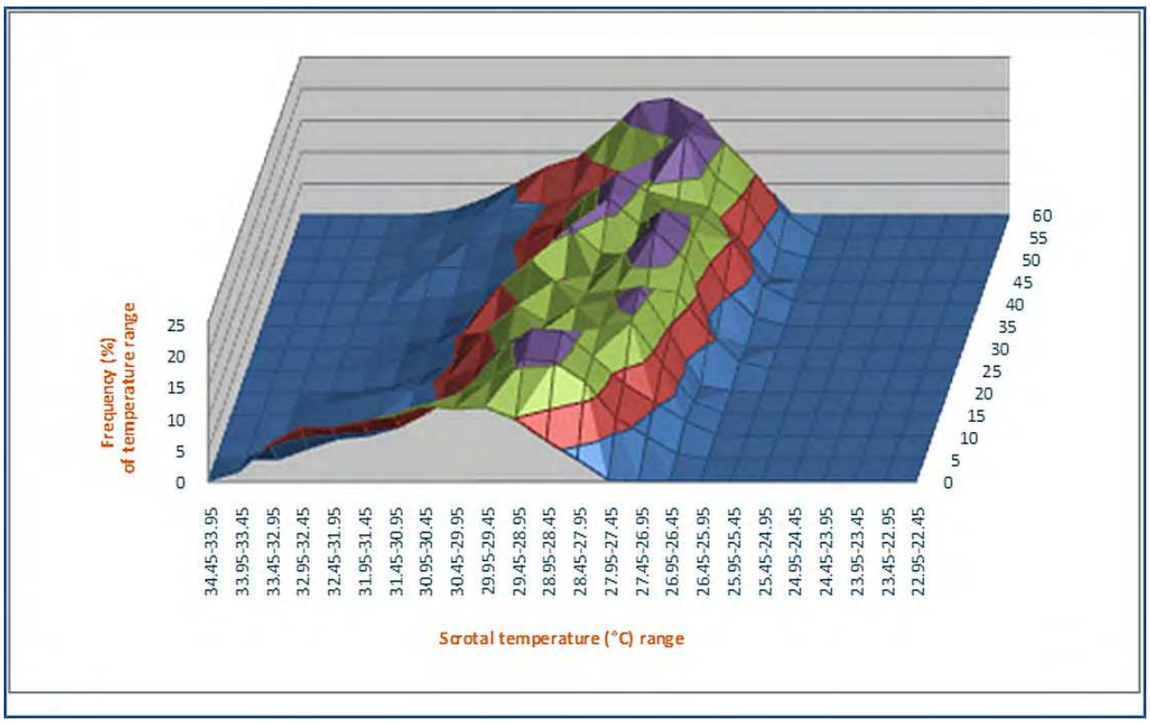
females 2 months before. Trial 2 (T2): males were exposed to females without mounts (3 weeks; two times each week for 15 min). Trial 3 (T3): males were exposed to females with mounts (3 weeks; two times each week, 15-20 minutes). Thermometric measurements of scrotal surface were carried out using an infrared-camera (P25, Flir System) every 5 minutes during each GnRH test (Figure 1.). Thermografic images were analysed using a specific software (ThermaCam Researcher Basic 2.08, Flir System). Testosteronemia was determined using a chemiluminescent method previously validated. Data obtained were analysed through ANOVA for repeated measures, using the GLM procedure of the statistical software SIGMASTAT 2.03, taking into consideration the trial as independent variable while ST and testosteronemia as dependent variables; besides Pearson correlation coefficients were calculated for the variables considered.

GnRH administration influenced testosteronemia and scrotal temperature during the monitoring time (60 minutes). Comparisons among monitoring times are reported in Table 1.

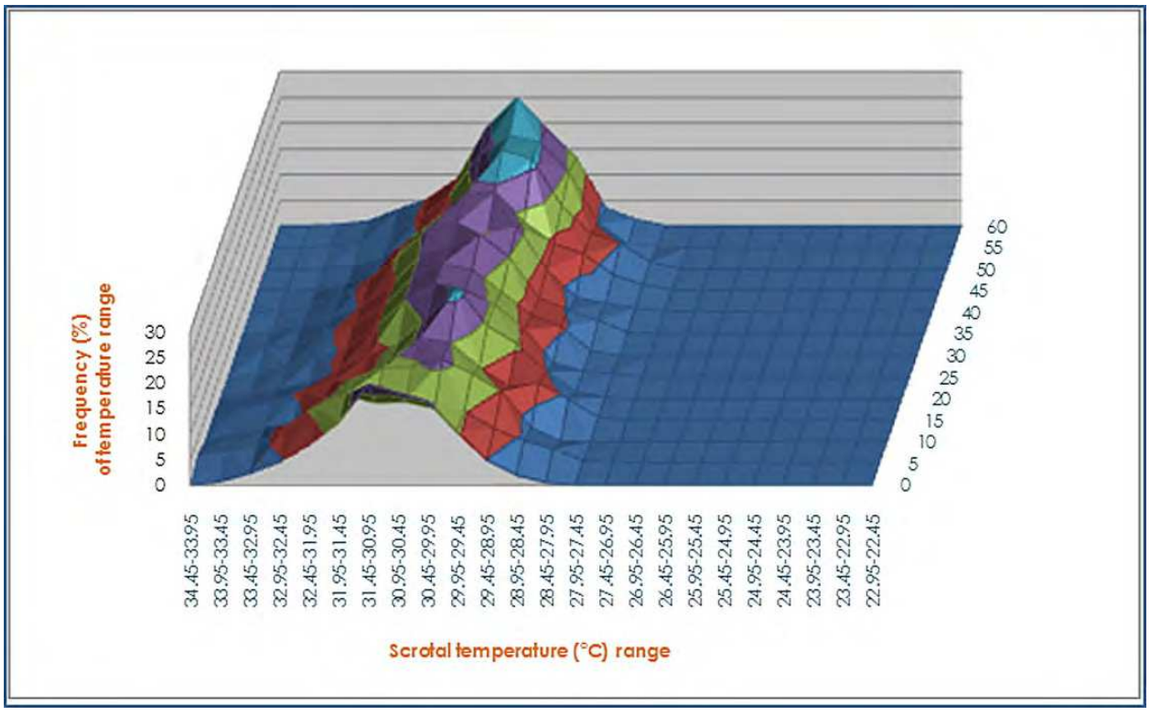
Time after GnRH administration	Trial 1	Trial 2	Trial 3	S.E.M.
0	30.790	30.900	30.270	
5	29.460^{a}	30.970^{b}	29.620 ^{ac}	
10	29.130^{a}	30.800 ^{b}	29.350 ^{ac}	
15	29.080^{a}	30.700 ^{b}	29.540 ^{ac}	
20	28.870^{a}	30.490 ^{b}	29.720 ^{ac}	
25	28.700^{a}	30.620 ^{b}	29.420^{c}	
30	28.700^{a}	30.680 ^{b}	29.740^{c}	0.195
35	28.460^{a}	30.600 ^{b}	29.250a^{c}	
40	28.280^{a}	30.570 ^{b}	29.190a^{c}	
45	28.620^{a}	30.310 ^{b}	29.410a^{c}	
50	28.460^{a}	30.030 ^{b}	29.250^{b}	
55	28.480^{a}	30.260 ^{b}	29.290^{ac}	
60	28.940^{a}	30.250 ^{b}	29.330^{ac}	

Table 1. Variation of scrotal temperature during the three GnRH tests.

