
UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

# VARIATIONS IN THE VULVAR TEMPERATURE OF SOWS AS DETERMINED BY INFRARED THERMOGRAPHY AND ITS RELATION TO OVULATION

VASCO JORGE GASPAR SIMÕES

CONSTITUIÇÃO DO JÚRI

Doutora Graça Maria Leitão Ferreira Dias

Doutora Luísa Maria Freire Leal Mateus

Doutor Fernando Jorge Silvano Boinas

Doutor Guy-Pierre Martineau

ORIENTADOR

Doutor Guy-Pierre Martineau

CO-ORIENTADOR

Doutor Fernando Jorge Silvano Boinas

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DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA

| CONSTITUIÇÃO DO JÚRI | ORIENTADOR |
|---|---|
| Doutora Graça Maria Leitão Ferreira Dias | Doutor Guy-Pierre Martineau |
| Doutora Luísa Maria Freire Leal Mateus | |
| Doutor Fernando Jorge Silvano Boinas | CO-ORIENTADOR |
| Doutor Guy-Pierre Martineau | Doutor Fernando Jorge Silvano Boinas |

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*To my grandfather Abílio.*
*May I, one day, be the man he once was.*

# Acknowledgements

Firstly, to Prof. Dr. Guy-Pierre Martineau, my supervisor and professor in swine medicine in the National Veterinary School of Toulouse (ENVT), France, for accepting me as an intern, for his enthusiastic way to face every challenge and for the will to share all his knowledge. A truly inspiring reference, from both a professional and personal point of view.

Secondly, to Prof. Dr. Fernando Boinas, my co-supervisor and professor in the Faculty of Veterinary Medicine, Technical University of Lisbon, for all his support in the elaboration of this thesis. His professionalism and dedication honor our institution.

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# Abstract

VARIATIONS IN THE VULVAR TEMPERATURE OF SOWS AS DETERMINED BY INFRARED THERMOGRAPHY AND ITS RELATION TO OVULATION.

The productive results of a pig's herd are closely related to the reproductive performance of their animals. Although in the last years several techniques were implemented to improve the reproductive efficiency of pig production, such as artificial insemination (AI) and estrus synchronization, the prediction of ovulation continues to be made with some degree of uncertainty due to the lack of an accurate, practical and fast technique.

In this experimental study, we tested the applicability of infrared thermography (IRT) for ovulation prediction, based on the variations observed in the vulvar skin temperature (VST) during the proestrus and estrus period. The group tested was composed by 36 crossbred Large White x Landrace females, of which 6 gilts and 30 multiparous sows. Estrus detection was performed twice daily in the morning and afternoon, starting one day after weaning (day 1). Temperature measurements were performed every 6 hours at 0000h, 0600h, 1200h and 1800h, from day 1 to day 7. Temperature was obtained from the vulvar area and from two marked spots in the gluteal area (GST), which worked as a control. A third variable (VGT) was obtained from the differential temperature between VST and GST. Ovary ultrasonography was performed in days 5 and 6, in order to detect ovulation; however, the exams were inconclusive and so a theoretical diagnosis of ovulation had to be established based on the weaning-to-estrus interval and the duration of estrus.

The statistical analysis focused mainly in the VGT of two sub-groups of animals, starting estrus at days 4 and 5. The VGT increased progressively during the proestrus ($p = 0.003$ and $p=0.017$), reaching a peak $61 \pm 10.8$ h and $82 \pm 6.6$ h before expected time of ovulation (eOv) in group D4 and D5, respectively. After this point, it decreased significantly ($p = 0.002$), reaching a lowest point $25 \pm 10.8$ h and $28 \pm 6.6$ h before eOv. Although the occurrence of ovulation could not be determined but only estimated, we believe the variations found in the VGT reflect the variations in the estradiol blood levels that will, indirectly, lead to the occurrence of ovulation. Even if no statistical relationship between vulvar temperature and ovulation could be established, the results suggest that these temperature variations may be indirectly related to the occurrence of ovulation.

Keywords: Swine, estrus, ovulation, temperature, thermography, reproduction.

# Resumo

VARIAÇÕES NA TEMPERATURA VULVAR EM PORCAS DETERMINADAS POR TERMOGRAFIA DE INFRAVERMELHOS E A SUA RELAÇÃO COM OVULAÇÃO

Os resultados produtivos de uma exploração de suínos estão intimamente relacionados com o desempenho reprodutivo dos seus animais. Apesar de nos últimos anos várias técnicas terem sido implementadas com vista a melhorar a eficiência deste sector, como inseminação artificial e sincronização do estro, prever a ocorrência de ovulação continua a envolver um certo grau de incerteza, dada a inexistência de uma técnica precisa, prática e rápida.

Neste estudo experimental foi testada a aplicabilidade da termografia de infravermelhos (IRT) para predição da ovulação, tendo por base as variações de temperatura registadas ao nível da região vulvar (VST) ao longo do pró-estro e estro. O grupo-teste era composto por 36 porcas cruzadas Large White x Landrace, das quais 6 eram marrãs e 30 porcas multíparas. A detecção do estro iniciou-se 1 dia após o desmame (dia 1), sendo realizada duas vezes por dia, de manhã e à tarde. As medições de temperatura foram realizadas a intervalos de 6 horas, às 0000h, 0600h, 1200h e 1800h, entre o dia 1 e dia 7. Foi avaliada a temperatura vulvar e de 2 pontos marcados na região gluteal (GST) que desempenharam o papel de controlos. Uma terceira variável (VGT) foi obtida a partir do diferencial de temperatura entre a VST e GST. Com vista a detectar a ovulação, foram realizadas ecografias aos ovários durante os dias 5 e 6; no entanto, os resultados foram inconclusivos, pelo que a ocorrência de ovulação foi estimada a partir do intervalo desmame-estro e duração do estro.

A análise estatística centrou-se sobretudo na variável VGT de dois sub-grupos, compreendendo animais com início de estro no dia 4 e dia 5. A VGT aumentou durante o pró-estro ($p=0.003$ e $p=0.017$ para os grupos D4 e D5), atingindo um pico $61 \pm 10.8$ h and $82 \pm 6.6$ h antes da ocorrência estimada de ovulação (eOv). De seguida, diminuiu significativamente ($p=0.002$), atingindo um valor mínimo $25 \pm 10.8$ h e $28 \pm 6.6$ h antes da ocorrência estimada da ovulação. Ainda que a ovulação não possa ter sido determinada com rigor, entendemos que as variações observadas na VGT reflectem as variações nos níveis sanguíneos de estradiol que, indirectamente, vão levar à ovulação. Assim, apesar não ter sido demonstrada uma relação inequívoca, os nossos resultados apontam para uma possível relação indirecta entre as variações de temperatura vulvar e a ovulação.

Palavras-chave: Suínos, estro, ovulação, temperatura, termografia, reprodução.

# Table of contents

# List of Figures

# List of Tables



# List of equations

# List of Abbreviations and Symbols

AI – Artificial insemination

AVG – Average

ºC – Celsius degrees

CH – Corpora hemorrhagica

CL - Corpora lutea

cm – centimeters

CNS - central nervous system

Cp – Change-point

DD – Differential diagnosis

DE – Duration of estrus

eOv – Expected time of ovulation

f – Frequency of wave

FSH – Follicle Stimulating hormone

g – grams

GST – Gluteal skin temperature

h – hour

ICC - intra-class correlation coefficient

ID – Animal identification number

IgA - immunoglobulin A

IgG - immunoglobulin G

IR – Infrared radiation

IRT – Infrared thermography

LH - Luteinizing hormone

n – sample size

OATD - Osteoarthrosis tarsi deformans

OE – Onset of estrus

Ov - Ovulation

P – P-value

PGF2α - Prostaglandin F2α

PSE – Pale, soft and exudative meat

r – Pearson product-moment correlation coefficient

$R^2$ - Coefficient of determination

$r_i$ – Repeatability index

RT – Room temperature

RTU – Real-time ultrasonography

SD – Standard deviation

SE – Standing estrus

SEM - Standard error of the mean

TAU – Transabdominal ultrasonography

Tg_avg – Average gluteal temperature

Tg_max – Maximum gluteal temperature

Tg_min – Minimum gluteal temperature

TRU – Transrectal ultrasonography

Tv_avg – Average vulvar temperature

Tv_max – Maximum vulvar temperature

Tv_min – Minimum vulvar temperature

US – Ultrasonography

VER – Vaginal electrical resistance

VGT – Difference between VST and GST

VST – Vulvar skin temperature

WEI – Weaning to Estrus interval

Z – Acoustic impedance

ε – Emissivity

λ - wavelength

# 1. Internship report

In order to fulfill the requirements of the Integrated Master in Veterinary Medicine from the Faculty of Veterinary Medicine, Technical University of Lisbon, I've made an internship in the National Veterinary School of Toulouse, France, for 7 months, between September 5, 2011 and March 30, 2012, in a total of 1040 hours, of which results this report.

During this period, I worked in the swine medicine department, under the supervision of Prof. Doctor Guy-Pierre Martineau. During the first 4 months, the majority of work consisted in swine clinic's consultancy. Regarding this consultancy work, we were contacted by practitioners that were facing clinical cases of difficult resolution, usually related to reproductive or nutritional issues. We asked an exhaustive description of these cases, and also the G3T (reproductive performance data) and GTT (technical management data) records of the last 2 or 3 years. I was then responsible for the analysis of this data, establish possible causes for this particular problem and propose corrective measures. After this preliminary analysis was concluded, the team held a meeting to discuss my conclusions and suggest other improvements. In the next phase, when we had a better understanding of the problem, we visited the problematic farms, confirm or deny some of our hypothesis and suggest corrective measures. One month after, the farms were re-evaluated to assess the success of the implemented measures.

Besides this, several other activities were also developed. I gave support to the students during their swine medicine practical lessons, namely in the resolution of clinical cases and in the farms' visits. I visited the SPACE from 13 to 16 September 2012, an international livestock trade show, with a strong presence of pharmaceutical companies, veterinarians and genetics companies, and had the opportunity to share different point of views regarding the swine industry and gather contacts for future projects. I attended the annual congress of the French Association of Veterinarian Swine Medicine, in Paris, between 1 and 2 December 2011, under the subject "Updates in swine pathology" and several other seminars promoted by Pfizer under the swine health subject. Between 31 October and 11 November 2011 I worked with Dr. Jean-Luc Sevin, a pig practitioner working for Triskalia in Brittany, France. During this period, I followed his daily work visiting several pig herds, spent some days with the technicians to learn further about swine nutrition and management and collaborated also with the LDA22, a national laboratory, performing pig's necropsies. Between February and March 2012, I collaborated with Pfizer in the Power Learning Project, creating video tutorials

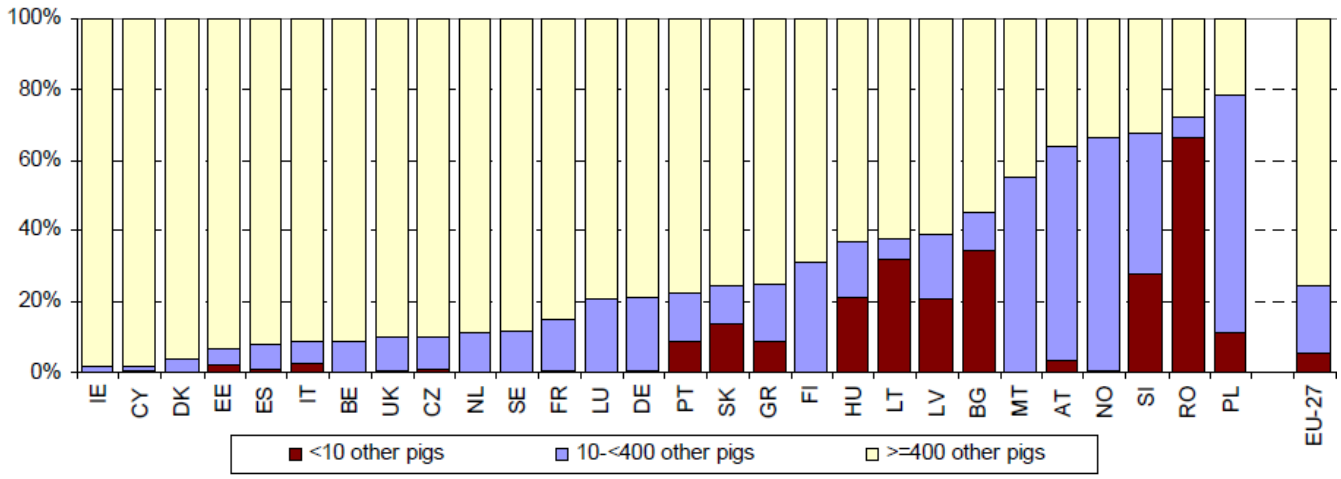
regarding swine medicine issues, based on the principle of the Kahn Academy, a non-profit organization that advocates free knowledge access in a worldwide scale, offering more than 3,000 free micro lectures via video tutorials on their website (www.khanacademy.org), covering various areas like mathematics, history, medicine, biology, astronomy, economics, etc.

In November 2011, with the support of a working team composed by Prof. Dr. Guy-Pierre Martineau, Prof. Dr. Agnès Waret Szkuta, Prof. Dr. Nicole Hagen-Picard, Prof. Dr. Veronique Gayrard and Prof. Dr. Faouzi Lyazrhi, I started the planning of the project that would lead, ultimately, to this thesis. The project took place from January 25, 2012 to February 2, 2012. From February until the end of March, I worked on the results of this project and, at the end of my internship, made a global presentation for my department with the preliminary conclusions of this study. After the conclusion of this internship, I wrote an article regarding the results of this project which is waiting to be peer-reviewed and will be further published in a refereed journal.

# 2. Introduction

In the last 30 years, the pork industry has gone through several major changes. The small and familiar production units, once profitable, now struggle to stay afloat. Market globalization, decreased profit margins and the rule of economies of scale led to a shift in the structure of this sector, with a decrease in the number of farms along the development of bigger production units, with more animals per unit, dilution of production costs and a strong investment in automated systems. In 2010, 1.5% of the largest fatteners were responsible for 75% of the total pork production of the European Union (Eurostat, 2010). This fact is particularly noticeable in countries where pig production has a special relevance like Denmark, Germany, France or Spain (figure 1). Additionally, new improvements in nutrition and genetics and the application of new husbandry practices like artificial insemination, estrus synchronization and ultrasound diagnosis of pregnancy lead to a progressive increase in the productivity of a sector where every small detail counts.

**Figure 1:** Distribution of numbers of fattening pigs by herd size in the European Union. Note the particularly high percentage of pigs concentrated in big herds in countries where pig production is especially relevant like Denmark (DK), Germany (DE), France (FR) or Spain (ES). Adapted from Eurostat, 2010.



## 2.1. Reproductive management as a key factor in the pig's production

The reproductive performance of the sows is one of the key factors for the farm's economic viability. It is usually evaluated based on the number of piglets produced per sow per year or the number of farrowings per sow per year. Other factors that should also be considered include litter size, fertility, fecundity and number of non-productive days (Ate & Oyedipe, 2011).

Optimal fertilization results are achieved when insemination is performed in the 24 hours period prior to ovulation (Soede, Wetzels, Zondag, de Koning and Kemp, 1995; Cassar, 2006). However, timing of ovulation vary largely among animals (ranging from 10 h to 85 h after onset of estrus, according to the Soede and Kemp, 1997) and the available methods to successfully predict its occurrence are still difficult to perform and of limited applicability in the farm daily routine, as they are very time-consuming and involve advanced training by their operators.

Given this, the timing of ovulation is usually estimated based on the weaning-to-estrus interval (WEI) and relies on an accurate detection of heat by the farmer and the boar. A deep knowledge and understanding of the behavioral and physical signs associated with the OE is of the upmost importance, so females are not erroneously classified. Nevertheless, this method still involves a certain level of uncertainty, as a slight variation in the occurrence of ovulation is always expected, even in females with similar WEI.

If AI is performed more than 24 hours before ovulation, the semen's viability at ovulation will be reduced, resulting in a lower fertilization rate and therefore a lower number of normal embryos (Soede, Langendijk and Kemp, 2002). To avoid this, multiple inseminations are employed as a standard swine practice, so that at least one matches that 24 hours period. This obvious has its drawbacks, since it is a time-consuming task and involves the risk of performing post-ovulation inseminations, which will induce a uterine inflammatory response and consequently early embryonic loss, prolonged estrus interval and also an increase of abnormal vaginal discharges (Rozeboom, Troedsson, Shurson, Hawton & Crabo, 1997; Kaeoket, Tantasuparuk & Kunavongkrit, 2005). The critical point is, thus, to find a simple, fast and inexpensive method that can not only detect ovulation, but above all predict its occurrence so the AI can be performed at ideal time.