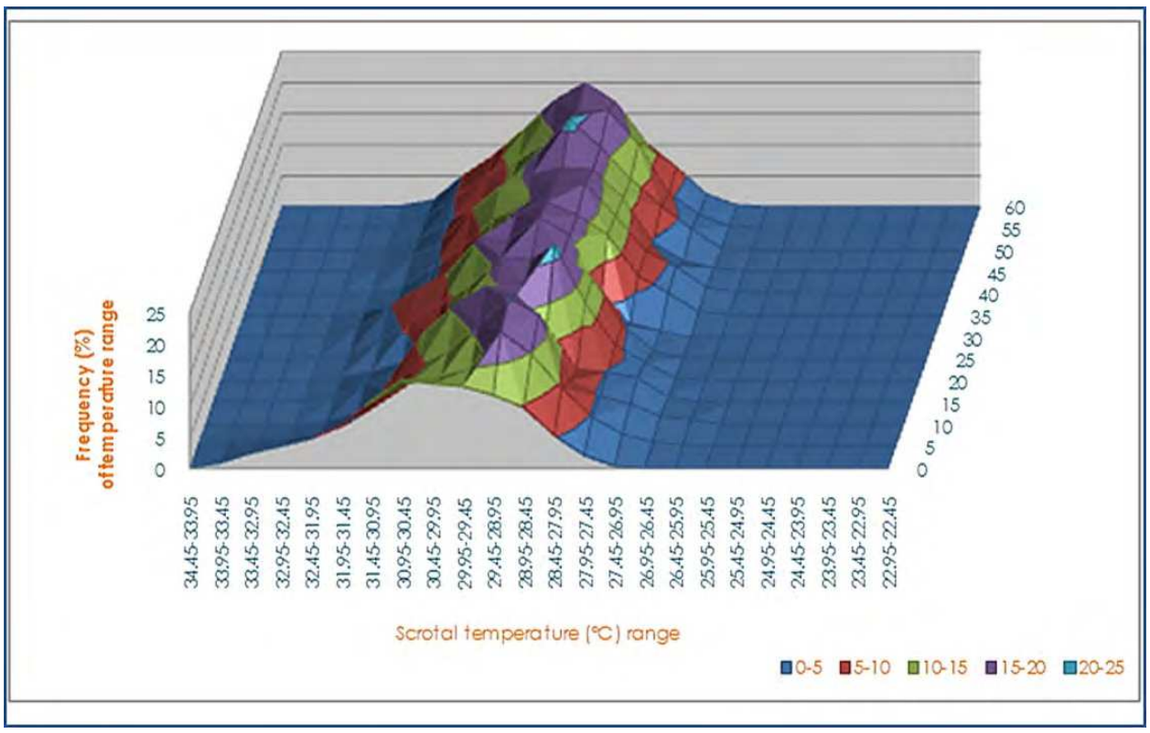
Frequency variations of temperature ranges are reported in graphics 1, 2 and 3 for 1st, 2nd and 3rd test respectively. ST decrease during the monitoring period (60 minutes) after GnRH administration with the higher variation before the female exposure.



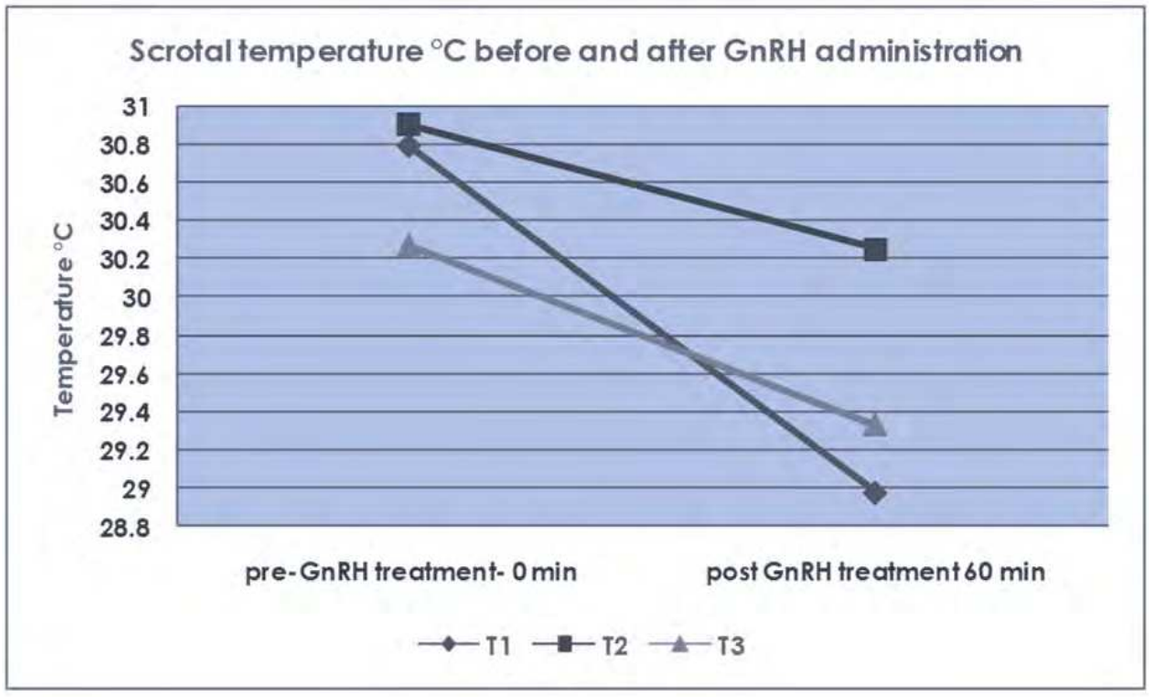
Graphic 1. Distribution of frequencies of scrotal temperature range during GnRH test after isolation from female



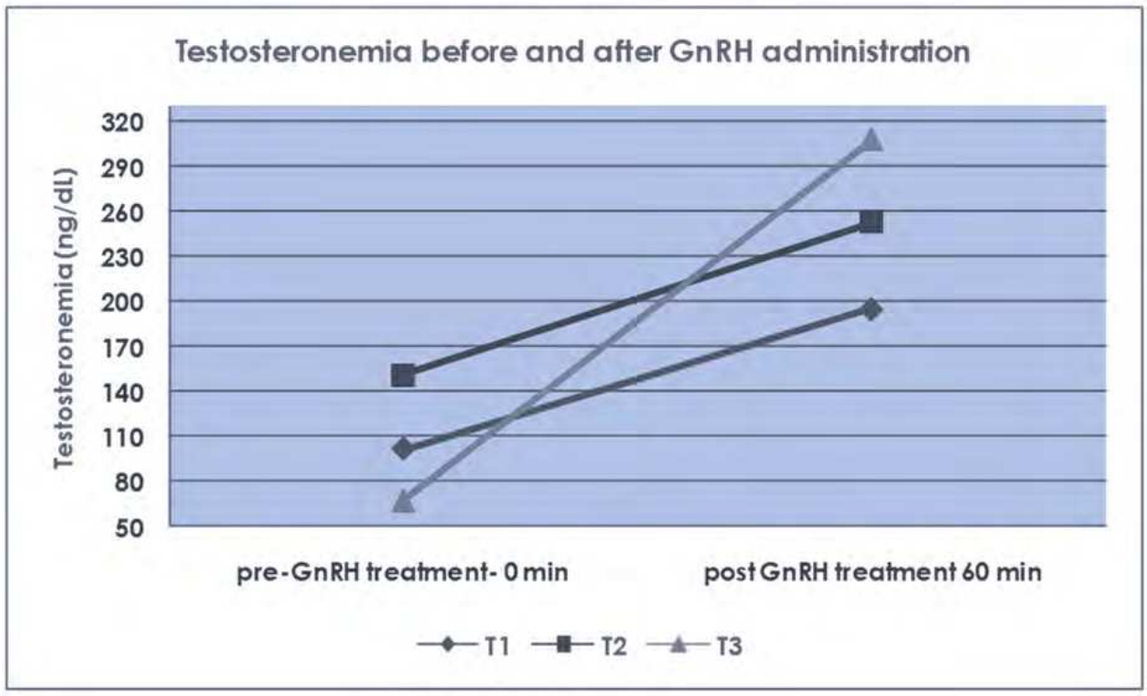
Graphic 2. Distribution of frequencies of scrotal temperature range during GnRH test after female exposure



Graphic 3. Distribution of frequencies of scrotal temperature range during GnRH test after mounts



Graphic 4.



Graphic 5.

	Scrotal Temperature				Testosteronemia			
	T1	T2	T3	s.e.m.	T1	T2	T3	s.e.m
0	30,79*	30,90	30,27*		101.52	151.34	67.64*	
				0.27				30.19
60	28,98^{a**}	30,25^{b}	29,33^{ab**}		195.32^{a}	253.2^{ab}	308.2^{b**}	

Table 2. Comparisons for scrotal temperature and testosteronemia (mean ± s.em.) during the three GnRH tests.

Testosteronemia increase during the monitoring time with the higher variation after mounts.

Pearson correlation coefficients were 0.77, -0.638 and -0.378 ($P<0.05$) for testosteronemia-times, ST-times and testosteronemia-ST respectively. ST decrease starting 5 minutes after GnRH administration during 1st and 3rd test. The stability of ST after the female exposure could be related to the primary activation of the local hormonal control of the testis. It was notable individual response to the work schedule, despite the significant mean differences, probably due to hierarchical behaviour among males.

The effect of GnRH administration on scrotal surface temperature may be assessed by thermography. The correlation between temperature and testosteronemia could be indicative of the stimulating local effect of the GnRH.

3.2 Effect of food intake and first digestive phase on superficial temperature estimated by thermography in dairy cattle

Regulation of the skin circulation is also the main mechanism to control the preservation or dispersion of core temperature above all during physiological phenomena like digestion. The cutaneous sympathetic thermoregulatory neural function regulates vasomotor activity within dermal arterioles and capillaries. Different methods has been adopted to study the dynamic temperature response i.e. indirect or direct cooling of body parts (stress thermography) and re-warming time as correlation index of certain pathological conditions[2]. Blood flow in the skin can be measured by the changes in skin temperature, washout technique, laser Doppler flowmetry, ulcer healing process, or the tissue PaO2. Thermography is non-invasive, simple, safe and can be monitored remotely[21,22]. In spite of such advantages, little evidences concerning physiological skin temperature changes for bovine has been reported. The objectives of this study were to investigate the influence of the first digestive phase (neural and hormonal mechanisms) on skin temperature variation in three different classes of dry cows, divided depending on the distance from the presumed delivery data and drying days, using the analysis of thermographic sampling before and after the Total Mixed Ratio (TMR) administration.

Twenty dry Holstein cows were considered during four times of thermographic monitoring. The cows were housed in a tie-stall during all observation time. Thermographic images were taken considering a poligonal area traced from ileum wings to sacral, caudal, gluteal and perianal areas. Two thermographic sessions (5 scans every 5 min for each one), before and after total mixed ration (TMR) distribution, were performed. All images were scanned using a hand-held portable infrared camera (ThermaCam P25, FLIR Systems, Limbiate, Italy). Temperatures were recovered by processing the thermographic images in ThermaCAMResearcher Software.

Data collected were divided taking in consideration the distance from the beginning of the dry period (class 1: 0-20 days; class 2: 21-40 days; class 3: >41 days) and the distance from the predicted parturition date (class 1: 0-20 days; class 2: 21-40 days; class 3: >41 days). Data were analyzed using a two ways ANOVA (sampling period: before and after TMR distribution; classes of distance from predicted parturition date or/and beginning of the dry period) with the GLM procedure of the software SigmaStat2.03. Moreover data were analyzed using a digital infrared imaging software package to collect the temperature ranges frequencies in the selected area and to study the variations between before and after TMR administration.

Skin temperatures were dependent on the time of measurements (before and after TMR administration). Results indicate a ΔT °C of about 1.5 °C in classes of animals with more than 20 days from the predicted delivery date (Table 3 and 4).

The analysis of the temperature ranges frequencies (% of 0.25 °C ranges from 20°C to 35°C) showed a substantial frequencies variation between before and after TMR administration (graphics 6-8).

The distributions of frequencies are different in classes considered and the variations due to the TMR intake are different among the classes considered. Another interesting finding was the possibility of individual thermal mapping during different thermographic sessions.

Item	Classes of distance from the predicted parturition date					
	0-20 days	21-40 days	>40 days	*P* =		
T °C	27.55 ± 0.65	27.97 ± 0.43	28.72 ± 0.47			
ΔT °C	1.064	1.463	1.510	0.311	0.014	0.028

Table 3. Effect of distance from the predicted parturition date on skin temperature (mean ± SEM) and the difference (ΔT) between before and after TMR distribution.

Item	Classes of distance from the beginning of dry period					
	0-20 days	21-40 days	>40 days	*P* =		
T °C	28.60 ± 0.48	28.45 ± 0.50	27.38 ± 0.46			
ΔT °C	1.592	1.336	1.250	0.030	0.028	0.084

Table 4. Effect of distance from the beginning of dry period on skin temperature (mean ± SEM) and the difference (ΔT) between before and after TMR distribution.

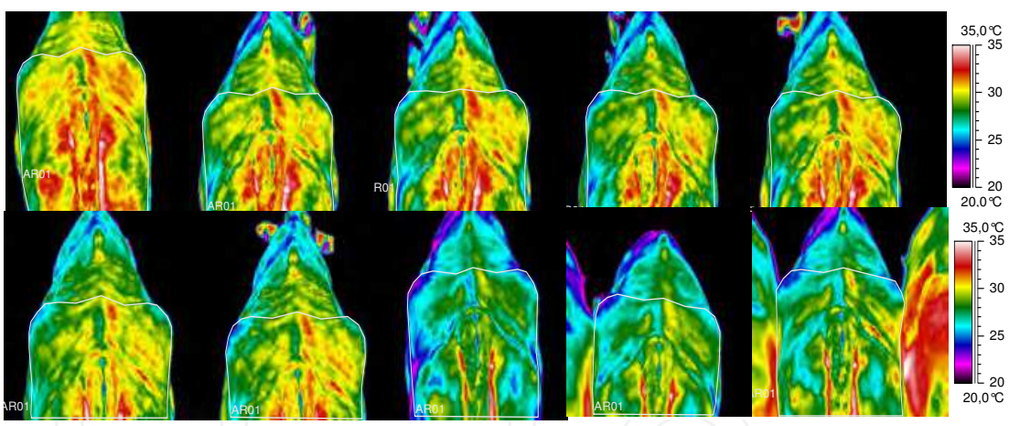
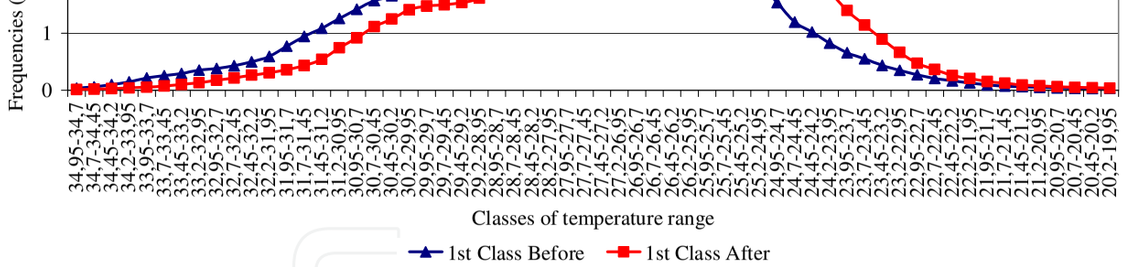
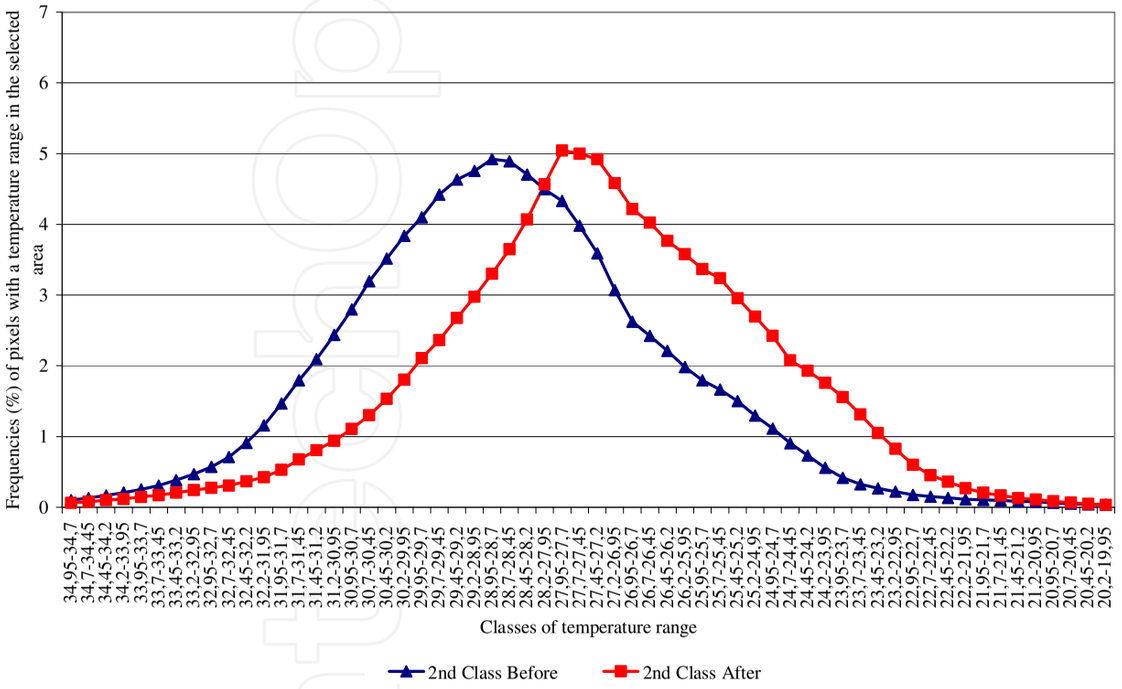


Fig. 2. Example of thermographic images of cows during the first digestive phase.