In this context, infrared thermography (IRT) may prove to be a valuable tool. IRT is a noninvasive and accurate technique through which surface temperatures are monitored and

recorded. It can detect slight changes in the superficial body temperature without inducing stress in the animals, as it does not require contact with the object to perform these measurements. It is intuitive and does not require advanced knowledge to be performed.

During the proestrus and early estrus, the vulvar area goes through several physical changes, becoming noticeably more red and swollen during proestrus, signs that begin to subside 24 to 36 hours before standing estrus (Worwood, 2007). These signs are closely related to hormonal changes, particularly the estrogen levels (Stelletta, Gianesella, Vencato, Fiore and Morgante, 2012). Since these changes are due to an increased local blood flow, resulting in a concomitant increase in the local temperature, IRT might detect these temperature fluctuations and, indirectly, infer the variations in the hormonal levels that will eventually lead to ovulation.

## 2.2. Goals of this study

With this study, we intended to answer several questions, with special focus on the following points:

- Assess the applicability of infrared thermography to evaluate the vulvar skin temperature of pigs.
- Assess variations in the vulvar skin temperature of pigs during proestrus and estrus.
- Establish a possible relationship between these temperature variations and ovulation.
- Evaluate the usefulness of this technique to predict the occurrence of ovulation under the farm's daily routine.
- Compare the accuracy and applicability of transabdominal and transrectal ultrasonography to visualize the ovaries of sows and gilts and detect ovulation.

# 3. Literature Review

## 3.1. Anatomy of the reproductive organs

The reproductive tract of the sow is composed by a pair of ovaries, two uterine tubes (also called oviducts), the uterus, the vagina and the vulva (figure 2). These organs present important morphological variations depending on the sows's age, breed and phase of the estrous cycle (Martinat-Botté, Renaud, Madec, Costiou & Terqui, 1998). In table 1, the differences between immature and mature females are evident.

**Figure 2:** Anatomy of the reproductive tract of a sow and adjacent structures (adapted from McCracken, Kainer & Spurgeon, 1999).

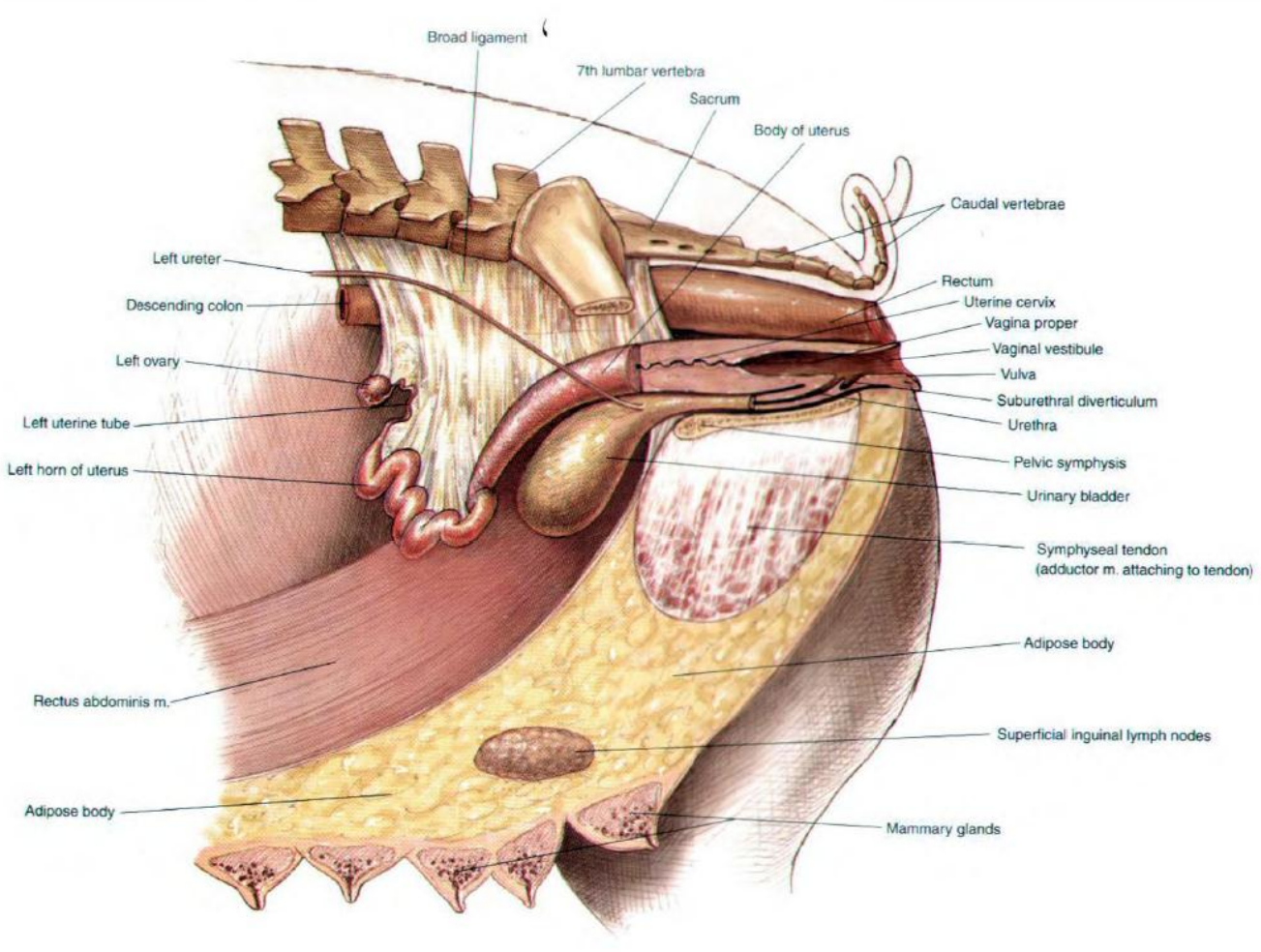


**Table 1:** Average weight and length of reproductive organs at different stages of the sows' sexual life (adapted from Martinat-Botté et al., 1998).

| Parameters | Immature | Prepubertal | Pubertal-Cyclic |
|---|---|---|---|
| **Weight of both ovaries (grams)** | 6.3 | 6.5 | 10-20 |
| **Length of the uterine horn (centimeters)** | | | 20-55 |
| **Uterus Weight (g)** | 66 | 90 | 350-900 |
| **Uterus Length (cm)** | 104 | 106 | 220-430 |
| **Length of the uterine cervix** | | 21 | 25 |
| **Vagina Weight (g)** | 60 | 66 | 67 |
| **Vagina Length (cm)** | 21 | 21 | 21 |

### 3.1.1. Ovaries

The ovaries are the primary organs of reproduction in the female. They have both endocrine and gametogenic functions as they produce hormones, such as estradiol and progesterone, and ova, which are released from the surface of the ovary during ovulation (Frandson, Wilke & Fails, 2009). The aspect of the ovary changes significantly depending on its physiological state, being its surface generally uneven because of the many protruding ovarian follicles and/or corpora lutea. Prior to ovulation several finely vascularized pre-ovulatory follicles measuring between 0.8 and 1 cm in diameter are visible on the surface of the ovary while 2 days after its surface is characterized by purple corpora lutea with visible follicle rupture points (Kyriazakis & Whittemore, 2006).

The ovaries are surrounded by a dense connective tissue capsule, the tunica albuginea, and are composed by medulla and cortex. The medulla, or central portion, consists of connective tissue and extensive vascular and nervous systems, while the cortex, or outer portion, consists largely of dense, irregular connective tissue interspersed with follicles and/or corpora lutea at various stages of development or regression (Frandson et al., 2009).

### 3.1.2. Uterine tubs

The two uterine tubs (previously known as fallopian tubes or oviducts) are responsible for conducting the ova from each ovary to the respective horn of the uterus and are the usual site of fertilization of ova by the spermatozoa (Frandson et al., 2009). They have a long, convoluted shape and are composed by three functional segments: infundibulum, the free cranial extremity funnel-shaped opening near the ovary; ampulla, a slight dilated section that accounts for about half the length of the oviduct; and finally the isthmus which connects the uterine tubs with the uterine lumen (Kyriazakis & Whittemore, 2006).

### 3.1.3. Uterus

The pig uterus is composed by two uterine horns, body and cervix, consisting of three different layers, from the outside towards the inside: serosa (perimetrium), muscular (myometrium), and mucosa (endometrium, the internal secretory layer) (Dyce, Sack & Wensing, 2009). The uterine horns contain many folds and may be up to 2 meters long, converging in the short and narrow uterine body. The cervix, longer than the uterine body, connects the uterus and the vagina. Its internal wall delimits the cervical canal, which contains a corkscrew arrangement of ridges or annular rings well adapted to the spiral twisting of the boar's penis. The cervix has a sphincter-like function, being usually tightly closed except during estrus and parturition (Martinat-Botté et al., 1998; Kyriazakis & Whittemore, 2006).

### 3.1.4. Vagina, vestibule and vulva

The vagina is about 20 cm long and has thick walls that get thinner towards the vestibule, its caudal portion in which the urinary meatus is located (Martinat-Botté et al., 1998). The mucosa is lined by a stratified squamous epithelium, with the glands confined to its cranial portion. In pigs, a short blind sac called suburethral diverticulum can be found ventral to the opening of the urethra (Dyce et al., 2009).

The vulva is the external part of the female's reproductive tract and consists of major and minor labia, meeting dorsally and ventrally to form the dorsal and ventral commissures. The ventral commissure is usually somewhat pendulous and conceals the clitoris (Frandson et al., 2009). At the onset of estrus, the high estrogen levels promote the swelling and reddening of the vulva and clitoris, typical signs of upcoming standing heat (Worwood, 2007).

## 3.2. Physiology of the estrous cycle

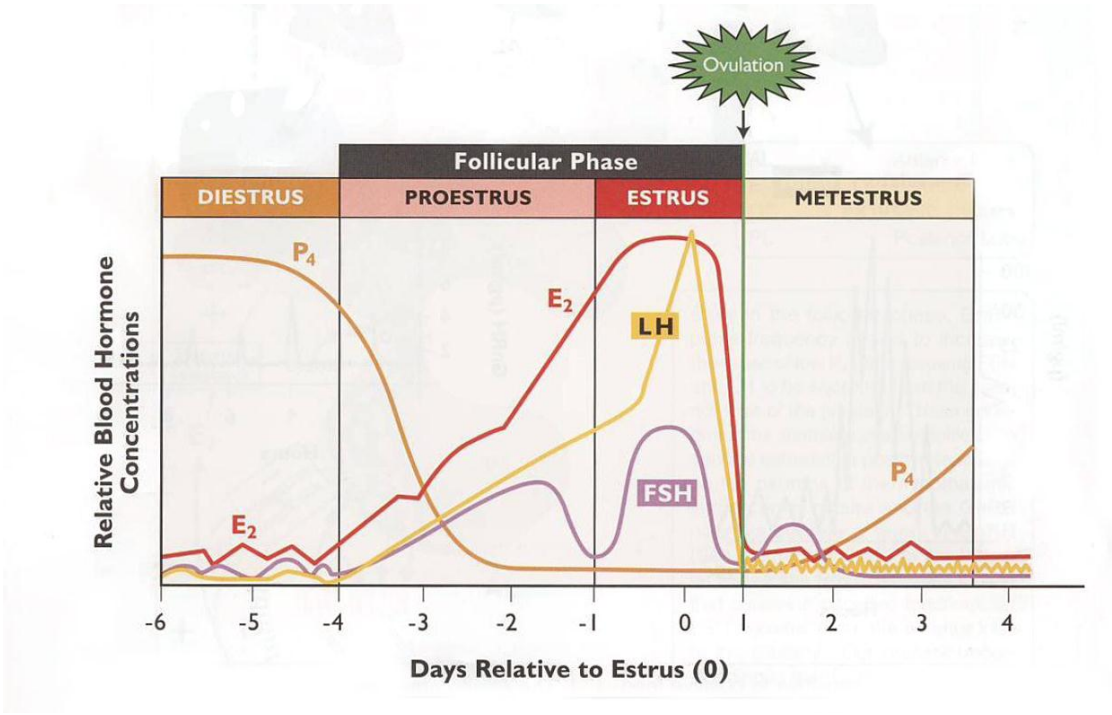
The domestic sow is a polyestrous animal with an average length of the estrous cycle around 21 days (Kyriazakis & Whittemore, 2006). Contrary to their wild ancestors which tended to mate in early winter and farrow in late spring, the nowadays sow doet show a striking seasonal infertility, although some negative effects in farrowing rate, weaning-to-estrus interval and age of puberty in gilts have been reported during late summer and early autumn (Peltoniemi, Love, Heinonen, Tuovinen & Saloniemi, 1999).

According to Tummaruk, Tantasuparuk and Kunavongkrit (2008), the age of puberty in gilts is usually defined as "the time of the first estrus and ovulation with a continuation of regular estrous cycles". It largely differs among breeds, with the average being highest in Duroc and lowest in Meishan (Bidanel, Gruand & Legault, 1996; Evans & O'Doherty, 2001). Other factors influencing the attainment of puberty include nutrition, live weight, boar exposure, confinement, season and disease (Faillace, Biggs & Hunter, 1994; Tummaruk, Tantasuparuk, Techkumphu & Kunavongkrit, 2004). In average, it occurs between 5 and 8 months of age in the different European breeds (Martinat-Botté et al., 1998). Although the physiological mechanisms responsible for the onset of puberty remain partially unclear, the luteinizing hormone (LH) appears to play a key role in the control of ovarian development and, hence, the age at puberty in gilts, with a progressive increase in mean LH concentrations and LH pulse frequency before puberty, leading to the final maturation of ovarian follicles and, ultimately, the first ovulation (Evans & O'Doherty, 2001). Evidences suggest that the adenohypophysis is capable of releasing FSH and LH before GnRH becomes available to stimulate their release, however contrary to the LH, no relationship was found between the levels of FSH and the onset of puberty (Evans & O'Doherty, 2001; Frandson et al., 2009).

### 3.2.1. Phases of the estrous cycle

The estrous cycle can be divided into two distinct phases according to the dominant ovarian structure: the follicular phase (approximately from day 1 to day 7) and the luteal phase (approximately from day 8 until day 21) (Kyriazakis & Whittemore, 2006). The follicular phase, incorporating proestrus and estrus, starts with the regression of the corpora lutea and extends until ovulation. The luteal phase, from ovulation until corpora lutea regression, incorporates metestrus and diestrus and is characterized by the active secretion of progesterone by the corpora lutea (figure 3) (Martinat-Botté et al., 1998; Senger, 1999).

**Figure 3:** Phases of the estrous cycle and hormonal profiles (adapted from Senger, 1999).



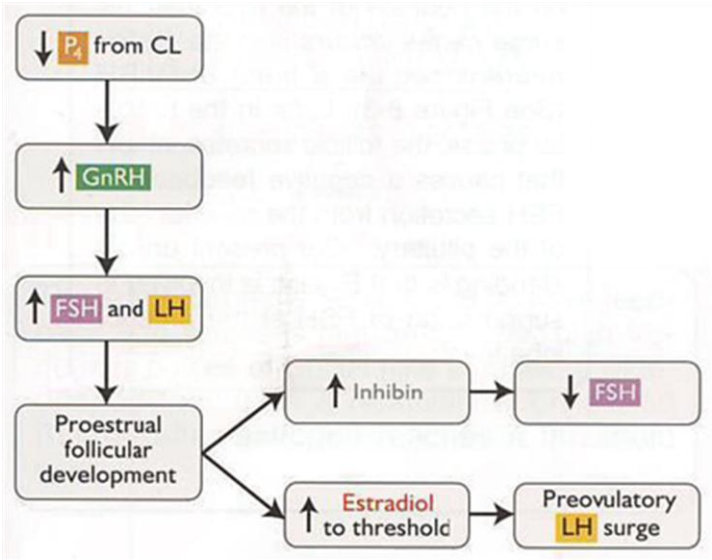

### 3.2.1.1. Proestrus

The first phase of the estrous cycle (proestrus) is characterized by a rapid follicular growth. Right after the lysis of the corpora lutea at the end of diestrus, by the influence of prostaglandin F2α (PF2α), the progesterone levels start to subside, which releases the hypothalamus from its negative feedback inhibition (figure 4). Consequently, GnRH is released from the hypothalamus with increasing amplitude and frequency, which will stimulate the adenohypophysis to produce follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Pineda & Dooley, 2002). These hormones will promote the growth of the follicles, which by their turn will produce increasing amounts of estrogen, responsible for the onset of sexual receptivity and several physiological changes like thicken of the vaginal walls and reddening and swelling of the vulva (Coffey, 1997; Cochran, 2011).