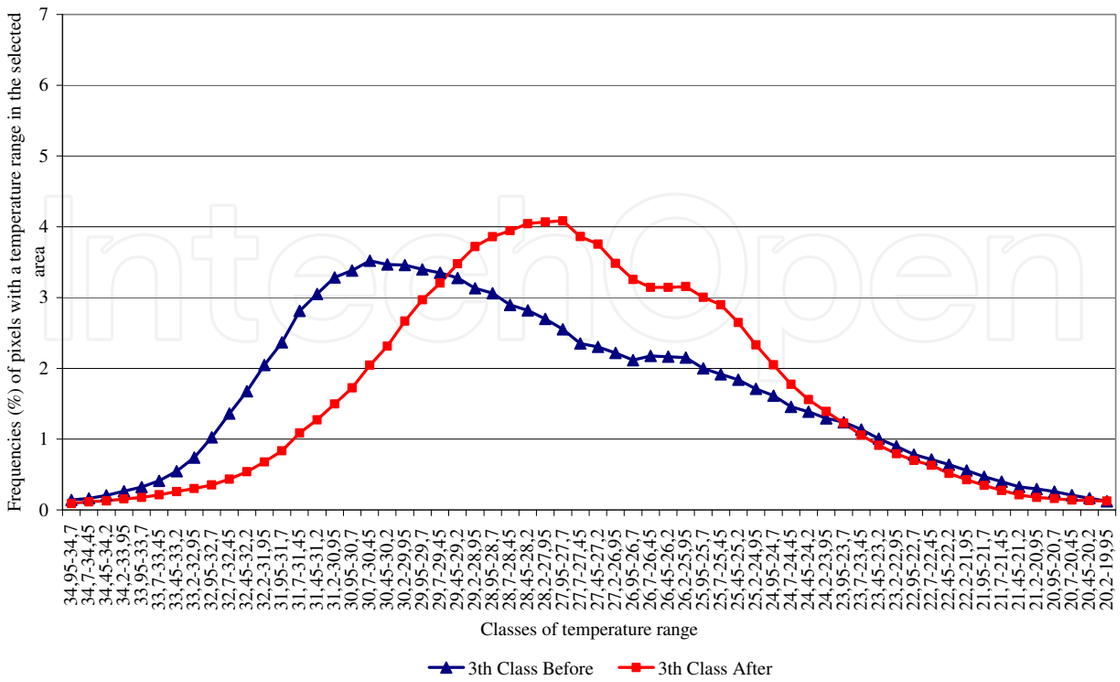
The body surface temperature is determined by the local metabolism, blood circulation underneath the skin, heat exchange between the skin and its environment. Variations in any of these parameters may induce alteration from the normal temperature or heat flux range at the skin surface. Therefore these changes may be reflecting the physiological and pathological state. In our trial, it was established that physiological changes due to the first phase of digestion may be investigated through the thermographic scanning. The choice of dry cows as animals for thermal scanning was almost imperative because it is clear the fetus influence on feed voluntary intake and the physiological changes which may be observed during the last two months of pregnancy. These changes are strictly linked to the reached maturity of fetal adrenal gland for corticosteroid production, to the hormonal variations during the last pregnancy time and, not less important, the fetal mass which limits the quantity of feed intake. Cows thermal mapping is possible even if hair length can influence



Graphic 6. Temperature ranges frequencies (%) in the 1st class (0-20 days) of distance from the presumed delivery data (red line)



Graphic 7. Temperature ranges frequencies (%) in the 2nd class (21-40 days) of distance from the presumed delivery data



Graphic 8. Temperature ranges frequencies (%) in the 3th class (>41 days) of distance from the presumed delivery data

the skin emissivity of infrared rays and, therefore, a error margin have to take into consideration. In our trials, the cows skin emissivity was fitted to 0.95. This value is similar to previously used values for bovine's skin[2,3,4].

Thermal imaging camera appear to be reliable under field condition. In cows as other animals the body surface temperature depends by blood flow and metabolism rate of the underlying tissues. The physiological state of the underlying cells could be studied by measuring skin temperature using thermography. This study enable us to quantify, in terms of skin temperature ranges frequencies in a selected area, the redistribution of the blood during the first phase of digestive processes. In cows these variations were different during the dry period. The voluntary feed intake, controlled by numerous neural and hormonal mechanisms, changes during the last period of pregnancy because fetus body encumbrance occur. Also during the dry period there is an increment of the subcutaneous fat content which could influence the heat radiation. Hormonal variations typically evidenced to partum preparation could also changes the tissues heat transfer capability. In conclusion thermography may have potential as a study tool for specie-specific heat transfer equation. However, more data about the relationship among others physiological events affecting cows skin temperature are required.

3.3 Thermographic study of the perivulvar area in estrous and anaestrus ewes

The aim of the present study was to detect skin temperature differences of perivulvar area between ewes in estral and anestral phase. Twenty four dairy ewes - 16 in estral phase and 8 in anestral phase - were investigated. Estruses were synchronized by using intravaginal

progestagen-impregnated sponges (fluorogestone acetate, FGA) for 14 days and after sponges removal ewes were treated with PMSG. Thermography sessions were carried out 50 hours after sponges removal at a distance of a meter from the vulvar area. A skin emissivity of 0.95 was assumed. The control subjects with no synchronization treatment were in a seasonal anestral period. The analysis performed on the acquired thermograms were: qualitative and quantitative analysis taking into account the mean perivulvar area temperature, quantitive analysis of temperature differences between unfleeced adjoining areas, quantitative analysis of frequencies of intervals temperature of 0,2 °C. A significant difference between the two groups in values expressed as mean temperature of perivulvar area ($P < 0.05$) was observed. The subjects in estrus and in anestrus ranging a temperature from 35,9 ° C to 37,7 °C with an average of 36,9 ± 0,5 °C and from 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 the 2% of the regional hematic flow and so this area may be used as control for other adjoining areas because the emitted heat increase is proportional to the parenchymal hematic flow. The animals in estrus show an increase in the hematic flow to the genital apparatus and hormonal changes. These variations are able to explain the increase of heat emitted by the unfleeced skin of posterior areas. Thermography is a tecnique useful to point out the different skin's ability of heat transmission from underlying areas. The subjects with estrus induced by synchronization have shown a different thermal behavior that can be detected by thermograpy sessions in adjoining areas receiving blood from common arteries (internal iliac –pudendal artery).

3.4 The use of thermography on the slaughter-line for the assessment of pork and raw ham quality

Several studies have been carried out to measure the surface temperature of pigs to evaluate the effects of environmental conditions[1,23] and to predict the pork quality of pigs immediately before they are slaughtered[24,25]. The aim of this preliminary study was to examine the possibility of applying the IRT directly on the slaughter-line for the evaluation of pork quality and ham suitability to be processed as dry-cured ham.

Thermographic images were collected on 40 carcasses of heavy pigs at 20 min after stunning. The Infrared Thermal Camera (ITC) (Flir System, Model ThermaCam P25) was placed after carcass splitting, thus left and right caudal and dorsal surface images were kept for each half carcass. The settings of the camera were as follows: emissivity of pig's skin 0.98; reflected air temperature 22°C; distance between camera and skin surface m 2.5. Temperatures were recovered by processing the thermographic images of a squared area located in the centre of the caudal side of ham using the ThermaCam Researcher Basic Software (Flir System). The pigs, consisting of commercial hybrids, were supplied from one farm located 35 km from the slaughter plant and were stunned by electronarcosis (250 V, 1.25 A) after a lairage that lasted 2 hours. At 90 min post mortem the pH (pH1) was measured on the semimembranous (SM) muscle of each left ham. After 24 hours of chilling at a temperature of 0-4°C, the measure of pH (pHu) was repeated together with the objective colour assessment (CIE Lab system, Minolta Colorimeter Cr300) after 30 min of bloom time. Moreover, a subjective evaluation of some characteristics of ham such as the veining defect (4-point scale, 1=none, 4= serious), the redness of skin defect (3-point

scale, 1=none, 3=serious) and the fat cover (3-point scale, 1=insufficient, 3=excessive) was carried out on the trimmed left thighs destined to be processed as Parma dry-cured-ham[26]. The temperature range frequencies (% of 0.25°C ranges from 27°C to 30°C) and the means of the obtained distributions were calculated. In order to evaluate the possible relationship between the surface temperature of ham and the meat quality, pH and L* colour values were arranged in the following classes (pH1, < 5.80, ≥ 5.80 < 6.00, ≥ 6.00; L*, < 45, ≥ 45 < 50, ≥ 50). The means and the measures of variability of the surface temperatures and meat and ham quality traits are reported in Table 1. The distributions of the average surface temperatures on left and right hams showed a total range from 27.3°C to 29.2°C with mean values of 28.3°C and 28.1°C, respectively. Although all carcasses were processed in the same way along the slaughter chain, a variation of 1.8°C for the left hams and of 1.5°C and for the right hams were found. The widest ranges of surface temperatures using ITC were found on live pigs before stunning at the level of the back by Schaeffer et al.[25] (from 17°C to 34°C) and by Gariepy et al.[24](from 21°C to 29°C), and after sticking at the level of the ears by Warriss et al.[1] (from 27°C to 35°C). A largest range of surface temperatures, from 16°C to 21°C, was also found on pig carcasses[25]. The pigs used in the present study produced meat characterized by a large variability in terms of glycolysis speed, as demonstrated by the range of pH1 values, but also a similar extent of acidification, as confirmed by the narrow range of the ultimate pH values (Table 5). The L* colour co-ordinate values showed that final meat colour of the examined pigs was included from normal to slightly pale.