The dynamics of antral follicles are composed by four processes: recruitment, selection, dominance and atresia (Senger, 1999). By the start of proestrus the numbers of small and medium follicles rapidly decline and a cohort (group) of about 50 antral follicles with 1-6 mm is recruited for further growth under FSH stimulation and estradiol production (Knox, 2005; Schwarz, Kopyra & Nowicki, 2008). After the recruitment phase the follicles will go through a selection, in which the dominant follicles, 3-4 mm bigger than the subordinate ones, start to produce inhibin which will inhibit the FSH secretion from the adenohypophysis. The drop in

the FSH levels, coupled with a reduced blood supply to the smaller follicles, results eventually in atresia of the non-dominant follicles. Only the bigger follicles receiving a large blood supply and, thus, higher levels of gonadotropin, will continue to grow (Senger, 1999). Another key factor in the dominance dynamic is the shift on the follicle's dependence from FSH to LH. Along with the decrease of the FSH levels due to the influence of inhibin, there is a simultaneous increase in the number of LH receptors on the granulosa cells of the dominant follicles. This way, these follicles shift from an FSH-dependency to an LH-dependency state, while the smaller follicles, with fewer LH receptors, eventually go into atresia (Zhenzhong, Garverick, Smith, Smith, Hamilton & Youngquist, 1995; Driancourt, 2001).

**Figure 4:** Events occurring during proestrus that lead to the preovulatory LH surge (adapted from Senger, 1999).




### 3.2.1.2. Estrus

Estrus is, by definition, the period of sexual receptivity. It is characterized by a number of morphological and behavioral changes, induced by the high estrogen levels, which will be discussed in further detail on Chapter 3.3 "Estrus expression". The duration of estrus in sows can vary from 24 to 96 h (Weitze, Wagner-Rietschel, Waberski, Richter & Krieter, 1994; Soede et al., 1995), being influenced by several factors like weaning-to-estrus interval (WEI), parity, boar effects, season and stress (Weitze et al., 1994; Soede & Kemp, 1997).

At this phase, the dominant follicles are producing increasing amounts of estradiol and inhibin, responsible for suppressing the FSH secretion from the adenohypophysis, while the

LH is progressively increasing. When the estradiol levels reach a threshold in association with the low progesterone levels, the hypothalamus is stimulated to release a large amount of GnRH, which will by its turn induce a sudden LH surge secreted by the adenohypophysis (Senger, 1999). This surge occurs 26 to 34 h before ovulation and it is directly responsible for the onset of ovulation, involving a complex interaction of proteolytic enzymes, PGF2α, Interleukin-1, histamine, among others (Soede et al., 1994; Tsafriri, 1995; Senger, 1999).

Ovulation occurs usually 36 to 40 hours after onset of estrus, although it might range from 10 to 85 hours (Soede & Kemp, 1997; Gotszling & Baas, 1998), being affected by variables like season, parity, genotype and WEI (Knox & Zas, 2001; Belstra, Flowers & See, 2004). It might last from 1 to at most 7 hours, being in average 2 or 3 hours long (Soede, Hazeleger & Kemp, 1998).

#### 3.2.1.3. Metestrus

Metestrus is the transitional period between ovulation and the full development of corpora lutea (CL). After ovulation, the estrogen levels start to subside and progesterone levels increase progressively, produced by the CL under development. This will eventually switch the organism from an estrogen-dominance to a progesterone-dominance, characteristic of the luteal phase (Pineda & Dooley, 2002).

After ovulation, and as a result of the vascular rupture on the follicles, each follicle gives place to a new structure, the corpora hemorrhagica (CH), which appear as small, hemorrhagic structures at the surface of the ovary. Under the influence of LH, the granulosa cells and some theca cells of these structures go through a process known as luteinization (Senger, 1999). At the end of metestrus the fully functional CL had lost their hemorrhagic appearance and produces actively progesterone, the dominant hormone of the luteal phase.

#### 3.2.1.4. Diestrus

Diestrus is the longest stage of the estrous cycle, extending from about day 3 through day 13-15 post-ovulation. It is characterized by the presence of fully functional CL and high levels of progesterone (Senger, 1999).

After the complete maturation of the CL under the influence of LH, the progesterone levels keep increasing, reaching a peak 11 to 13 days after ovulation (Kyriazakis & Whittemore, 2006). The high levels of progesterone found during this stage play a crucial role in the

preparation for the upcoming pregnancy. On the central nervous system (CNS) exerts a negative feedback in the hypothalamus, suppressing the release of GnRH and, indirectly, FSH and LH; at the uterus, stimulates the production of glandular secretions from the endometrial glands and inhibits the contractibility and tone of the myometrium; finally, stimulates the development of the alveolar growth of the mammary gland, allowing initiation of lactation (Senger, 1999).

At the end of diestrus, if a successful pregnancy is not established, the CL will go through a regression process called luteolysis due to the action of $PGF_{2\alpha}$ produced by the endometrium. The CL decrease in size, the capillaries undergo degeneration and the lutein cells are replaced by fibroblasts. The remaining structure named corpus albicans is visible as a connective tissue scar at the surface of the ovary (Pineda & Dooley, 2002; Miyamoto & Shirasuna, 2009).

With the regression of all CL, the progesterone levels fall and the inhibition of the hypothalamic-pituitary axis is disrupted, allowing the start of a new cycle.

## 3.3. Estrus expression

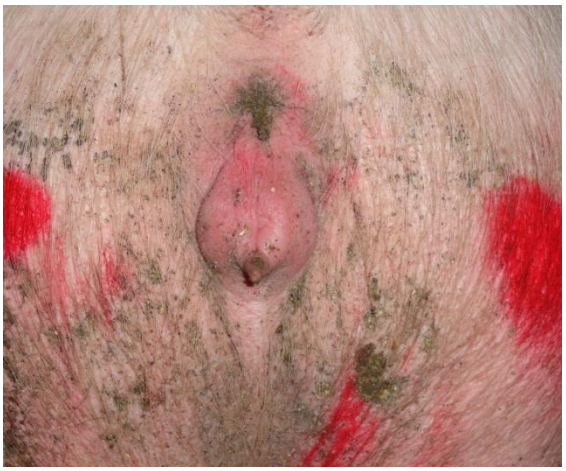
Detection of estrus is one of the most critical components of a successful swine breeding program. With the widespread use of artificial insemination (AI), the responsibility of detecting estrus has shifted from boar to breeding technician. An accurate detection of estrus will ensure an increase in the herd's reproductive performance and a decrease in the non-productive days. To achieve this, a good knowledge of all the typical signs related to estrus expression is fundamental.

Estrus is, by definition, "the time during the reproductive cycle in animals when the female displays interest in mating and will stand to be mounted" (Blood & Studdert, 1999). The physiological and behavioral changes observed during this period are closely related to the high estrogen and low progesterone levels (Pederson, 2007; Cochran, 2011). During the mid to late proestrus, as the production of estrogen increases from the growing follicles, several physiological changes occur in the reproductive tract due to an increase in the local blood flow, causing genital swelling and change in tissue electrical conductivity, increased mucosal secretion, uterine gland growth and increased uterine tone (Senger, 1999).

### 3.3.1. Physiological Signs

The swelling of the vulva is, perhaps, the most noticeable sign of the upcoming estrus in pigs (figure 5). Reddening and swelling of the vulva appears 2 to 5 days before standing heat and begin to subside in the 24 to 36 hours prior to standing heat (Worwood, 2007). It is due to an increased local blood flow that increases the local capillary pressure and causes lymph to buildup in the external genitalia, causing a local retention of fluids. These signs might be less pronounced in older parity sows since, although the described physiological changes occur the same way, the change in color and size is masked by their loose, flaccid skin around the vulva, as a result of the numerous farrowings (Diehl, Day & Flowers, 1998). These changes extend also to the clitoris, causing it to protrude outward, engorged with blood, and to the cervix, ensuring an effective "lock" for the boar's penis and, thus, an effective insemination into the body of the uterus (Diehl et al., 1998; Senger, 1999).

**Figure 5:** Vulva of gilt in proestrus, with obvious reddening and swelling. Original image.



A clear and sticky mucus discharge is often present just before and during standing heat. As the estrus approaches, this mucus increases in thickness, conductivity and pH (Belstra, Flowers, See & Singleton, 2008). This mucus acts as lubrication during copulation and promotes sperm penetration of the utero-cervical junction; additionally, it has also an important role on the local immunity, since it acts as a barrier to contamination of uterus by flushing out foreign material introduced during copulation and by supporting phagocytic activity of the neutrophilic granulocytes (Branscheid & Holtz, 1988; Senger, 1999).

In the uterus, estrogen causes increased tone due to myometrial contractions and also an increased motility of the muscularis, partially responsible for sperm transport to the uterine tubes (Senger, 1999).

A special reference must be made to the regulatory role of estrogens in the uterine local immunity. Several studies proved the positive impact of estradiol in the uterine immunity, due to an increase in the concentration of immunoglobulin A and G (IgA and IgG) on the uterine lumen (Wira & Sandoe, 1980; Wira & Sullivan, 1985; Wira & Stern, 1986). This increased local immunity, associated with the above-mentioned functions performed by the uterine mucus, prepares the uterus for the upcoming fertilization removing all the unwanted agents that might threaten the success of fertilization and implantation.

Finally, under the influence of estrogen, the uterine tube's epithelium increases its secretory rate and their ciliated cells increase their motility, facilitating gamete and fluid transport (Senger, 1999).

### 3.3.2. Behavioral Signs

According to Beach (1976), the estrus in female mammals can be divided in two components: the proceptive and the receptive behavior. Proceptive behavior corresponds to the "various reactions by the female toward the male which constitute her assumption of initiative in establishing or maintaining sexual interaction", while the receptive component connotes "female responses necessary and sufficient for the male's success in achieving intravaginal ejaculation" (Beach, 1976).

The first behavioral sign that sows are coming into heat is an increase in activity and vocalization, increased restlessness and reduced appetite (Diehl et al., 1998; Almond, Flowers, Batista & D'Allaire, 2006). When housed in crates, sows often increase their forward-backward or lateral movements and attempt to nibble or nose the adjacent females. Additionally, they might paw at the front door of the crate and "chant" to animals in front of them; when housed in pens, typical activities include sniffing and nuzzling the rear and fore flanks (Diehl et al., 1998).

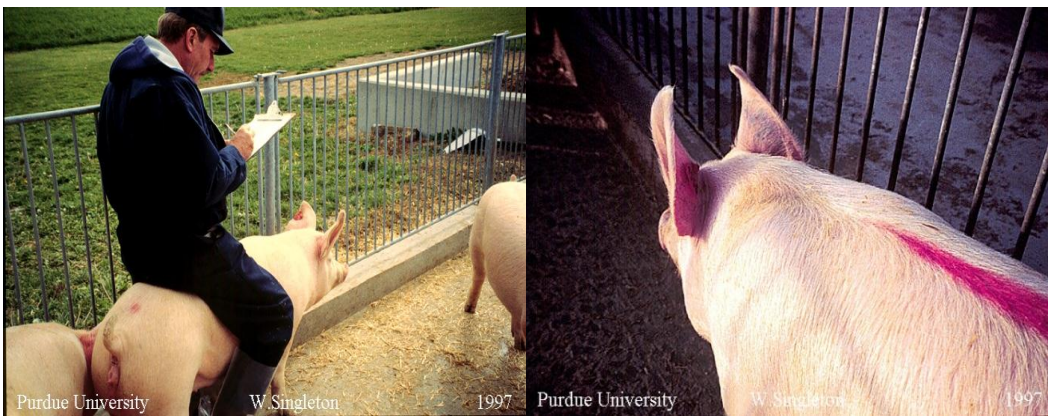
A typical sign of sows coming into or going out of estrus is trying to mount and ride other females. The behavior of the mounted female may differ: if not in standing heat, she will not tolerate being mounted and will vocalize loudly and try to escape; if she's already in standing heat, she will stand immobilized (Belstra et al., 2004). These behavioral changes have

presumably the purpose of attracting the attention of the boar and stimulate his sexual activity, as already demonstrated in goats (Shearer & Katz, 2006).

When in presence of a boar, the female will actively try to approach him and if possible, will spend more time close to his pen (Pedersen, 2007). The visual, olfactory, auditory and tactile stimuli of the boar are one of the critical factors responsible for a full and effusive expression of estrus by the female (Zink & Diehl, 1984; Langendijk, van den Brand, Soede & Kemp, 2000; Kemp et al, 2005). The male pheromone 5-α androsterone, produced in the testis and salivary glands, is one of the responsible components for the female's sexual receptivity, triggering one of the most unequivocal signs of estrus, the standing reflex (Diehl et al., 1998; Gerritsen, Langendijk, Soede & Kemp, 2005). When exposed to a boar, females will assume a rigid, motionless stance, in anticipation of being mounted known as standing reflex or immobilization response. Their hind limbs start isometric muscle contractions, their back arches slightly and in some breeds the ears are hold erected, known as "ear popping" (figure 6) (Belstra et al., 2004). The standing reflex assumes a particular importance in heat detection, as it is the reference point upon which most breeding protocols are based. In order to detect the standing reflex, a method called "back-pressure test" is used, which consists in pushing down on the animal's loin with both hands or sitting on the loin (figure 7); if the female is in heat and standing reflex is present, the animal will not try to avoid this contact and the above-mentioned signs might be visible (Worwood, 2007). This test should be performed ideally with the presence of the boar, if possible with nose-to-nose contact, as it increases significantly the expression of these signs, improving by 30% to 40% the chance of identifying females in estrus. If a boar stimulus is not provided, the standing reflex may not be detected, especially in the early and late estrus (Diehl et al., 1998).

**Figure 6 (right):** Female in estrus showing "ear popping" (adapted from Singleton, 1997).

**Figure 7 (left):** Female in standing reflex (adapted from Singleton, 1997).



Since this immobilization response requires a great expenditure of energy to support the muscle contractions, this state will last in most animals only 15 to 30 minutes, after which muscles become fatigued and the animal enter a refractory period that last for a few hours (Levis & Hemsworth, 1995). If back-pressure test is performed during the refractory period, it will result in a negative diagnosis and this animal will be erroneously classified as not in estrus. To avoid this, the contact of the male should be restricted to a small number of females, either 5 to 10 crates or 1 to 2 pens, so the estrus detection can be made simultaneously with the boar stimulation (Knox, 2008).

## 3.4. Alternative methods for estrus detection

The classical and most used method to detect females in standing heat is looking for all the signs already mentioned, using simultaneously a mature boar to stimulate the exhibition of these signs by the females. The success of this method is greatly dependable on the experience of the herdsman recognizing the estrus behavioral signs and on a correct management of the boar. Sometimes females will cycle and ovulate without showing obvious behavioral signs (known as silent estrus) and so a good knowledge of the physiological changes is also required (Napel, Kemp, Luiting & Devries, 1995). When the first signs of estrus are detected in a female, some producers opt, additionally, to take her individually to the boar pen, in order to allow a stronger stimulation and thus confirm the diagnosis (Senger, 1999). Estrus detection should be performed twice daily, as close as possible to 12 hours intervals; this will eliminate most cases of false estrus, since sows that are not truly in estrus will rarely stand for two consecutive exams (Worwood, 2007).

All these procedures are obviously very laborious and, with the continuous increase of the pigs' herd size, they turn to be very time-consuming and labor-demanding. Moreover, these methods are particularly difficult to apply in sows housed in pens or open spaces, due to the difficulty to restrain the animal movements and identify these behavior signs.

Thus, other methods to identify females in estrus were developed, some of them perfectly tailored to use routinely, while others more focused on experimental trials. Both non-automated and automated methods will be briefly discussed, with more emphasis given to the latter due to their significantly higher importance in the pig industry.

### 3.4.1. Non-automated methods

#### 3.4.1.1. Measurement of Vaginal Electrical Resistance

In the last decades, several reports have described significant changes in the electrical resistance of the vaginal mucus (VER) associated with day of the estrous cycle (Ko, Evans & Hopkins, 1989; Szenci, Sima, Hartmann & Keresztes, 1990). However, several authors stand that although VER increases slightly during estrus, there is a considerably variation between animals and this method cannot be used as a stand-alone reliable tool to fix the proper time to inseminate (Harbison, Kirkwood, Aherne & Sather, 1987; Charuest, Dufour, Savoie & Richard, 1990; Stokhof, Soede & Kemp, 1996).

#### 3.4.1.2. Ultrasonography

The use of ultrasonography in the porcine reproduction field brought a great understanding of the follicle dynamics and its relation with estrus expression. Although mainly used as a pregnancy diagnosis tool, ultrasonography is frequently applied in experimental studies to monitor follicle development or detect ovulation (Knox & Althouse, 1999; Kauffold & Althouse, 2007). This subject will be discussed in further detail in chapter 3.5.4 “Applications of ultrasonography in swine industry”.

### 3.4.2. Automated methods

The automated estrus detection methods assume special relevance in the current pig production industry given the progressive increase in the size of the herds and the growing need for methods that can reduce the dependence of human labor. The recent developments in legislation about animal welfare and the increasing awareness of the consumer for this question as led to a rise in the group-housing facilities, bringing additional difficulties to the classical estrus detection methods and giving space to improvements in the detection methods. The use of automated mechanisms may alert the producer, for instance, for a sow that is returning to estrus in a group of served females, leading this way to a precocious detection of fertilization failure and, thus, avoid a greater increase in the non-productive days.

#### 3.4.2.1. Accelerometer

An accelerometer is a device that detects movement and measures the accumulated distance over time.

One of the first signs of a sow approaching heat is an increased activity, restlessness and exploratory behavior (Diehl et al., 1998). When applied an accelerometer to a female (in pigs, usually in a neck collar) this device will detect the increased movement when the animal is getting close to heat and thus the producer can focus its attention in the suspected animals.

Bressers (1993) tested this method in group-housed sows and reported that this method could reduce 10 to 15% of the check-ups when compared to no automated system. In 1995, Geers et al. (cited by Cornou, 2006) tested its applicability in crate-housed sows and reported physical activity to be 10 times higher the day before estrus.

#### 3.4.2.2. Infrared sensor

Freson, Godrie, Bos, Jourquin and Geers (1998) tested the use of infrared sensors to quantify the movement of sows housed in crates. These sensors were installed 50 cm above the sow's shoulders and the movement was detected based on a change in position of the body in relation to the sensor. The authors reported that 86% of the sows were correctly classified when using the mean daily activity as the selection parameter, with 79% sensitivity and 68% specificity.

#### 3.4.2.3. Visit to the boar pen

One of the components of the sexual behavior of the estrous female is, as already mentioned, the proceptive behavior, which can be described as "the tendencies to 'search for the boar', 'stay close to the boar' and 'present' in front of the boar" (Cornou, 2006). Based on this, some automated estrus detection systems have been developed to evaluate the interest of the females by the boar and the time spent close to him. The boar is housed in a separate pen from the sows but nose-to-nose contact is possible. When a female approaches the boar to establish contact, a transmitter–receiver device reads their individual identification and sends to a central computer the frequency and length of the visits. Using this method, Bressers, Te-Brake and Noordhuizen (1991) were able to detect 95% of the sows that came into estrus, while Ostersen, Cornou and Kristensen (2010) reported a sensitivity of 87.4% and a

specificity of 99.4%. Several other studies confirmed the usefulness of this method as a complementary tool in estrus detection (Blair, Nichols & Davis, 1994; Korthals, 1999).

A major limitation of the methods based on behavioral traits may be related to motility problems such as lameness and hierarchical conflicts observed within group housed sows. These factors may either increase or decrease the physical activity and the frequency of visits to the boar pen and therefore impair the results of these methods (Cornou, 2006).

#### 3.4.2.4. Body Temperature

Several studies reported significant changes in the body temperature around the time of estrus, but their results are often discordant and so the applicability of this method remains unclear.

The temperature records are usually obtained from implants placed in the base of the ear or inside the vagina. Junge and Holtz (1984) measured intravaginal temperature of sows and cows and found a defined pattern in relation to estrus, with the temperature reaching the lowest value 2 days before standing estrus (SE) and the highest value 2 days after SE. Regarding the ear base temperature, Geers et al. (1995) found a significant rise in 75% of the sows until 2 days prior the standing reflex.

Henne (1991, cited by Soede, Hazeleger, Broos & Kemp, 1997), by his turn, measured the rectal temperature twice daily and found a large variability between animals: in 30% of the gilts the temperature rose at onset of estrus, in 20% remained stable and declined in 50% of the animals.

Given the still remaining variations in terms of results between different studies, the monitoring of body temperature is not used yet to detect estrus in swine herds.

## 3.5. Ultrasonography

### 3.5.1. Historical background

Diagnostic sonography, or simply ultrasonography (US), is a diagnostic imaging technology used to visualize internal organs using high frequency sound waves, usually between 2 and 18 Mhz (Burk & Feeney, 2002). The use of ultrasound in the human medical field dates back to 1940, when it was used not only as a diagnosis tool, but also as a therapy for several conditions, such as arthritic pain, gastric ulcers, eczema or urinary incontinence (Woo, 2002).

In fact, this technology was seen almost as a "cure-all" remedy, despite the lack of scientific evidences. Karl Dussik (1908-1968), a neurologist from the University of Vienna, was the first physician who employed ultrasound in medical diagnosis, in order to locate brain tumors and the cerebral ventricles (Man & Karmakar, 2006; Suetens, 2009).

In 1980, Palmer and Driancourt reported for the first time the use of an ultrasound imaging system as an early pregnancy diagnosis method in mares (Kähn, Volkmann & Kenney, 2004). This was the first step for the use of ultrasonography in animal reproduction.

Today, ultrasonography is routinely used in many species for reproductive and obstetric exams. In pigs, it assumes particular importance as a pregnancy diagnosis tool, being nowadays an absolute requisite for the modern pig industry (Williams, Piñeyro & de la Sota, 2003; Boulot, 2010; Medan & Abd El-Aty, 2010).

### 3.5.2. Basic principles

Ultrasonography is based in the pulse-echo principle, similar to the echolocation used by bats, whales and dolphins. A pulse of high-frequency sound (ultrasound) is transmitted into the body by a probe, which travels through the body until it reaches a boundary between tissues of different physical properties. When it hits this boundary, part of the sound waves are reflected back to the probe, while others are transmitted and keep travel on further until they reach another boundary and get reflected. The reflected waves (echoes) are picked up by the probe and transmitted to the central unit which estimates the position of the reflecting structure based on the gap in time between the transmission of these sound waves and the return to the probe, usually on the order of millionths of a second (Arbona, Khabiri & Norton, 2010). This is possible since it is assumed the sound travels at a fairly constant speed in the body tissues, around 1,540 m/s (Gorgas, 2011). The ultrasound unit displays then a two dimensional image in the screen representing not only the distance at which a sound wave was reflected, giving thus a visual representation of the organs' anatomical relationships, but also its intensity, which will vary from a bright image for a highly reflective, hyperechoic surface like the lungs to a dark image representative of a poorly reflective, hypoechoic structure like the urinary bladder (Burk & Feeney, 2002; Arbona et al., 2010).

### 3.5.3. Instrumentation

With the advances in ultrasonography technology, lighter and more affordable ultrasound units were developed. Currently, a wide range of models are available in the market to fulfill the demands of the clinics, from heavy, bulky units with great resolution to light, portable units to use under field conditions. The basic ultrasound unit is composed by a transducer probe, responsible for sending and receiving the sound waves, a central processing unit that processes all the information and contains the electrical power supplies, transducer pulse controls that allow the operator to change the amplitude, frequency and duration of the pulses emitted from the transducer probe and a display that shows the image resulting from the processed echoes (Martinat-Botté et al., 1998).

The transducer is the main component of the ultrasound unit. It can differ in terms of frequency and design, so special attention shall be paid to the selection of this component. Usually, each transducer can only operate in a specific frequency, with higher frequencies more suited to visualize superficial organs and lower frequencies used for deeper examinations, although with a significant loss in the image resolution. (Merritt, 2011). As a general guideline, 5 MHz probes will image properly to a depth of 15 cm, 7.5 MHz will image up to 7 cm and 10 MHz will only image well to a depth of 4–5 cm (Mannion, 2006). Recently, multifrequency transducers have been developed in order to allow the selection of a specific frequency within a certain range, which allows the simultaneous imaging of the near and far fields with sound waves of different frequencies without the need to change the probe (Nyland et al., 2002).

Concerning their design, transducers can be classified as array or phased array. The first ones produce a rectangular image, as wide as the array, clear and without a significant loss of information on the edges (Rizk, 2010). They are especially useful when the acoustic window is large, such as in obstetrics exams. Phased array transducers produce a pie-shaped image and are mainly used when the acoustic window is small, like in echocardiography due to the presence of the ribs, and also to visualize deeply located structures in bigger animals. However, it has a limited value for scanning superficial structures and its image shows a slightly distortion on the edges (Martinat-Botté et al., 1998; Gorgas, 2011).

### 3.5.4. Applications of ultrasonography in swine industry

Although the use of ultrasound to evaluate biological tissues dates back to the 1940s, the widespread of this technology in livestock species was somewhat slow, mainly due to the high investment costs involved (Moeller, 2002). One of the first studies regarding the use of ultrasound in pigs was conducted by Temple, Stonaker, Hovry, Posakony and Hazaleus (1956, cited by Szabo et al., 1999) who reported the applicability of this technology in the evaluation of backfat thickness in live pigs. Several studies followed, with Price, Pearson, Pfost and Deans (1960), Urban and Hazel (1965) and Moody and Zobrisky (1966) confirming the accuracy of this technique.

In the latter years, its use has greatly increased due to advances in the ultrasound hardware, with the onset of lighter, portable and less expensive equipment, suited to use under field conditions. Among several advantages, this method is non-invasive and harmless to the animal, there is no risk of abortion in pregnant sows, the results are immediate and can be stored for further analysis (Martinat-Botté et al., 1998). It has, though, some limitations: the exam might be difficult to perform if the animal is not still and the skin must be perfectly cleaned and coated with ultrasound gel (Martinat-Botté et al., 1998). In fat animals might be difficult to perform transcutaneous ultrasonography, while in prepubertal gilts the transrectal approach is not always possible (Waberski, Kunz-Schmidt, Neto, Richter & Weitze, 2000).

Given the importance of this technique in the pig production, some of its applications will be briefly reviewed.

#### 3.5.4.1. Pregnancy diagnosis

Early and accurate identification of nonpregnant sows improves reproductive efficiency in commercial swine farms by reducing number of non-produtive days and, thus, increasing the number of farrows per year (Pequeno, Zúniga & Wilschral, 2009).

With Doppler or A-mode ultrasound, the initial financial investment is lower but the sensibility and specificity of these methods are not very high at the beginning of gestation, which make them not very useful before 30-35 days of gestation (Williams et al., 2003).

With B-mode, real-time ultrasonography (RTU) better results can be achieved. Using this technique, the earliest detection of pregnancy reported varies among the sources, but is usually described between 12 and 16 days of gestation (Knox & Althouse, 1999; Resnis, Bigliardi, Parmigiani & Peters, 2000; Romero, 2005). Nevertheless, sometimes it may be

quite difficult to have a correct diagnosis so soon using the routine transcutaneous RTU with a 5.0 MHz probe, so usually this exam should be performed after 21 days post artificial insemination (AI) (Flowers, Armstrong, White, Woodhard & Almond, 1999). Using transrectal scanning and a 7.5 MHz probe, it is possible to anticipate a few days, being an embryo usually detected between days 18 and 20 of gestation (Miller et al., 2003).

#### 3.5.4.2. Monitoring of follicle dynamics and ovulation

The application of ultrasonography to the ovaries visualization greatly increased our understanding of follicle development and its dynamics along the estrous cycle. When performed in complement with blood assays for hormonal measurements, several authors were able to establish a relationship between FSH, LH, estradiol and progesterone levels and the follicle development (Dalin, Nanda, Hultén & Einarsson, 1995; Madej, Lang, Brandt, Kindahl, Madsen & Einarsson, 2005; Madej, Brandt & Einarsson, 2009). Through US several external factors were identified as having influence on the follicle development post-weaning, including high ambient temperature (Barb et al., 1991), low energy feed intake during lactation (van den Brand, Dieleman, Soede & Kemp, 2000), parity and body condition score (Bracken, Lamberson, Safranski & Lucy, 2003).

Ovary US is also a valuable tool to detect ovulation, being currently the gold-standard technique to determine its occurrence (Boulot, 2010). Several authors believe the implementation of US as a routine method for monitoring ovulation would be economically viable due to a more precise AI program, a decrease in the number of AI per animal and the absence of post-ovulatory inseminations (Knox & Althouse, 1999; Waberski et al., 2000; Kauffold & Althouse, 2007). According to Knox & Althouse (1999), only the transrectal technique can provide the required resolution and sensitivity to successfully visualize the ovarian structures and identify follicles, corpora hemorrhagica (CH) and corpora lutea (CL). The precision of this diagnosis in terms of timing of ovulation can go up to 4 hours, depending on the frequency of the examinations. A positive diagnosis of ovulation is established when sequential examinations showed the disappearance or a significant decrease in the number of pre-ovulatory follicles, followed, but not mandatorily, by the visualization of CH (Kemp & Soede, 1996; Kauffold & Althouse, 2007)

### 3.5.4.3. Determination of puberty status in gilts

The diagnosis of puberty is based on the evaluation of both ovaries and uterus. Follicles ranging from 2 to 5 mm in size, absence of any luteal structures in the ovary and an average cross-sectional area of the uterine horns of $\leq 1$ $cm^2$ is a typical finding for pre-pubertal female; in pubertal females, there was evidence of follicles around 7-8 mm, presence of CL and cross-sectional areas of $\geq 1.2$ cm2 (Kauffold, Rautenberg, Richter Waehner & Sobiraj, 2004). Kauffold & Althouse (2007) reported an accuracy of 91% and 100% in the pubertal and pre-pubertal diagnosis, respectively.

### 3.5.4.4. Detection of reproductive disorders

In the last years, ultrasonography has been successfully used to identify causes of reproductive failure. Among the responsible factors for culling rate, reproductive disorders are usually the one with the biggest weight (Stein, Dijkhuizen, D'Allaire & Morris, 1990; Moreira, Pilati, Reis, Dick & Sobestiansky, 2006).

Ovarian cysts are single or multiple fluid-filled structures with diameter greater than 11 mm, resulting from unovulated or luteinized follicles and might cause permanent or transitory anestrus, smaller litters, decrease in the farrowing rate and higher frequency of returns to estrus (Knox & Althouse, 1999; Waberski et al., 2000; Pequeno et al., 2009). Follicular cysts, originated from unovulated follicles, usually appear as fluid-filled bubbles surrounded by a thin wall while luteal cysts, originated from a developed CL, have a thicker wall and a distinct lumen. In 2008, Rodríguez, Puche, Vale and Camacho reported an incidence close to 13% of ovarian cysts in culled sows, while Waberski et al. (2000) found these structures in 30% of the scanned animals. Thus, US assumes an important role in the diagnosis of these structures, supporting the decision to either treat or cull the animal.

Inactive ovaries, characterized by several follicles of predominantly smaller size that fail to ovulate and persistent corpora lutea due to embryonic mortality or exposure to zearalenone are some of the other ovarian malfunctions that can be detected with US.

Regarding uterine disorders, a special reference should be made to endometritis which sometimes is not followed by obvious clinical signs, making difficult the diagnosis. This uterine infection might have an acute or chronic evolution and the symptoms might range from very discrete to severe. The ultrasound diagnosis is based on the analysis of uterine

echotexture, size and the presence of intrauterine fluid (Kauffold, Rautenberg, Hoffmann, Beynon, Schellenberg & Sobiraj, 2005).

## 3.6. Infrared thermography

Infrared thermography (IRT) is a process that detects infrared energy emitted from an object, converts it to temperature and displays it as an image of temperature distribution called thermogram. It is a modern, noninvasive and safe technique to measure the surface temperature of an object, with wide application in almost every scientific field (Knízková, Kunc, Gürdil, Pinar & Selvi, 2007).

### 3.6.1. Historical background

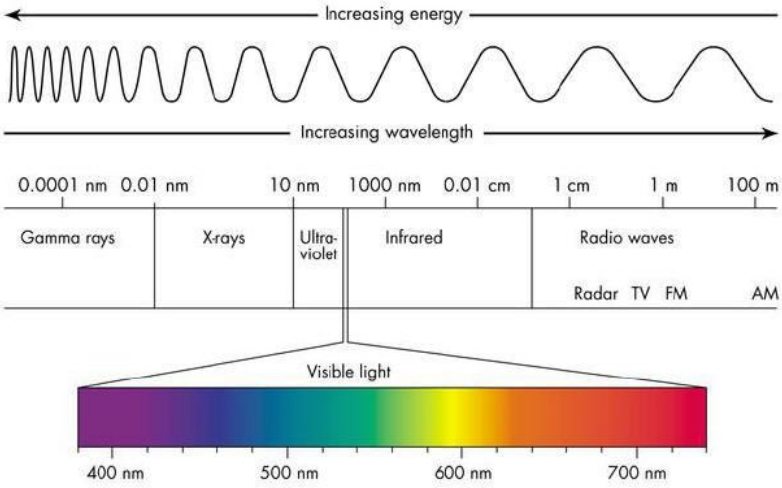
The discovery of infrared radiation is attributed to the astronomer William Herschel (Fluke, 2009). In 1800, Herschel passed sunlight through a glass prism to create a spectrum and, using a sensitive mercury thermometer, measured the temperature of each color. Herschel discovered that the temperature increased from the violet to the red part of the spectrum and, curiously, it was even higher in a control thermometer placed beyond red light, an area he referred to as "dark heat" (Gaussorgues, 1994). With this experiment, Herschel identified for the first time the existence of radiation outside the visible spectrum and described the "dark heat", the radiation nowadays known as infrared. However, only 30 years later the first detectors for this type of radiation were developed, as a result of the collaboration between two Italian physicists, Leopoldo Nobili and Macedonio Melloni (Rogalski & Chrzanowski, 2002). In 1880, the American astronomer Samuel Langley invented the bolometer, which relies in the change in electrical resistance related to the change in temperature; with this new device, Langley was able to measure the body temperature of a cow over 300 meters away (Fluke, 2009).