	Mean	Standard Deviation	Min.	Max.
Average left ham surface T°	28.3	0.43	27.40	29.20
Average right ham surface T°	28.1	0.36	27.30	28.80
pH_1	6.13	0.26	5.56	6.63
pH_u	5.42	0.05	5.33	5.57
L*	48.2	2.90	40.7	55.6
Veining score	2.14	0.80	1	4
Skin redness score	2.25	0.60	1	3
Fat cover score	2.11	0.57	1	3

Table 5. Means and measures of variability of the surface temperatures and meat and ham quality.

Least-squares means of surface temperature in the classes of meat and ham quality are presented in Table 6. In both hams, the differences of surface temperature among pH_1 classes were extremely small and non significant. The temperature of both hams were also very similar and not significantly different in the L* colour co-ordinate classes. These results are consistent with previous findings of Schaeffer et al.[25] showing an absence of relationship between these meat quality traits and the skin surface temperature. The veining and the red skin defect classes were not significantly related to a variation of the skin surface a

temperature; although, in both hams there was a tendency for the latter defect to decrease with an increase in the surface temperature. Significant differences of temperature in both hams according to the fat cover score were found. An increase of temperature was found in hams with a decreasing of fat cover, particularly in the right hams. It is suggested that lower thermal insulation due to a thinner subcutaneous adipose tissue might be responsible to of the higher skin surface temperature. The relationship between the fat cover score of ham and the surface temperature suggests that infrared thermography could be a valuable, fast and non-invasive method to estimate its fatness. Thus, the preliminary results achieved here showed a possible application of this technique to better select the raw hams destined to the successive dry-cured processing.

	Left ham	Right ham
pH_1		
< 5.80	28.40 ± 0.09	28.12 ± 0.09
≥ 5.80 < 6.00	28.40 ± 0.19	28.26 ± 0.18
≥ 6.00	28.32 ± 0.22	28.08 ± 0.21
L*		
< 45	28.41 ± 0.18	27.99 ± 0.18
≥ 45 < 50	28.32 ± 0.10	28.19 ± 0.10
≥ 50	28.54 ± 0.15	28.12 ± 0.15
Veining score		
1	28.32 ± 0.17	28.14 ± 0.17
2	28.53 ± 0.11	28.19 ± 0.11
3+4	28.24 ± 0.13	28.05 ± 0.13
Skin redness score		
1	28.77 ± 0.27	28.57 ± 0.26
2	28.44 ± 0.10	28.19 ± 0.09
3	28.23 ± 0.13	27.96 ± 0.12
Fat cover score		
1	28.82 ± 0.19^{a}	28.75 ± 0.16^{A}
2	28.35 ± 0.09^{b}	28.08 ± 0.08^{B}
3	28.22 ± 0.15^{b}	27.90 ± 0.13^{B}

^{a,b}= $P<0.05$. ^{A,B}= $P<0.01$.

Table 6. Least squares means and standard errors of surface temperature in the classes of meat and ham quality.

3.5 Use of thermography during monitoring of estrous and ovulation times in the mare

Essential prerequisites for successful artificial insemination is accurate estrus and ovulation detection, which is classically perfomed by teaser, transrectal palpation and ultrasonographic examination of reproductive tract. Alternative method could be the measurement of electrical impedance of vaginal mucus and perivulvar and vulvar temperature[27,28]. Report on using infrared thermography in detection of estrus in mare is limited. Therefore, the aim of this study was to assess perivulvar and vulvar temperature using infrared thermography as a non invasive method for the monitoring of estrous cycle in the mare.

Animals used were nine trotter mares, five with foal and four without foal. The monitoring was done in three stages : T1(follicle with Θ > 3cm), T2 (follicular growth), T3 (ovulation). During each moment, the thermography was performed first on perivulvar and vulvar regions by Thermacam P25, followed by transrectal palpation and ultrasonographic examination of reproductive tract by 8Mhz probe, and finally, the measurement of electrical impedance was done with endovaginal Draminisky probe. Ten blood samples were collected from five mares to measure serum progesteron and estrogen concentration in stages T2 (5) and T3 (5).

	T1	T2	T3
PMinT (°C)	28,6±0,80	28,84±0,32	27,72±0,54
PMaxT (°C)	34,2±0,38 ^{ab}	34,41±0,22 ^{a}	33,43±0,13 ^{b}
PMT (°C)	31,98±0,51 ^{a}	31,54±0,15 ^{ab}	30,91±0,21 ^{b}
VMinT (°C)	27,95±0,22	27,17±0,61	26,85±0,28
VMaxT (°C)	33,72±0,46 ^{ab}	34,12±0,18 ^{a}	33,14±0,14 ^{b}
VMT (°C)	31,40±0,30 ^{a}	31,31±0,19 ^{a}	30,31±0,20 ^{b}
ΔPVMT (°C)	0,58±0,23	0,23±0,16	0,60±0,19
DGF (cm)	4,31±0,28 ^{a}	4,72±0,21 ^{a}	1,10±0,13 ^{b}
EFW	2,30±0,12 ^{a}	2,45±0,11 ^{a}	1,0±0,00 ^{b}
FC	1,58±0,07 ^{a}	1,79±0,04 ^{b}	1,0±0.00 ^{c}
UE	1,50±0,29 ^{a}	1,39±0,14 ^{a}	0,92±0,05 ^{b}
EI (mOhm)	320,00±21,99	391,11±23,12	366,67±22,11
PG (ng/ml)	O,2±0,00	0,23±0,01	0,31±0,03

Different letters among groups mean a significant difference (P<0,05)
PerivulvarMinimum 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).

Table 7. Monitoring parameters considered during estrous and ovulatory times in mare

Thermographic analysis was performed using an established range of temperature (15°-35°C°) by the software Thermacam Researcher. The data was statistically analyzed by software Sigmastat. Parameters were evaluated with R>0,4 and P<0,05.