With the beginning of the 20$^{th}$ century, several improvements were made in this technology, but the biggest developments were achieved in the 40's and 50's, due to the investment in this technology for military purposes in the World War II (Rogalski & Chrzanowski, 2002). Only in the 70's IRT started to be used in civil applications, with progressive improvements in portability, accuracy and image quality (Santos, 2006). Nowadays, IRT is widely used in many areas such as civil engineering, medical and veterinary imaging, industrial process control, building temperature monitoring and military technology.

### 3.6.2. Physical principles

IRT is based on the principle that any body above absolute zero (-273.15 °C) emits infrared (IR) radiation as a function of its temperature and emissivity (Speakman & Ward, 1998). In the electromagnetic spectrum, IR is located between radio waves and visible light (figure 8) and, like all electromagnetic radiations, is characterized by its wavelength (λ) and intensity (Q). The wavelength of the emitted radiation is closely related to the temperature of its source: the higher the temperature, the faster the vibration of its atoms, and thus the shorter the wavelength that results from this vibration (Rogalski & Chrzanowski, 2002). This is the fundamental principle underlying IRT: a body releases IR with different wavelengths according to its temperature, the infrared camera will detect these radiations, analyze their wavelengths and convert this information into a color image called thermogram, where the colder regions are represented in blue and the warmer ones in red (Speakman & Ward, 1998).

**Figure 8:** Electromagnetic spectrum (adapted from Fordham, 2011)




As stated before, the radiation measured by an infrared camera does not depend only upon the temperature of the object, but is also a function of the emissivity (ε). Emissivity is, generally speaking, the relative ability of a given material to emit energy by radiation compared to a theoretically perfect emitter, ranging from 0 to 1 (Williamson Corp, 2008) (see annex 12.2). A perfect emitter is called blackbody and has an emissivity value of 1, while a regular body that emits only 60% of the theoretical amount of infrared energy has an emissivity value of 0.6. The emissivity value of an object is influenced by the material, its surface condition, its reflectivity and its opacity (Williamson Corp, 2008). Since this variable can significantly

influence the temperature measurements, the emissivity of the object under study must be always taken into account to obtain accurate results.

### 3.6.3. Pros and cons of IRT

The use of IRT in a wide range of different areas is proof of its numerous advantages. IRT does not require direct physical contact with the object, which allows the measurements to be made from a safe distance; this is particularly useful with wild animals that might be life-threatening, animals susceptible to stress due to human contact or to monitor a specific spot in a building that is out of reach. The results are displayed as an image, which allows comparisons between different areas. This technique possesses an excellent accuracy (that can go up to 0,1 ºC, depending on the camera model) and excellent resolution, with each pixel in the image corresponding to a single temperature, and suited for a wide range of temperatures that can go from -50 ºC to above 2000 ºC (FLIR, 2011). It is possible to measure a specific point of the object or an area and record the corresponding maximum, minimum and average temperatures. The images are saved in a memory card and can be stored to be analyzed later and used in work reports.

However, some limitations also need to be considered. The price of infrared cameras is still a limitative factor for the widespread use of this technology, with values above 5000€ for a decent quality camera. From the technical point of view, the object measured must be protected from direct sunlight and wind drafts, hair coats should be free of dirt, moisture or foreign material and the effect of weather conditions, circadian and ultradian rhythms, time of feeding, milking, laying and rumination should be also considered, since they can affect considerably the temperature obtained with this technique (Knízková et al., 2007). The emissivity of the material must be known as it might influence significantly the temperature recorded and only the surface temperature can be monitored, but not the core temperature (Garnaik, 2005).

### 3.6.4. Applications of IRT

Infrared thermography has been widely used in several areas, from civil to aerospace engineering, from surveillance in security to medical and veterinary imaging. In 2007, Mccafferty reviewed the applications of IRT in studies of thermal physiology, veterinary diagnosis of disease or injury and population surveys on domestic and wild mammals and

compiled a total of 71 studies since 1968. This considerable amount of publications illustrates the increasing interest for this technology and its potential in the veterinary sphere. This work will focus on the studies directed to livestock, briefly reviewing the ones that showed particular applicability under field conditions.

#### 3.6.4.1. Bovine

Several studies have been performed in cattle using IRT. In 2004, Schaefer et al. used IRT as an early detection method for identifying animals inoculated with type 2 bovine viral diarrhea virus, concluding that there were significant changes in eye temperature several days to one week before other clinical signs of infection, which suggests IRT can be used in developing an early prediction index for infection. Alsaaod and Büscher (2012) tested the applicability of IRT as an early detection tool of foot pathologies in dairy cows and reported a significant increased temperature in the coronary band region in animals with hoof lesions of the hind limbs, when compared to animals with no lesions.

IRT can also be used as a diagnostic tool for assessing udder function, as reported by Colak et al. (2008), who concluded that IRT shows potential as an early detection method for mastitis. Hellebrand et al. (2003), by their turn, concluded that the external pudendum temperature is a good estimator of the core body temperature, which makes this technique suitable for estrus climax determination.

In bulls, it was successfully used to diagnose testicular inflammation and degenerative disease, with an increase in 2.5 to 3 ºC in the compromised testicle, when compared to the contralateral testicle without lesions (Purohit et al, 1985). Several studies followed, always confirming a change in the scrotal temperature pattern related to testicular function disorders (Lunstra, 1993; Kastelic, Cook, & Coulter, 1997; Lunstra & Coulter, 1997; Gabor, 1998).

#### 3.6.4.2. Equine

In the equine field, IRT has been mainly used as a diagnosis tool in foot and leg problems, namely lameness, navicular disease, abscesses and ligament injuries, proving to be an efficient tool detecting these conditions in an early phase or when physical and radiographic findings were inconclusive (Eddy, Van Hoogmoed & Snyder, 2001; Turner, 2001; Embaby, Shamaa & Gohar, 2002; Turner, 2010). It was also successfully applied in the diagnosis of

thoracolumbar lesions (Fonseca et al., 2006) and pleuropneumonia (Costa, Bogossian & Sobrinho, 2009).

Recently, Bowers, Gandy, Anderson, Ryan and Willard (2009) tested IRT as a pregnancy diagnosis tool in mares and found a significant increase in the flank temperature in pregnant mares, concluding this technique can be used as a trusted noncontact method for mid- to late-gestation pregnancy diagnosis.

#### 3.6.4.3. Swine

In pigs, IRT has also been widely studied in numerous fields. Numerous studies had already proved the usefulness of IRT in predicting the incidence of meat quality defects in pigs prior to stunning. Gariepy, Amiot and Nadai (1989) were, perhaps, the first ones to release a publication about this question. In their study, they found a correlation between incidence of meat quality defects and increased skin surface temperature of pigs prior to slaughter, concluding IRT can be used as a practical and rapid method to detect animals especially predisposed to present meat quality defects. On that same year, Schaefer, Jones, Murray, Sather and Tong (1989) performed a similar study using pigs with known genotypes for stress susceptibility, although without achieving clear results; nevertheless, from their results they concluded that a higher drip loss and percentage of pale, soft and exudative meat (PSE) could be expected in pigs with a lower superficial temperature. Several studies followed, with a generalized consensus about the utility of this technique to predict pork quality based on the superficial body temperature prior to slaughter (Owen, Montgomery, Ramsey & Miller, 2000; Lawrence et al., 2001; Dikeman, Spire, Hunt & Lowak, 2003).

Šabec & Lazar (1990) used it to detect osteoarthrosis tarsi deformans (OATD) in Swedish Landrace boars, with higher tarsus temperature obtained in the affected animals; Loughmiller et al. (2001) reported positive results when evaluating the ability of IRT to detect febrile responses in pigs following intranasal inoculation with *Actinobacillus pleuropneumonia*.

In some recent studies, IRT showed promising results in the swine reproductive management. Scolari, Clark, Knox, Manoel and Tamassia (2011) demonstrated that the vulvar skin temperature (VST) of sows measured by IRT changes significantly during the periovulatory period, which opens perspectives for the use of this technique as a predictor for ovulation. Sykes et al. (2012) tested IRT in gilts to discriminate animals in estrus and diestrus and reported significantly higher VST in the estrus period. Thus, this technique can presumably be

used for early detection of the onset of estrus, with particular interest in animals with silent estrus.

#### 3.6.4.4. Animal welfare

Growing public concern regarding animal welfare forced the animal industry to provide better environment conditions to their animals and develop methods to measure their levels of stress. However, many of the techniques used to measure stress are, *per se*, stress-inducers, as they frequently involve restraint and invasive procedures such as collection of blood samples, which may themselves cause a stress response, affecting the results obtained. The use of noninvasive methods is, thus, of the utmost importance, in order to achieve more accurate results.

When an animal becomes stressed, its hypothalamic-pituitary-adrenal axis is activated, leading to an increase in catecholamines and cortisol concentrations and consequently an active peripheral vasoconstriction (Mormède et al., 2007). These changes in the peripheral blood flow can be assessed by IRT as stated by Stewart, Webster, Schaefer, Cook and Scott (2005), who conclude IRT can be used as a reliable, accurate and noninvasive method to assess the levels of stress and, thus, animal welfare, based on the measurement of the eye temperature.

IRT was also successfully applied to the study of heat stress in cows (Knízková, Kunc, Novy & Knizek, 1996; Coppola, Collier & Enns, 2002; Knízková, Kunc, Koubková, Flusser & Dolezal, 2002) and pigs (Adamec, Kunc, Knizkova, Dolejs & Toufar, 1997, cited by Knízková et al., 2007). In these studies, different cooling systems were tested and their efficacy to keep animals in a thermal comfort status assessed, by continuously monitoring their body surface temperature. Furthermore, it was possible to characterize different superficial temperature profiles according to their level of thermal comfort, which might be useful in the future to easily identify animals in heat stress.

# 4. Materials & Methods

## 4.1. Animals and Housing

This study took place in the GIE Villefranche Grand Sud, Pôle de Formation de Bernussou, located in Villefranche de Rouergue, France, from January 25, 2012 to February 2, 2012. The experimental group comprised a total of 36 females, of which 30 multiparous sows with average parity of 4.1 ± 2.3 and lactation length of 28.3 ± 0.7 days and 6 gilts (animals without any farrow) with, in average, 249 days of age. The females were crossbred Large White x Landrace, housed individually in crates. The boar used for heat detection was a 2.5 years old Duroc, housed in a pen located in the same room of the females.

The animals were kept in a controlled temperature (around 19 $^{o}$C) and humidity (60 to 70%) environment, being the air temperature evaluated 4 times per day based on the electronic ventilation system's thermostats, every time thermal imaging was being performed. An independent hi-lo thermometer was placed inside the room to confirm the readings from the ventilation system thermostats and check for temperature fluctuations.

Animals were fed twice daily at 0600 h and 1800 h with a liquid feed, according to the INRA recommendations, with the following plan: at day 0 (day of weaning), 2 kg of food per female per day, increasing until day 4 reaching 4 or 4.5 kg depending on the body condition; at day 5 starts decreasing again until reaching 2.8 kg at day 10. An extra dose of water was given at 1200 h and 0000 h.

Gilts were synchronized using altrenogest, a synthetic progestational agent (Altresyn®, Ceva Santé Animale, Libourne, France), administered orally in a daily dose of 20 mg (5 mL) to each gilt during 14 days, with the last administration made 2 days before weaning the sows. In order to standardize all animals in time, the day in which the sows were weaned was established as Day 0 of the trial.

The floor was kept clean and the vulvar and gluteal area were cleaned twice daily between the thermal measurements, to avoid reading errors due to the presence of feces or due to the cleaning process.

## 4.2. Detection of estrus

At Day 0, the females were exposed to the boar in order to stimulate the onset of estrus (OE). Starting at Day 1, estrus detection was performed twice daily, in the morning and afternoon. The boar was placed in front of the females and his movements restricted to about 8-9 females, in order to avoid over-stimulation of too many animals at the same time, leading to a subsequent refractory period during which the females would not show signs of estrus. The OE was confirmed using the "back pressure test" and the observation of typical signs such as the reduction in the reddening and swelling of the vulva, presence of a clear and tacky vulvar discharge, vocalization, ear pricking, lordosis and tremble or flick the tail up and down (Progressive swine technologies, 1999).

In order to facilitate the analysis of the results, the heat detected in the morning were recorded as beginning at 0600 h of that same day, while the ones detected in the afternoon were recorded as beginning at 1200 h.

## 4.3. Thermal imaging

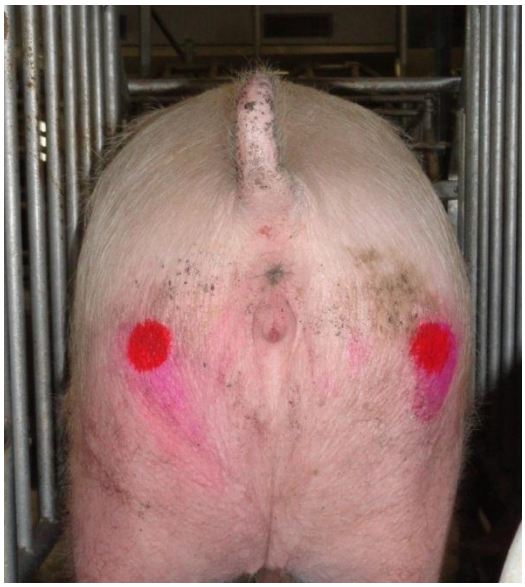
Temperature measurements were performed using an infrared thermal camera (Fluke® TiR 9HZ Thermal Imager, Fluke Corporation, Everett, USA) (specifications in annex 12.3). The thermal camera was calibrated with an emissivity value (ε) of 0.97, following the recommendations of Berbigier (1975) for thermal imaging the pig's skin. These images were acquired every 6 hours, at 0000 h, 0600 h, 1200 h and 1800 h, always from the same distance to the posterior end of the animal (1 meter) and the same angle of incidence to the vulvar area (90º). One pink spot was marked on each gluteal region (Figure 9.1), so that the temperature from this area could be measured always in the same location.

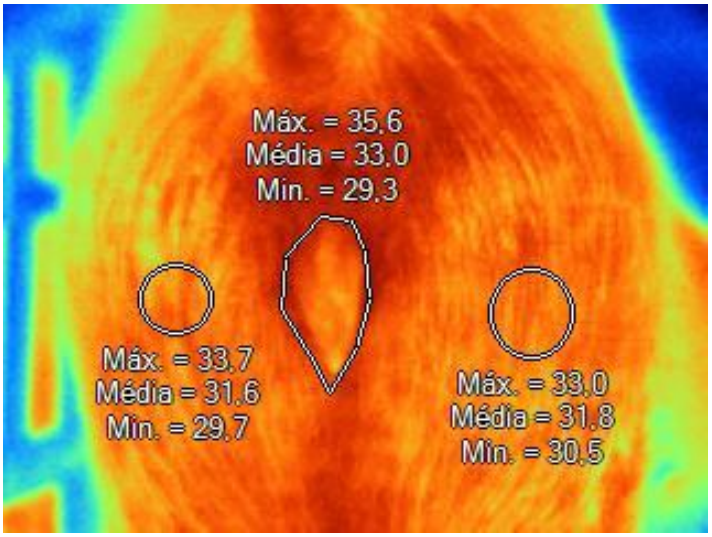
When the animal was standing up and still, after focusing the vulvar area, 2 sequential images were acquired. No cleaning was performed at this time to avoid disturbing the skin temperature, unless the level of dirtiness would make impossible to obtain a correct temperature reading. If so, careful cleaning with alcohol-based moist wipes was performed and the thermal imaging acquired 15 minutes later.

**Figure 9:** Comparison between a normal and a thermal image.

**Figure 9.1 (Left):** Posterior end of a female, with the auxiliary spots to measure the gluteal temperature marked in pink. Original image.