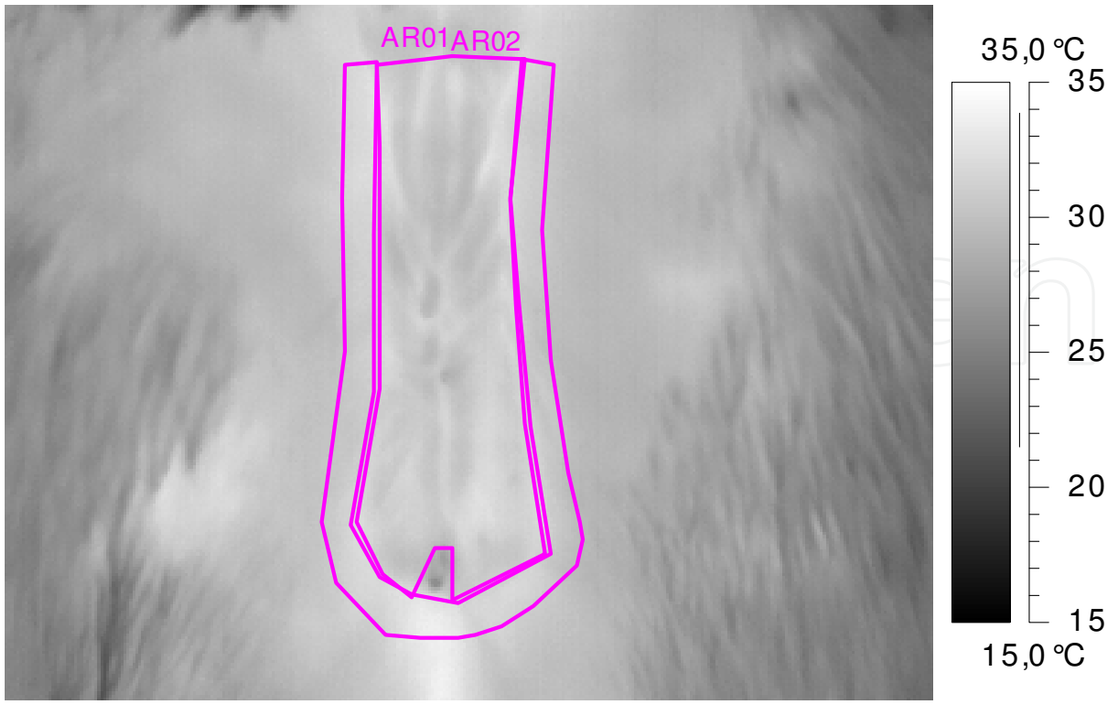


Fig. 3. Example of vulvar and perivulvar mare's thermography

The analysis showed a positive correlation within the thermographic parameters, which were Perivulvar Maximum Temperature (PMT), Perivulvar Mean Temperature (PMT), Vulvar Maximum Temperature (VMT), Vulvar Mean Temperature (VMT). There was a simultaneous increase of these parameters. These increased parameters positively correlated with Diameter of Greater Follicle (DGF) and Echotexture Follicular Wall(EFW), and negatively correlated with the presence of corpora lutea (CL). These data suggest an increase of maximum and mean perivulvular and vulvular temperature during follicular growth and a decrease of the same temperature during the establishment of CL. Probably that is because the mare under the influence of estrogen has an increase of hyperemia of the vulvar region[29]. A negative correlation was also existed between PMT and the values of Electrical Impedance (EI). So, the increase of PMT is associated with lower values of EI, which occur during the follicular growth. Moreover, the Perivulvar Minimum Temperature (PMT) was positively correlated with serum estrogen concentration and negatively correlated with serum progesteronconcentration which occur during the follicular growth and ovulation time respectively.

These preliminary results are in favour to a possible use of thermography as an auxiliary non invasive method during estrous cycle monitoring in mares.

3.6 Milking procedures and thermographic monitoring

The milking machine may affect both blood and lymphatic circulation in teat walls, because the radial and longitudinal stretching action exerted by the milking vacuum, particularly in correspondence of an inadequate massage phase. This can induce teat congestion and oedema, altering the defense mechanisms against bacterial penetration of teat duct. Milking

duration (overmilking), liner and cluster characteristics, pulsation parameters and vacuum level have been recognized as factors that can affect the integrity of teat tissues[30,31].

The effect of milking procedures and liners on udder and teat skin temperature was investigated in cows through thermographic scanning, showing that thermography can be a very useful tool to evaluate, estimate and differentiate short and longer-term tissue reactions to machine milking[2,3]. In dairy sheep different methods have been adopted to measure udder blood circulation and the effect of cold exposure and lactation on the distribution of blood flow[32].

The objective of this study was to evaluate the influence of the vacuum level on udder and teat temperature changes during milking procedure, monitoring the teat recovery via digital infrared thermography.

The experiment was carried out in a 1x24 side by side milking parlour, with a low pipeline milking system, using the same pulsation parameters (120 cycles/min and 60% ratio) and a medium weight milking unit (0.49 kg) equipped by plastic shells and cylindrical rubber liners (20 mm mouthpiece bore, 101.2 mm length mounted, 2,8% extended) (AlfaLaval 961403-01). Two groups of 24 ewes were milked twice a day at 28 and 42 kPa for a period of 8 weeks, applying a standard routine without udder preparation and stripping. Individual milking-on time was on average 55.7 s when the system vacuum was set at 42 kPa and increased to 67.1 s at 28 kPa. At the 9th week six ewes of each group were monitored during two consecutive evening milkings via infrared thermography.

Thermographic images (Flir System, ThermaCam P25, sensitivity of 0.08 °C) of posterior udder area (PUA) and teats were taken pre-milking (PM), during milking (M) (only for PUA), immediately after milking (IAM) and up to 2.25 minutes after milking (AM+). Mean temperatures of the different teat areas (teat base – TB; mid teat – MT and teat tip – TT) and the distribution of frequencies of temperature ranges (0.2 °C) were recovered by processing the thermographic images in ThermaCam Researcher Basic 2.8 SR-1 Software (Flir System).

Furthermore, digital pictures of each teat have been taken to examine teat apex condition and, after a classification in three classes of phenotype (Class 1, 2, 3), find any connection to the morphology and teat skin temperature (figure 5). The classification was based on teat cistern height and on sphincter protrusion, both linked to the intra-mammary pression before milking, to the teat end wall thickness and to papillary canal length.

Some IR images of the udder posterior area taken at low and high vacuum level are shown in figure 4, where the different thermal patterns of the teats before and after milking can be easily individuated.

Thermographic analysis of all different teat areas (teat base, mid teat and teat tip) shown that skin temperatures before milking were characterized by decreasing values between TB and TT. During milking the skin temperature had an average drop of about 2,2 and 1,9 °C, respectively at low and high vacuum level. After milking, temperature differences among all teat locations were more evident and specifically starting from IAM for high vacuum level group, while from AM+15s for the low ones (table 8).

The temporal variation of temperatures for each teat location is reported in graphics 9 and 10, for low and high vacuum level respectively. It was evident that low vacuum level

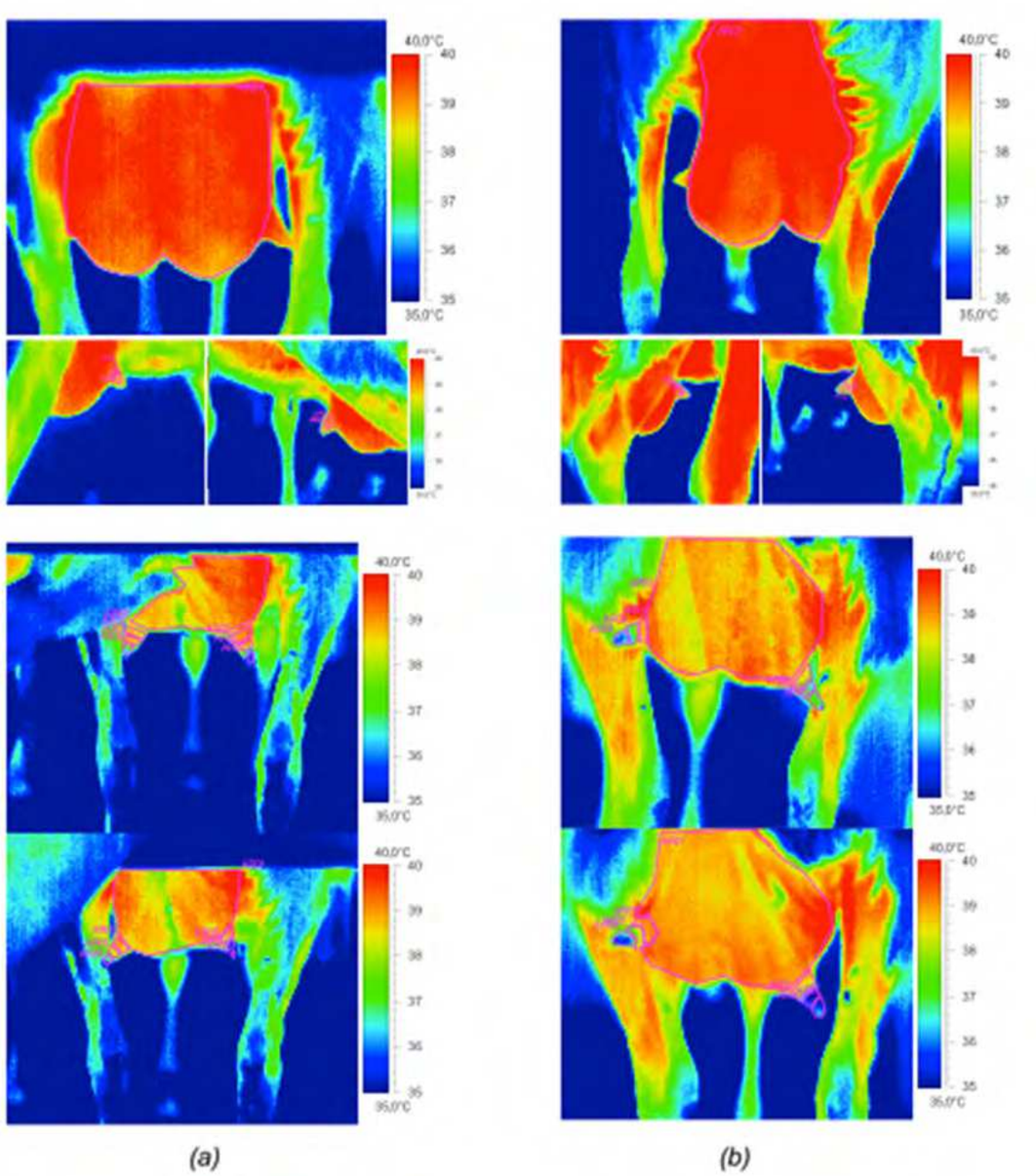


Fig. 4. Thermograms of the udder and teats milked with high (a) and low (b) vacuum level

maintains a higher difference among the teat locations. Graphic 11 reports the distribution of the frequencies of temperature ranges (0.2°C) for each teat location and both vacuum levels. The temperature recovery time was shorter (1 min vs 2 min)) at the teat base for the group milked at 28 kPa, and this can be attributable to a faster return to a normal blood flow, as a result of a lighter mechanical action exerted by the machine during milking at a low vacuum.