**Figure 9.2 (Right):** Thermogram showing the method used to obtain the temperature records. Original image.






In order to facilitate these measurements, the time of feeding was adjusted in the automatic distribution system so that it could coincide with these measurements, making easier to have the animals standing up and still. If the temperature was altered due to the animal's position prior to the measurements (as observed later in the repeatability test), the animal would be kept standing and the temperature obtained 15 minutes later.

These images were later downloaded to a laptop and visualized using the SmartView™ Version 2.0 software (Fluke Thermography, Plymouth, USA). The temperature was obtained from the first image recorded from every animal. If the image quality was not satisfactory (due, for instance, to a movement of the animal at the time of caption) and the readings unreliable, this image would be discarded and the temperatures would be taken from the second image. Concerning the method to collect these temperatures from the images, a polygonal area around the vulva was marked and the mean, maximum and minimal temperatures recorded. A circular area in the gluteal regions was also marked, being the mean, maximum and minimal values recorded too. The VST used in our analysis corresponded to the average value from the polygonal area, while the GST resulted from the mean of the averages of both circular areas (Figure 9.2). All temperature values are reported in Celsius degrees (ºC).

In order to validate this method, determine its repeatability and early detect possible flaws, a sample group of 3 sows and 2 gilts were tested in Day 0, with 5 measurements performed in each animal, one measure every 5 minutes. The reproducibility could not be assessed, as it implies the analysis of measurements performed by different operators, which was not the case in this project.

## 4.4. Real-time Ultrasound

Real-time ultrasonography was performed during Day 5 and 6 in a sample group of 14 sows and 6 gilts in order to detect ovulation. Both the transcutaneous and the transrectal technique have been performed, so the precision of both methods could be compared. To visualize the ovaries, an ultrasound device (Hospimedi MyLab$^{Tm}$OneVet, Esaoete®, Fontenay-sous-Bois, France) fitted with a linear 7 MHz to 10 MHz transducer used for the transrectal technique and a 3.5 MHz curvilinear transducer for the transabdominal approach.

To perform the transabdominal ultrasonography (TAU), the transducer was initially lubricated with ultrasound gel and then placed horizontally on the right ventro-lateral abdominal wall just dorsal to the last pair of teats, cranial to the hind leg and pointing to the spine (Kähn et al., 2004). If the ovaries were not visualized, the same procedure should be repeated on the left side.

For the transrectal technique, the linear probe was fitted into a rigid PVC adapter and then generously lubricated with ultrasound gel. The feces inside the rectum were removed by hand and the probe gently introduced into the rectum with its transducer turned down. The transducer was then rotated 45–90$^{o}$ in order to visualize the right and left ovary (Knox & Althouse, 1999).

Time of ovulation was defined as the first time of zero follicle count or when a noticeably lower count was noticed (Kemp & Soede, 1996), after a successful visualization of pre-ovulatory follicles in the previous exam.

## 4.5. Statistical Analysis

The repeatability test (intra-class correlation coefficient or ICC) was performed based on the one-way ANOVA method, as described by Measey, Silva and Di-Bernardo (2002). The repeatability index ($r_i$) was determined by equation 1, where $MS_{between}$ is the Mean Squares between groups, $MS_{within}$ is the Mean Squares within groups and n is the number of repeated measurements per animal.

**Equation 1:** Repeatability index based on Analysis of Variance (ANOVA). This equation establishes a ratio between the variation among individual and the variation within individual. A high ratio (closer to 1) corresponds to a highly repeatable method.

$$r_i = \frac{MS_{between} - MS_{within}}{(MS_{between} + (n-1)MS_{within})}$$

All the mean values are reported in terms of mean ± standard deviation (SD) unless otherwise states. The data collected was inserted in an Excel database (Microsoft Excel 2010, Microsoft® Corporation, USA). Simple comparisons between two samples (such as duration of estrus (DE) on gilts vs sows and temperature records at 0600 h vs 1800 h) were performed with Student's t test using SPSS Statistics 20.0 (IBM Corp.©, USA) and RStudio 0.95 (RStudio Inc, USA). A significant difference was reported for a p-value lower than 0.05.

Pearson's correlation tests were performed between weaning-to-estrus interval (WEI) and DE, room temperature (RT) and vulvar skin temperature (VST) and between RT and gluteal skin temperature (GST) using SPSS Statistics 20.0, in order to determine a possible relationship between two variables.

In order to better understand the variation in VST, GST and vulvar-gluteal temperature (VGT) along the trial, a method based in a 4-points average for the graphic representation was used, in which, for a given hour, 4 measurements were considered: the two previous (12 and 6 hours before), the one obtained at that same hour and the one obtained 6 hours after. This method allowed us to stabilize the temperature in 24h periods and minimize its daily fluctuations. It is important to note this method does not give us a statistical analysis of the records, but only a clearer visual representation about the variation trends of these records along the experiment.

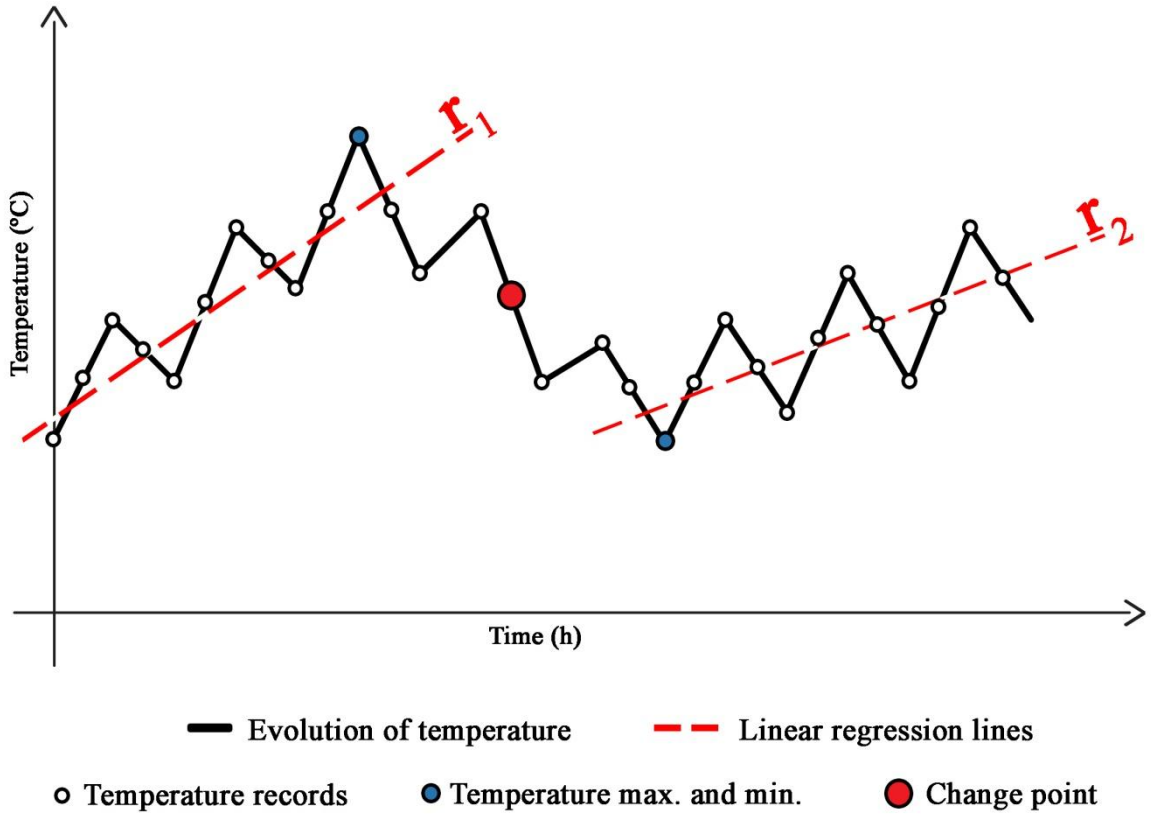
In order to statistically analyze the VST, GST and VGT, and given the considerable daily variation found, the records of each day were grouped and the difference between consecutive days analyzed using an ANOVA test in SPSS Statistics 20.0 and RStudio 0.95. The temperature was reported to be significantly changing when a p-value lower than 0.05 was found between consecutive periods.

The statistical analysis of VGT deserved special attention from us, as it was one of the fundamental points of our conclusions. Since we were dealing with small values and slight variations, it was used a method that could provide us a greater sensitivity on this analysis. This method – detection procedures for change points – was developed by Caussinus and Lyazrhi (1997) and consists in a multiple regression analysis in which a linear model with an unknown number of change-points (Cp) is tested (figure 10). It is used an R-source program (unnamed, Caussinus and Lyazrhi, France) that establishes, if any, an initial linear regression model (in the figure represented by the linear regression line $r_1$). This linear regression is somewhere broken due to a certain event but after some time a new linear regression is established (linear regression line $r_2$). Between the two regression models, a Cp is established (red dot). For each Cp the maximum (M) and the minimum (m) values were determined (blue dots), with the M corresponding to the point at which the linear regression was broken and the temperature started decreasing, while the m corresponding to the point at which the temperature stopped decreasing and the new linear regression is established.

It is important to note that these Cp are fundamental to statistically prove the occurrence of a significant change in the VGT trend but are of limited interpretation in terms of time of occurrence from a biological point of view, as they do not provide us, by themselves, the information about when did the changing event started and ended. The maximum and minimum values relative to each Cp have thus a special interest for our study, as they represent a biological parameter easily measured and interpreted.

Finally, two sub-groups were created, comprising animals with similar OE: Group D4, with 11 females starting estrus at Day 4, 0600 h and Group D5, with 7 females starting estrus at Day 5, 0600 h. This way it was possible to analyze the records of animals synchronized in time and exposed to the same external conditions. A thorough analysis of the VGT in these groups was conducted, following the model already described, leading to the main conclusions presented in this study.

**Figure 10:** Graphic representation with a theoretical temperature variation pattern. The evolution of temperature in time can be represented by two regression models ($r_1$ and $r_2$), set apart by a change point. The max and min values are also recorded to determine the start and the end of the changing-event (original image).



# 5. Results and Discussion

## 5.1. Validation of the skin temperature measurements

The records obtained for the repeatability test revealed a significant difference among animals (table 2). One of the animals (ID = 1126) presented a spread in the records significantly higher than the others, in both VST and GST (figures 11 and 12). This fact is proved by its higher standard deviation values (2.5 ºC and 1.1 ºC for VST and GST) when compared to the average of the other 4 animals (0.35 ºC for both VST and GST).

**Table 2:** Temperature records of the repeatability test, regarding the vulvar skin temperature (VST) and gluteal skin temperature (GST). Five animals were tested (563, 1106, 1126, 1177 and 1346). Temperature values reported in Celsius degrees.

| | ID | | | | |
|---|---|---|---|---|---|
| | **563** | **1106** | **1126** | **1177** | **1346** |
| **VST #1** | 35 | 32,7 | 28,8 | 33,1 | 33,3 |
| **VST #2** | 34,3 | 32,8 | 32,2 | 32,5 | 33,4 |
| **VST #3** | 34,2 | 33,5 | 33,8 | 33 | 34,1 |
| **VST #4** | 34,8 | 33,1 | 34,6 | 33,1 | 34,1 |
| **VST #5** | 34,2 | 33,1 | 35,3 | 32,5 | 33,3 |
| | | | | | |
| **GST #1** | 33,6 | 31,3 | 31,9 | 32,4 | 31,3 |
| **GST #2** | 33 | 31,6 | 32,6 | 32 | 31,4 |
| **GST #3** | 32,8 | 31,8 | 34,2 | 32,7 | 32 |
| **GST #4** | 33,5 | 32 | 34,2 | 32,6 | 32,1 |
| **GST #5** | 32,8 | 32,1 | 34,4 | 31,9 | 31,5 |

**Figure 11:** Boxplot for the vulvar skin temperature records of the repeatability test. Each box shows the interquartile range that contains values between the 25th and 75th percentile, the line inside the box shows the median and the two "whiskers" show the upper and lower values registered. The spread of records observed in the animal ID 1126 is significantly higher compared to the other animals.

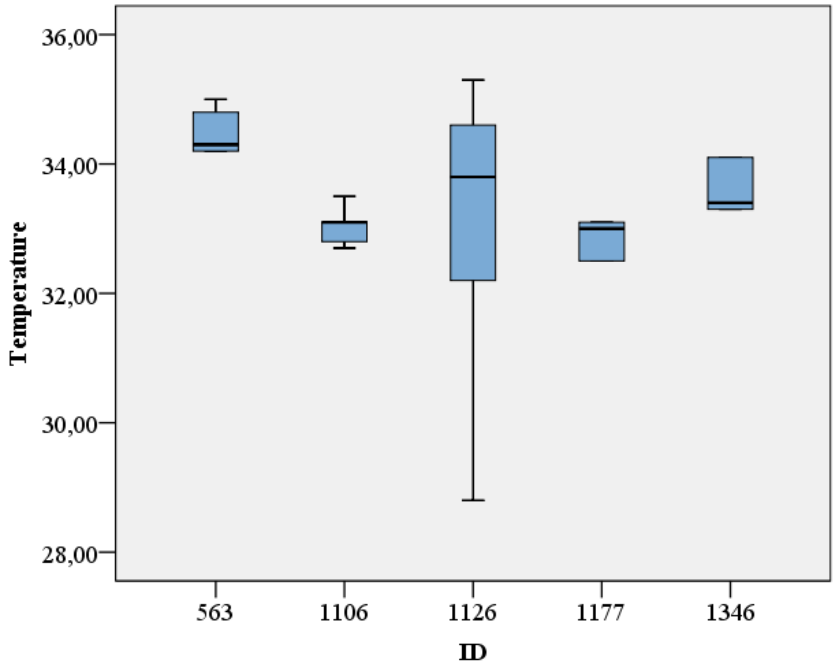
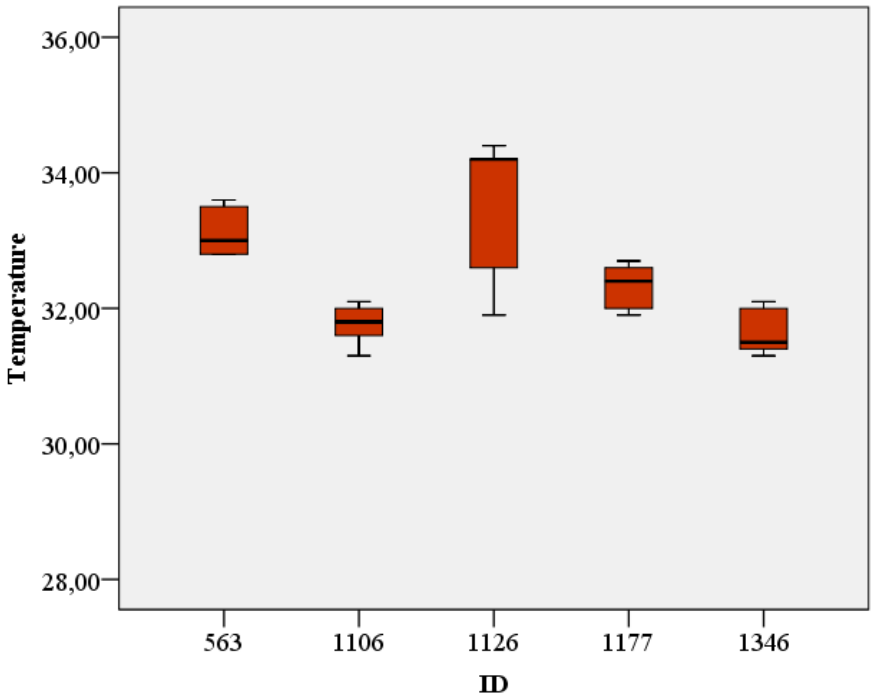



**Figure 12:** Boxplot for the gluteal skin temperature records of the repeatability test. Each box shows the interquartile range that contains values between the 25th and 75th percentile, the line inside the box shows the median and the two "whiskers" show the upper and lower values registered. The spread of records observed in the animal ID 1126 is significantly higher compared to the other animals.