The phenotypic teat classification, carried out independently from vacuum levels, gives more information about the influence of the teat conformation on the mechanical milking aptitude (figure 5). Class 1 was the group of animal which have had the lower temperature gap between PM and IAM to AM+60sec while the class 2 have had the slower recovery time.

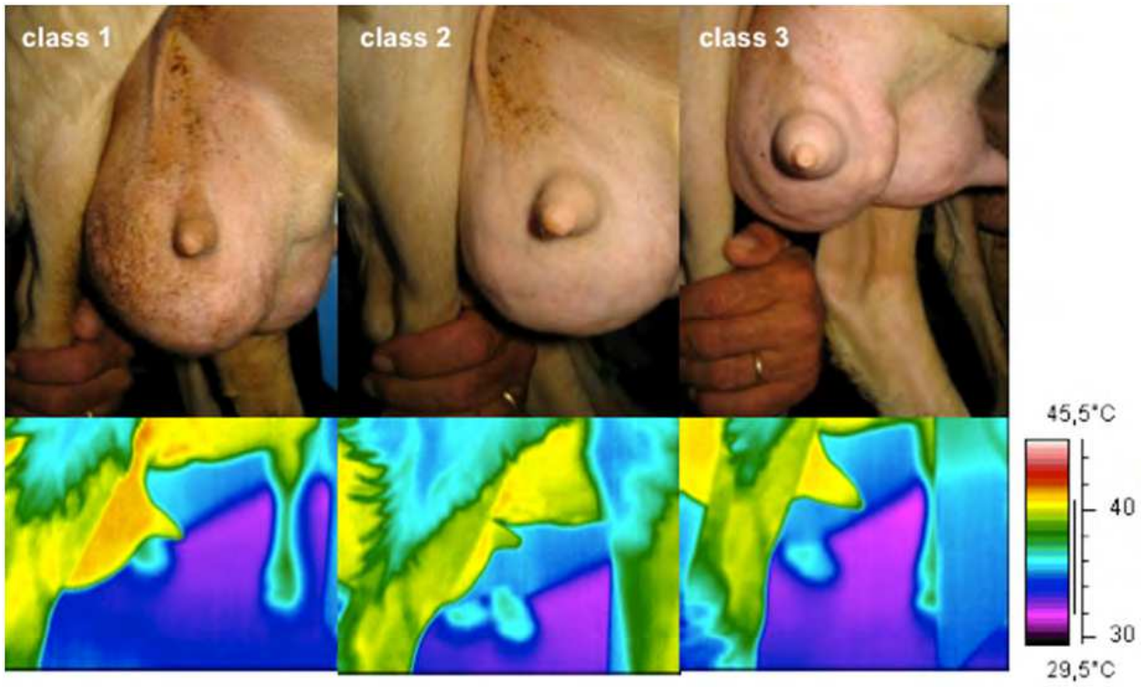
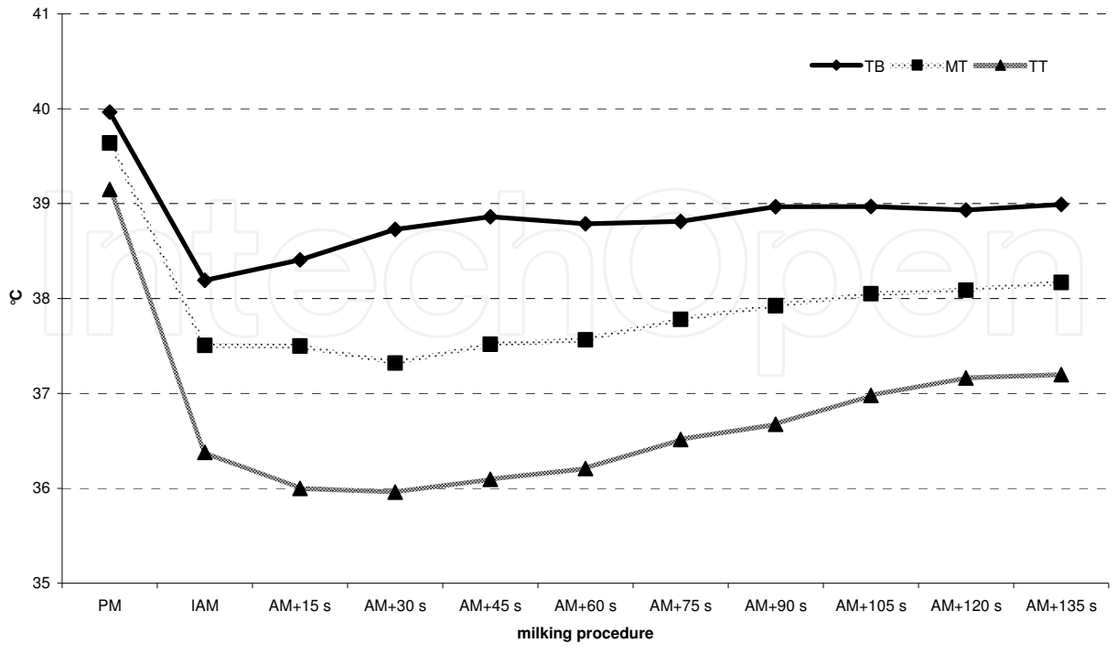


Fig. 5. Classification of the teat conformation

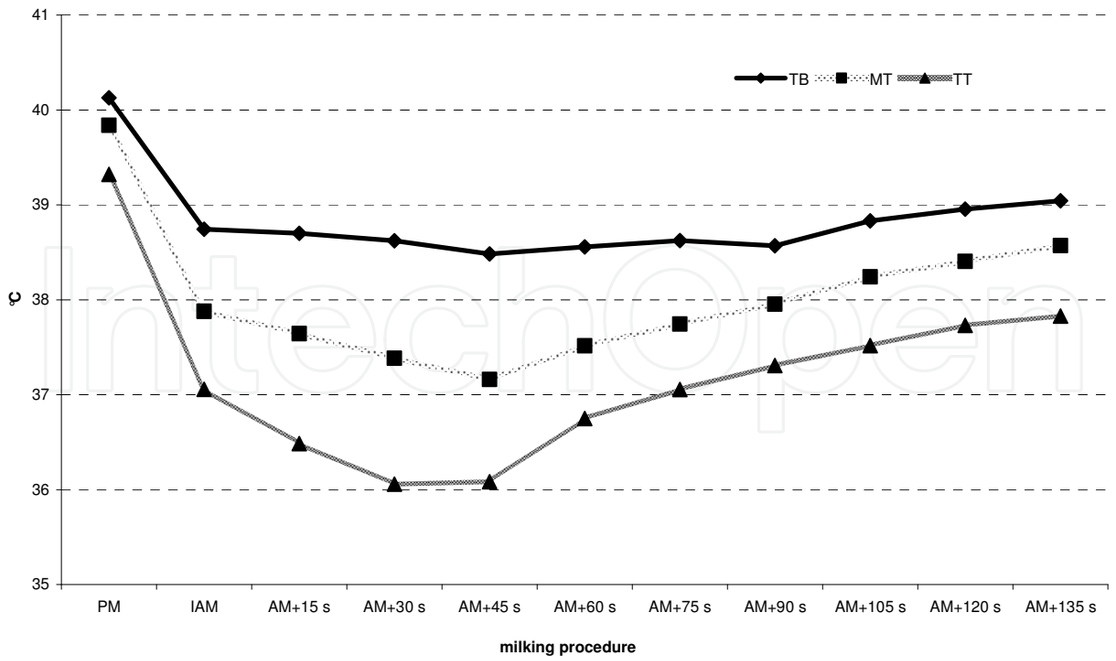
	Low Vacuum	High Vacuum	SEM	Contrast					
				Low			High		
				A	B	C	A	B	C
PM	39.59	39.76	0.23			*			*
IAM	37.36	37.89	0.23	*		*	*	*	*
AM+15 s	37.30	37.61	0.23	*	*	*	*	*	*
AM+30 s	37.34	37.35	0.23	*	*	*	*	*	*
AM+45 s	37.49	37.24	0.23	*	*	*	*	*	*
AM+60 s	37.52	37.61	0.23	*	*	*	*		*
AM+75 s	37.70	37.81	0.23	*	*	*	*		*
AM+90 s	37.86	37.94	0.23	*	*	*			*
AM+105 s	38.00	38.20	0.23	*	*	*			*
AM+120 s	38.06	38.36	0.23	*	*	*			*
AM+135 s	38.12	38.48	0.23	*	*	*			*

Contrast: A = TB vs MT; B = MT vs TT; C = TB vs TT *=P<0.05

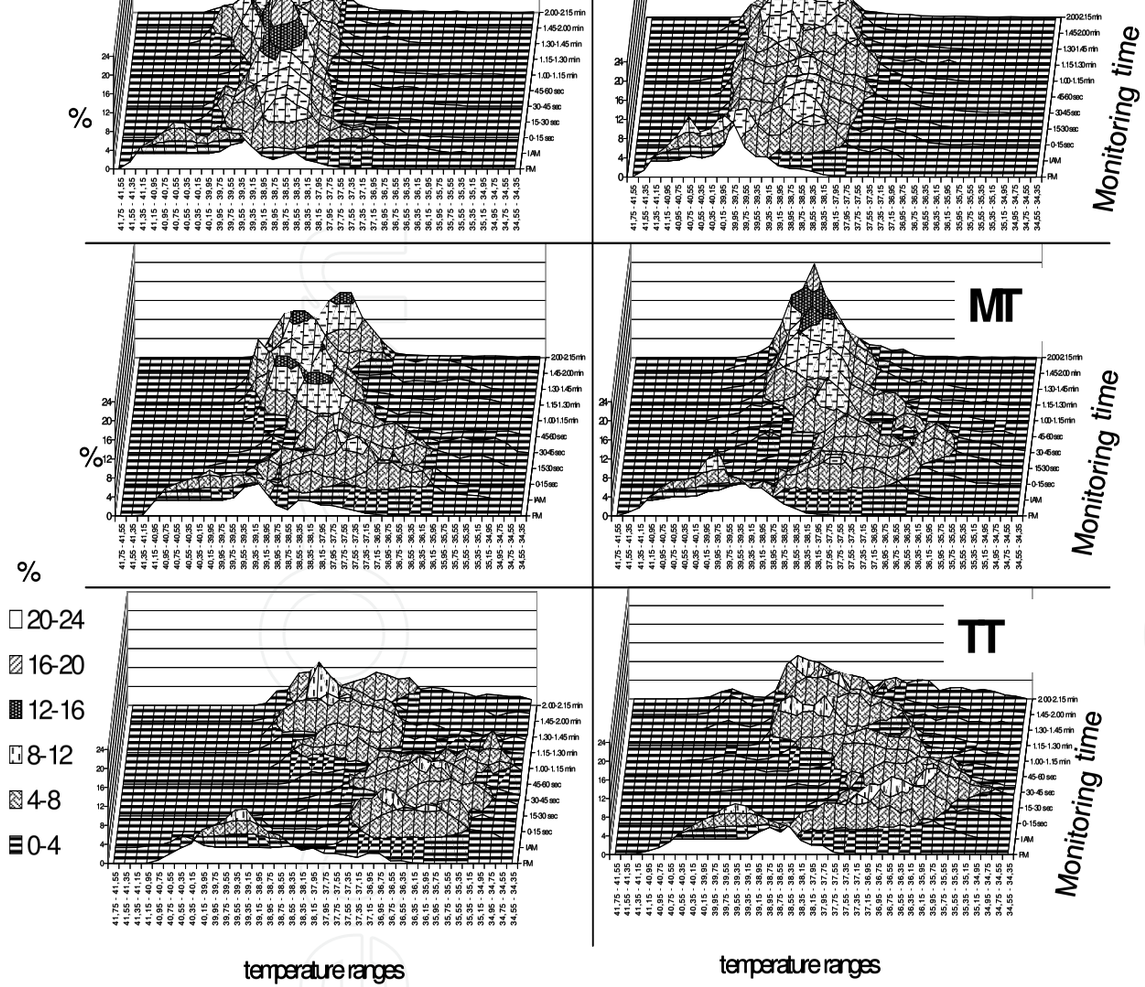
Table 8. Least Squares Means of temperatures of teats milked with low and high vacuum level during milking procedures (pre-milking-PM; immediately after milking-IAM; after milking AM+) and contrast among teat locations.



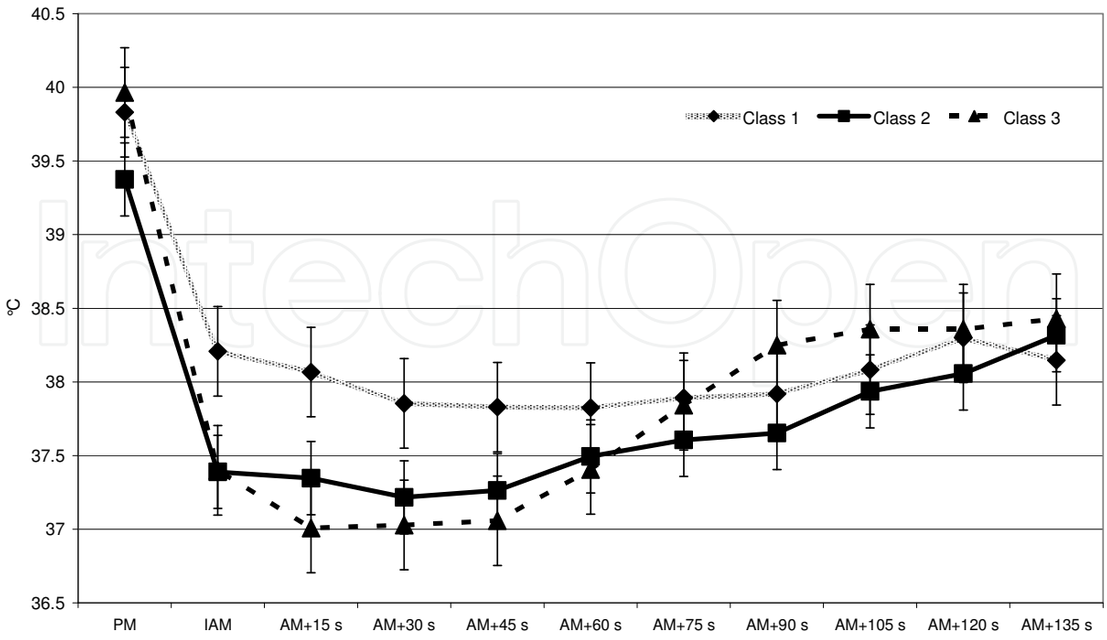
Graphic 9. Temperature variation of teat locations (Teat base-TB, Middle teat-MT, Tip teat-TT) during milking procedure at low vacuum level



Graphic 10. Temperature variation of teat locations (Teat base-TB, Middle teat-MT, Tip teat-TT) during milking procedure at high vacuum level.



Graphic 11. Distribution of frequencies of the temperature ranges (0.2°C) for the teat locations (teat base –TB; mid teat – MT and tip teat – TT) at low and high vacuum levelsduring the milking monitoring time.



Graphic 12. Temperature variation of three phenotypic teat classes during milking monitoring time.

Mechanical milking affects teat temperature of dairy ewes in a different way if compared to the effect on cows, where milking caused a marked increase in teat temperature after an initial drop due to preparation massage (3, 4). In dairy ewes there is no manual udder stimulation before milking and the decrease in teat temperature during milking can be comparable to the effect of the udder massage.

A low vacuum level seems to be more physiological than a high level because the faster recovery time and the more stable temperatures at both TB and MT after milking. Further surveys could provide more detailed information about the role played by machine milking parameters and teat morphology on circulation impairments.

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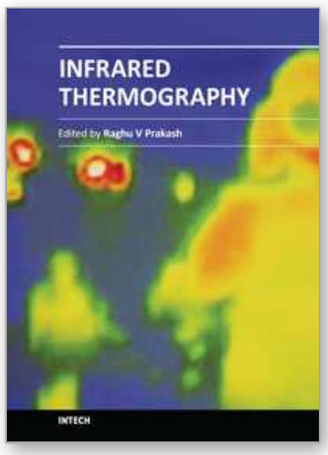

Infrared Thermography
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Infrared Thermography (IRT) is commonly as a NDE tool to identify damages and provide remedial action. The fields of application are vast, such as, materials science, life sciences and applied engineering. This book offers a collection of ten chapters with three major sections - relating to application of infrared thermography to study problems in materials science, agriculture, veterinary and sports fields as well as in engineering applications. Both mathematical modeling and experimental aspects of IRT are evenly discussed in this book. It is our sincere hope that the book meets the requirements of researchers in the domain and inspires more researchers to study IRT.

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