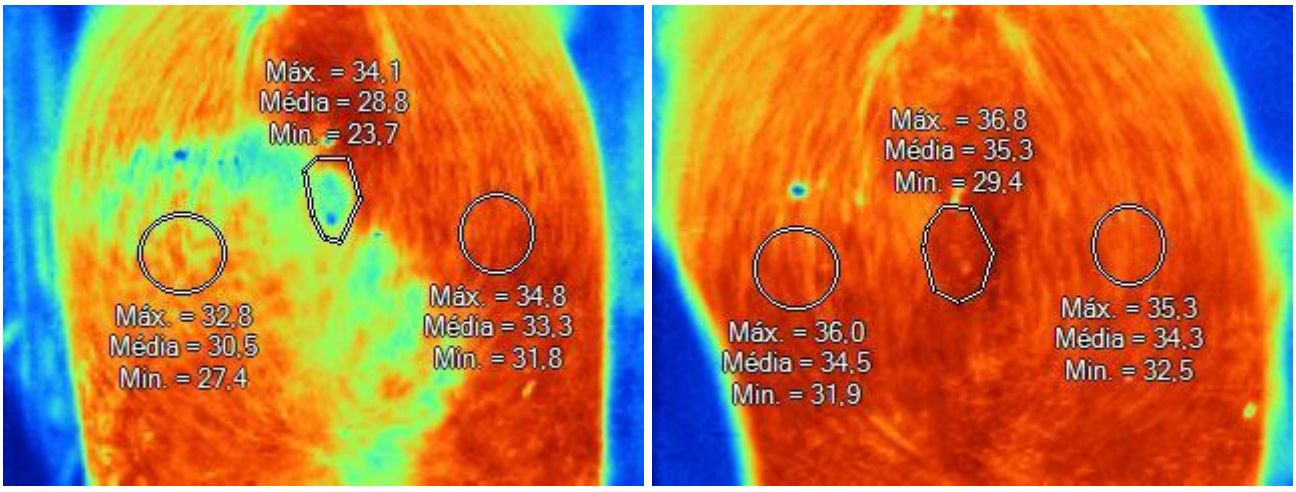
When looking further into the variation found in this animal, we conclude it was due to its different stance immediately before the measurements. While all the other animals were standing up when we started the measurements, this one was seated and the skin in contact with the floor was significantly colder. This is easily noticed when analyzing the first and the last thermograms. In the first one (figure 13.1), there is an obvious cold area, with the pattern of the cast iron floor, represented by colder temperatures (blue and yellow), comprising the vulvar region. In the last (figure 13.2), obtained 25 minutes later, this cold area had disappeared and the local temperature was higher, with the red tones prevailing in the image. The temperature increased significantly during the course of these tests, with the VST increasing from 28.8 ºC to 35.3 ºC and the GST from 31.9 ºC to 34.4 ºC. Since the temperature changed significantly during the 25 minutes of the repeatability test, one can assume the 15 minutes that we had defined in the materials and methods for the temperature to stabilize to normal values in animals with abnormal readings might not be sufficient.

Given the variation found in this animal was due to an external error that does not go in agreement with the principles of the repeatability tests (results obtained always by the same operator, with the same method under the same circumstances) (Slezák & Waczulíková, 2011) and violated the assumption of homogeneity of variances of the ANOVA test ($p<0.001$ in the Levene and Welch test), this animal was classified as outlier and its results excluded from this test. Nevertheless, this finding evidences the influence of external variables over the temperature measurements, a fact that shall be kept in mind.

**Figure 13:** Thermograms from sow ID 1126.

**Figure 13.1 (left):** First thermogram, obtained at t=0

**Figure 13.2 (right):** Last thermogram, obtained 25 minutes later. Note the absence of the cold area present in the previous image.



In the repeatability test with the remaining 4 animals, a $r_i$ (repeatability index) of 0.873 was obtained, which means 87.3% of the variability found is due to the differences between animals and only 12.7% is attributable to measurement errors. According to Measey et al. (2002), a $r_i$ between 0.7 and 0.9 represents a highly repeatable method. Given the high $r_i$ obtained, the temperature measurement protocol can be validated. The ANOVA results for the repeatability test are available in annex 1.

## 5.2. Characterization of estrus

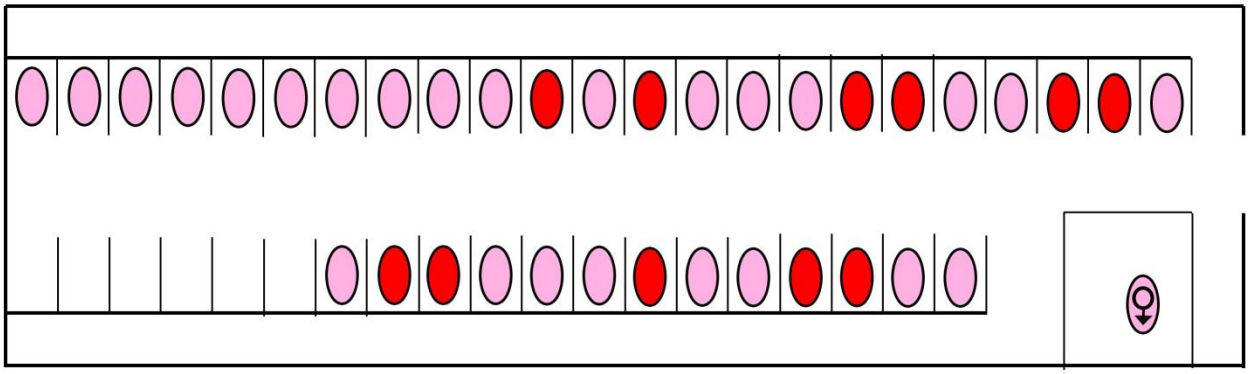
From the initial group of 36 animals, 2 of them (one sow and one gilt) did not show signs of estrus and their results were excluded from this trial. Several factors can lead to a persistent anestrus or an increase in the WEI. The first factor to look at is, obviously, the heat detection. The successful identification of sows coming into heat is highly dependent on the technician's experience to identify the behavioral and physical changes associated with the OE and a correct management of the boar to stimulate the exhibition of these signs. Signoret and Du Mesnil du Buisson (1961, cited by Gerritsen et al., 2005) reported that only 60% of the estrous sows showed a standing response to the back pressure test in the absence of a boar; when the boar was presented at a certain distance, providing olfactory and auditory stimuli, the detection rate increased to 90%; if visual and tactile contact was allowed, this rate reached 100%. Nevertheless, in this case we were in presence of experienced technicians, with a correct boar stimuli management validated by the veterinarian.

Another factor that can lead to an increased anestrus rate is the seasonal infertility. This phenomenon has been widely studied and it is generally accepted that is mainly due to heat stress and to the fact that pigs are inherently seasonal breeders (Peltoniemi et al., 1999; Rozeboom, See & Flowers, 2000). Nevertheless, this seasonal infertility has been observed only during late summer and early autumn (Peltoniemi et al, 1999; Peltoniemi, Tast & Love, 2000) and since this study was conducted during winter, seasonal infertility can be excluded. Lower parity females, especially primiparous sows, have longer WEI (Koketsu & Dial, 1997); shorter lactations (particularly less than 21 days) and an insufficient feed intake during lactation, especially in terms of energy and lysine, will also lead to an increase in the WEI (Knox & Zas, 2001). The social environment can also play an important role. When submitted to poor housing conditions or social stress resulting from constant hierarchy fights, the animals might have a silent estrus or even stop their follicle development (Madec, 2009). Zearalenone contamination at levels of 5 to 10 ppm in feed after weaning can induce a

prolonged cycle or even anestrus (Kanora & Maes, 2009). Since one of the non-cycling animals was a gilt, the puberty-factor must also be taken into account. In 1979, Christenson and Ford estimated that 10 to 40% of gilts did not show regular estrous cycles at 9 months, either due to delayed puberty or "behavioral anestrus". Since 1979, a great genetic improvement has been performed in our commercial breeds, namely regarding the sexual precocity, yet the delayed puberty must be considered. Finally, ovarian cysts can also lead to an important increase in the anestrus rate. According to Ebbert and Bostedt (1993), 75% of the sows with more than 10 cysts showed persistent anestrus, while this percentage decreased to 53% when less than 10 cysts were observed.

A curious fact observed was that in 19 animals (56% of the detected estrus) the OE occurred simultaneously with one of the animals from the adjacent crates. To make this idea clear, in figure 14 one can observe the heat detection performed during the morning of day 4, with an obvious tendency of the animals to come into heat in groups.

**Figure 14:** Plan of the experimental room, with the animals distributed by their individual crates. In red, the sows with detected onset of estrus in the morning of day 4. Note the tendency of animals to come into heat in couples.



This estrus synchronization has been reported in many species, pigs included. In 1992, Pearce and Pearce performed an experiment in which they compared the OE from three different groups after weaning: sows kept isolated, sows housed adjacent to an anestrous ovariectomized sow and sows housed adjacent to an ovariectomized sow in which estrus was induced by the administration of estradiol benzoate. In the latter two groups, sows had 10 minutes of physical contact per day. Estrus detection revealed that the latter group had a significant shorter WEI and an increased rate of synchronized OE. In a study performed by

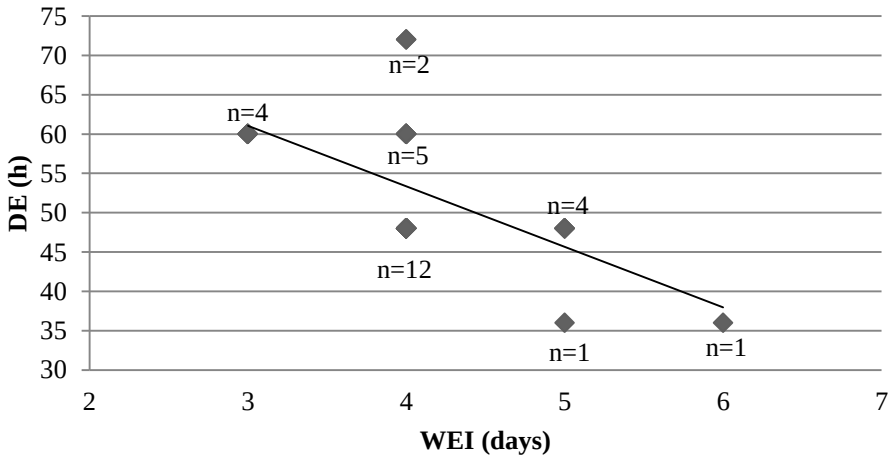
Delcroix, Mauget and Signoret (1990) during two years with female wild boars similar results were found, with a high degree of synchrony inside each social group. These studies suggest that this synchrony is probably due to the presence of female pheromones that, in combination with social stimuli (mounting, flank nosing etc.), may affect the estrous cycle.

In sows, the mean WEI was 83 ± 15.1 hours (mean ± SD), while gilts showed estrus 94.8 ± 10 hours after last administration of altrenogest. The WEI is smaller than the findings in other studies such the one performed by Kemp and Soede (1996), who reported a WEI of 92 ± 14.4 hours when making a retrospective analysis of 201 sows in commercial farms. The shorter WEI found in this farm illustrates their good reproduction management, housing conditions and nutrition, among other factors. Although a short WEI is desired, since it leads to a decrease in the number of non-productive days and an increase in the number of farrows per sow per year, a too narrow interval may also have some disadvantages. In 2006, Poleze, Bernardi, Amaral Filha, Wentz and Bortolozzo tested the influence of WEI on the reproductive performance and found that females with very short WEI (0-2 days) had higher return to estrus rate (23.8%) and lower farrowing rate (71.4%) when compared to sows whose WEI was between 3-5 days (4.6% and 82.9%, respectively). Thus, Behan and Watson (2005) suggested that delaying the boar stimulus until day 4 after weaning (delaying, this way, the OE), contrary to the classical approach of exposure since the day of weaning, can actually improve farrowing rate and litter size.

The duration of estrus was slightly longer in sows than gilts, with average values of 52.6 ± 8.6 hours and 48.0 ± 0 hours, respectively. Although the number of gilts was small (n=5) and, thus, of limited interpretation, these findings are in agreement with the study performed by Steverink et al. (1999) that analyzed the data from 12,794 sows and 2,389 gilts in 55 farms and reported that on average, sows had a longer duration of estrus than gilts (48.5 ± 1.0 h vs 40.8 ± 1.1 h [mean ± SEM], $p < 0.001$). In our study, the duration of estrus was slightly longer than in the study mentioned above, either in sows (52.6 ± 8.6 h vs 48.5 ± 1.0 h), as in gilts (48 h vs 40.8 ± 1.1 h). The difference observed between the two studies is probably due to a farm-effect. In fact, it was found that the differences in DE between farms accounted for 23% of the total variation in DE (Steverink et al., 1999), which might explain the differences found. Additionally, it has been proved the existence of a negative correlation between WEI and DE (Kemp and Soede, 1996; Belstra, Flowers and See, 2004), which will be discussed in further detail below. Since in our study we found, in average, a shorter WEI (83 ± 15.1 hours vs 129.6 ± 84 hours found by Steverink et al.), the increased duration of estrus is in agreement with that assumption.

A negative correlation (r = -0.59, annex 2) was found between the WEI and the OE, according to the following linear regression: DE = 84,13-7,6957xWEI (figure 15). For a WEI of 3 days, the mean duration of estrus was 60 hours, for a 4 days WEI 53.7 ± 8.1 hours, 45.6 ± 4.8 hours for a 5 days WEI and 36 hours for a 6 days WEI. Thus, the greater the WEI, the smaller the DE. Kemp and Soede (1996) showed that duration of estrus significantly decreased from 61 ± 12 hours for a WEI of 3 days to 38 ± 19.9 hours for 6 days. Viana, Silveira, Moretti and Rodrigues (1999) also found a negative correlation factor (r = -0.47) between these two variables, ranging the duration of estrus from 73.9 ± 10.4 hours for a 3 days WEI to 56.5 ± 12.6 hours for a WEI of 6 days. Given this significant variability in terms of DE and its strict relation with WEI, it can be easily assumed that the moment of ovulation will obviously differ according to the WEI and the DE. Soede, Helmond and Kemp (1994) found a strong correlation ($R^2$=0.89) between the timing of ovulation and the DE so it can assumed that the greater the DE, the longer the interval from the OE to ovulation. This is also supported by the findings of Kemp and Soede (1996), who detected ovulation 41 ± 6.1 hours after OE for a 3 days WEI (with a DE of 61 ± 12 hours) but only 27 ± 13.2 hours after OE for a WEI of 6 days (DE 38 ± 19.9 hours). This variation in time of ovulation is of the utmost importance, since maximum sow fertility is achieved when insemination is performed 24 hours prior to ovulation (Soede et al., 1995; Nissen et al., 1997; Cassar, Kirkwood, Poljak, Bennett-Steward & Friendship, 2005; Cassar, 2006). Therefore, pig producers follow complex insemination protocols adapted to the WEI in order to have, among the inseminations performed (usually 2 or 3), one of them hitting that 24 hours interval (see annex 12.4 for the insemination protocol used in this farm).

**Figure 15:** Relationship between weaning-to-estrus interval (WEI) and duration of estrus (DE) in 29 multiparous sows. A negative correlation was found (r= -0.59) between these two variables.



## 5.3. Temperature profiles

A statistically significant ($p < 0.001$, annex 3) daily variation pattern in VST was found from day to night (figure 16). The highest temperatures were obtained at 1800 h (average 32.5 ± 2 $^{o}$C) and the lowest ones at 0600 h (average 29.9 ± 2.6 $^{o}$C). At 0000 h and 1200 h, the mean temperatures recorded were, respectively, 31.6 ± 2.4 $^{o}$C and 31.9 ± 2.3 $^{o}$C (table 3). The same trend was found in the GST ($p < 0.001$, annex 4), ranging from 30.7 ± 1.9 $^{o}$C at 1800 h to 27 ± 2.6 $^{o}$C at 0600 h (table 4). Soede, Hazeleger, Broos and Kemp (1997) found a similar pattern when monitoring the vaginal temperature using internal temperature transmitters suited to measure the internal body temperature. Between 1500 h and 2100 h they reported an average vaginal temperature of 38.5 ± 0.03 $^{o}$C, while during the night, between 0300 h and 0900 h, this value decreased to 38.2 ± 0.03 $^{o}$C. It is important to that note these values refer to internal body temperatures, which explains their higher temperatures and their lower variation from day to night.

**Figure 16:** Daily variation in the average vulvar skin temperature (VST) and average gluteal skin temperature (GST) from all animals (n=34). The fluctuation pattern is obvious, with the highest temperatures obtained at 1800h and the lowest at 0600h.

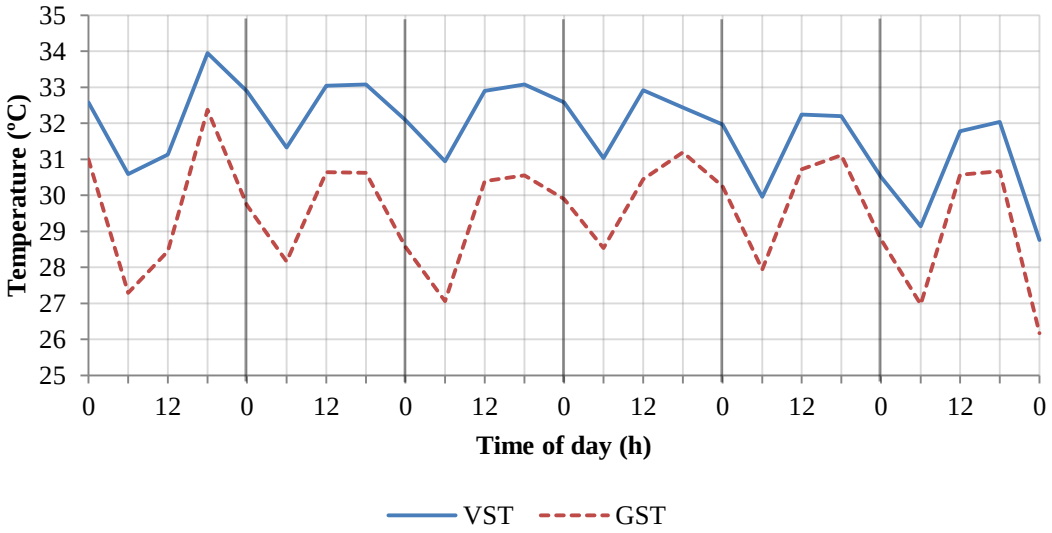


**Table 3:** Average vulvar skin temperature from all animals (n = 34) according to day and hour of measurement. A significant variation was found along the day, with the highest temperatures obtained at 1800h and the lowest ones at 0600h.

| | 0000h | 0600h | 1200h | 1800h |
|---|---|---|---|---|
| **DAY 1** | 32,6 ± 1,5 | 30,6 ± 2,1 | 31,1 ± 1,8 | 33,9 ± 1,3 |
| **DAY 2** | 32,9 ± 1,4 | 31,3 ± 1,7 | 33,0 ± 1,4 | 33,1 ± 1,4 |
| **DAY 3** | 32,1 ± 1,8 | 30,9 ± 1,8 | 32,9 ± 1,6 | 33,1 ± 1,4 |
| **DAY 4** | 32,6 ± 1,5 | 31,0 ± 1,7 | 32,9 ± 2,2 | 32,4 ± 1,9 |
| **DAY 5** | 32,0 ± 1,7 | 30,0 ± 2,4 | 32,2 ± 1,9 | 32,2 ± 1,3 |
| **DAY 6** | 30,5 ± 2,6 | 29,1 ± 2,1 | 31,8 ± 1,8 | 32,0 ± 1,4 |
| **DAY 7** | 28,8 ± 2,5 | 26,3 ± 2,4 | 29,1 ± 2,3 | 30,5 ± 2,7 |
| **AVG** | 31.6 ± 2.4 | 29.9 ± 2.6 | 31.9 ± 2.3 | 32.5 ± 2 |

**Table 4:** Average gluteal skin temperature from all animals (n = 34) according to day and hour of measurement. A significant variation was found along the day, with the highest temperatures obtained at 1800h and the lowest at 0600h.

| | 0000h | 0600h | 1200h | 1800h |
|---|---|---|---|---|
| **DAY 1** | 31,0 ± 1.9 | 27,3 ± 2.5 | 28,4 ± 2.4 | 32,4 ± 0.9 |
| **DAY 2** | 29,7 ± 2.3 | 28,2 ± 2.3 | 30,6 ± 1.4 | 30,6 ± 1.5 |
| **DAY 3** | 28,6 ± 2.4 | 27,1 ± 2.2 | 30,4 ± 1.7 | 30,6 ± 1.7 |
| **DAY 4** | 29,9 ± 2.2 | 28,5 ± 2.1 | 30,5 ± 2.0 | 31,2 ± 1.3 |
| **DAY 5** | 30,3 ± 2.2 | 27,9 ± 2.2 | 30,7 ± 2.1 | 31,1 ± 1.6 |
| **DAY 6** | 28,8 ± 2.3 | 27,0 ± 1.9 | 30,6 ± 1.6 | 30,7 ± 1.4 |
| **DAY 7** | 26,2 ± 2.3 | 23,5 ± 1.6 | 26,5 ± 2.2 | 28,4 ± 2.6 |
| **AVG** | 29,2 ± 2,6 | 27 ± 2.6 | 29,7 ± 2,4 | 30.7 ± 1.9 |

This daily temperature variation must be taken into account since, as we were looking for slight temperature variations during the estrous cycle, this regular fluctuations might mask the physiological variations we were trying to identify and, thus, negatively affect our results. Two scenarios were likely to give us a wrong interpretation of the results: first, considering that, hypothetically, we were expecting a physiological negative temperature peak 24 hours prior to ovulation. In animal A, 24 hours prior to ovulation corresponded to a measurement recorded at 0600 h, and so we actually noticed a significant decrease in the temperature recorded. On the other hand, in animal B, this measurement was obtained at 1800 h and, thus, only a slight, negligible drop in temperature was found, or no drop at all if the RT was particularly high that afternoon. In this case we would obtain a false negative and the physiological drop would be neglected. On the second scenario, a particularly cold night could cause a sudden drop in temperature from 1800 h to 0600 h, which would make us overestimate that variation, even if it was not due to a physiological event. This type of error, false positive, would make us consider a temperature variation that was not related to the estrous cycle.

Given the influence that these variations can have in our results, it is important to look for possible causes that can explain this phenomenon. The first factor to look at is, obviously, the RT. The trial was conducted, as mentioned before, between January and February in the south of France. The outside temperatures changed significantly from day to night but also during the course of the trial, ranging from a maximum of 7 $^{o}$C at day 0 to a minimum of -12 $^{o}$C at day 7. Although the building had a controlled ventilation system, these extreme external fluctuations had a considerable impact in the RT, either from day to night, with the lowest temperatures recorded at 0600 h (average 16.1 ± 2.2 $^{o}$C) and the highest ones at 1800 h (average 17.8 ± 1.6 $^{o}$C), but also during the course of the trial, with the highest daily average obtained at day 2 (20.0 ± 0.8 $^{o}$C) and the lowest one at day 7 (15.1 ± 0.9 $^{o}$C, table 5).

When comparing the VST, GST and RT, a clear correlation is found, either between VST and RT, either between GST and RT (figures 17, 18 and 19). The VSTxRT followed a linear regression model based on the equation VST = 22,76+0,51RT (r=0.61, p=0.001, figure 18 and annex 5), while for the GSTxRT interaction the following linear regression was found: GST = 20,75 + 0,49RT (r=0.50, p=0.007, figure 19 and annex 6). From these results, it is clear the RT had a strong influence in the VST and GST and can partially explain its daily variation.

**Table 5:** Room temperature during the trial. The highest temperatures were recorded at 1800 h and the lowest ones at 0600 h.

| | 0000h | 0600h | 1200h | 1800h | Day AVG |
|---|---|---|---|---|---|
| **DAY 1** | 18,8 | 17,6 | 20 | 20,8 | 19,3 ± 1,4 |
| **DAY 2** | 21 | 20,4 | 19,4 | 19,2 | 20,0 ± 0,8 |
| **DAY 3** | 17,6 | 14,6 | 15,8 | 17,2 | 16,3 ± 1,4 |
| **DAY 4** | 16,6 | 15,8 | 17 | 17,2 | 16,7 ± 0,6 |
| **DAY 5** | 15 | 14,6 | 15,4 | 17,4 | 15,6 ± 1,2 |
| **DAY 6** | 16,4 | 15,6 | 16,6 | 16,8 | 16,4 ± 0,5 |
| **DAY 7** | 15 | 14 | 15 | 16,2 | 15,1 ± 0,9 |
| **Hour AVG** | 17,2 ± 2,2 | 16,1 ± 2,2 | 17,0 ± 2,0 | 17,8 ± 1,6 | |

**Figure 17:** Evolution of room temperature along the trial compared to the average vulvar skin temperature (VST) and gluteal skin temperature (GST) from all animals. The red columns put on evidence the similar pattern between the room temperature peaks and the VGT and GST peaks.

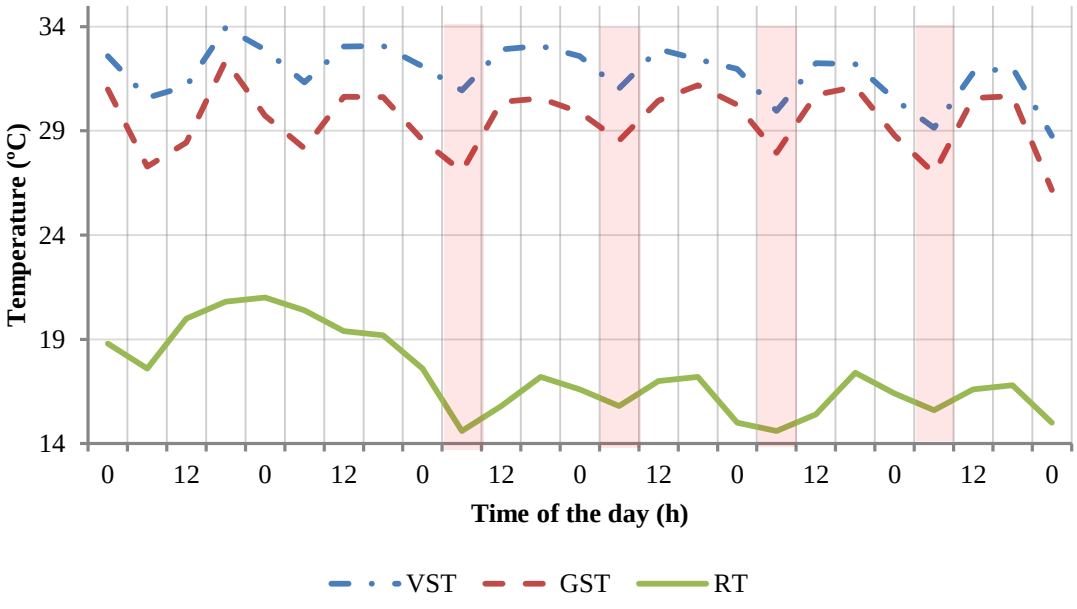


**Figure 18:** Scatter plot and linear regression line between room temperature (RT) and vulvar skin temperature (VST). A positive correlation was found (r=0.61) between these two variables, with the VST increasing with higher RT values.

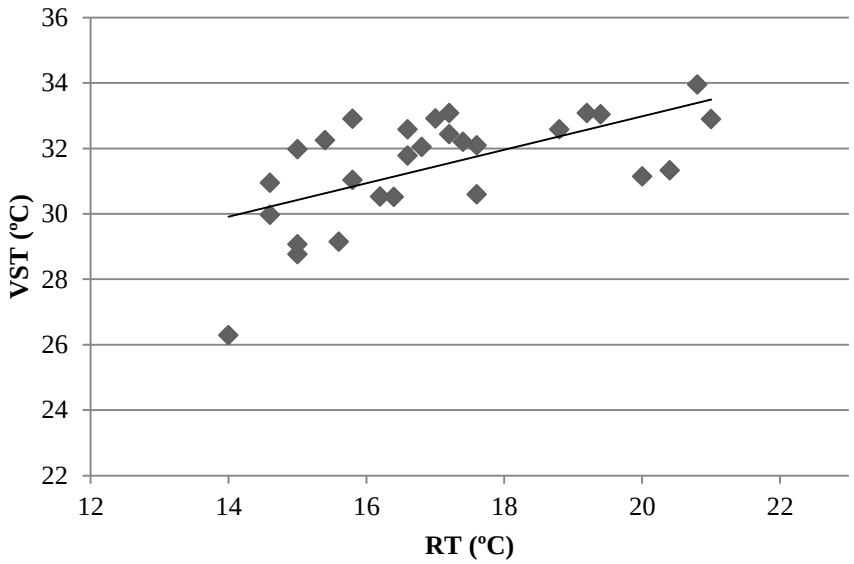
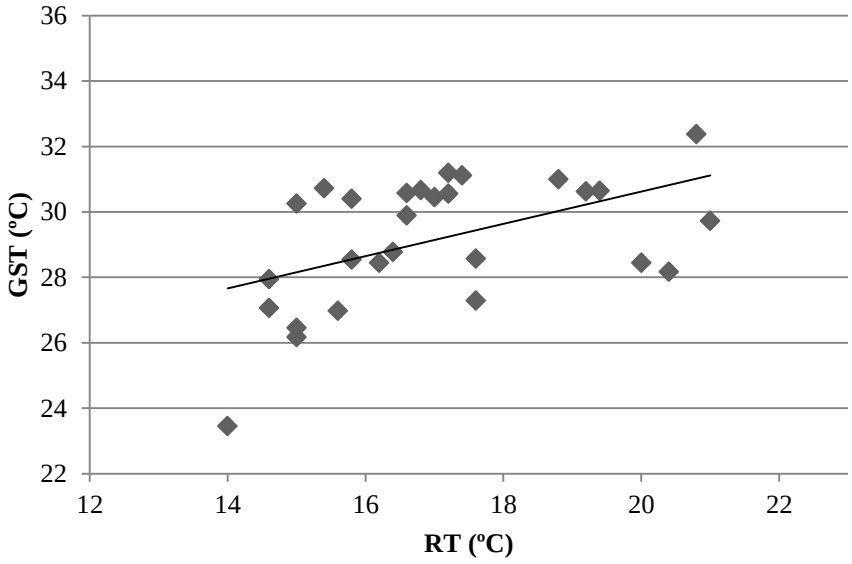



**Figure 19:** Scatter plot and linear regression line between room temperature (RT) and gluteal skin temperature (GST). A positive correlation was found (r=0.50) between these two variables, with the GST increasing with higher RT values.



Another factor that must be taken into consideration in these daily variations is the circadian rhythm of the body temperature. It is well known the body temperature changes along the day, showing some variation between waking and sleeping periods. These variations are controlled by a thermoregulatory center located in the hypothalamus and are closely related to levels of melatonin produced by the pineal gland: as the melatonin secretion increases in the evening due to the decrease in the amount of light, the body temperature falls, while in the morning as the light increases the melatonin levels decrease, leading to an increase in the body temperature (Kräuchi, 2002; Weinert, 2010) (see annex 12.5).

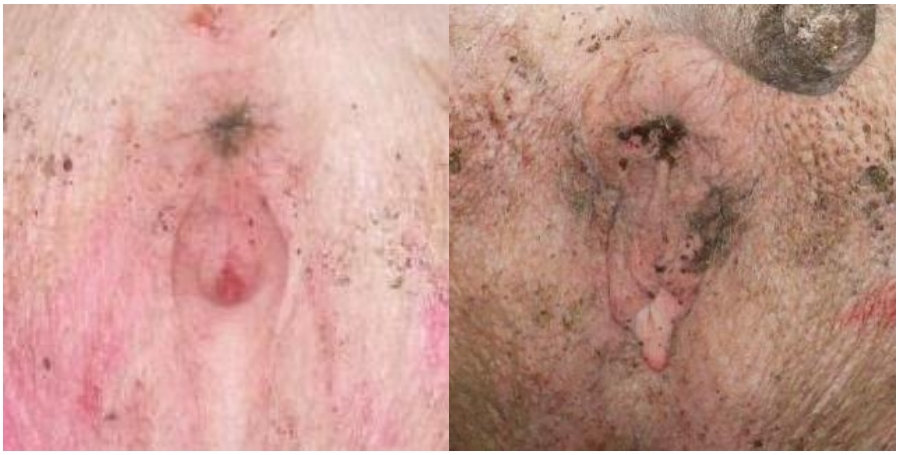
Apart from this endogenous thermal regulation, some exogenous factors must be also considered. The digestion or the physical and mental activity increase significantly the body temperature, while the sleep itself leads to its decrease, due to the change in body posture, into a more "relaxed" state (Deboer, Franken & Tobler, 1994; Kräuchi, 2007).

It can be assumed that during the day an increase in RT, level of physical activity and a decrease in the levels of melatonin result in a raise in the body temperature, reaching a peak at 1800 h in our measurements. As the evening approaches, the decrease of sunlight leads to an increase in the levels of melatonin, the RT and the level of physical activity starts to decrease and so the body temperature starts decreasing too. At 0600 h, just before the sunrise, the RT reached the lowest value and the melatonin blood levels the highest, which explains the lowest VST and GST recorded at that hour.

VST was significantly higher in gilts than in sows, either before estrus (32.9 ± 1.8 $^{o}$C vs 31.9 ± 1.9 $^{o}$C, $p = 0.002$, annex 7), either during estrus (31.7 ± 2.1 $^{o}$C vs 30.4 ± 2.5 $^{o}$C, $p = 0.01$, annex 7). Regarding the gluteal temperatures no difference was found between gilts and sows during the trial ($p = 0.3$, annex 8). This can probably be explained by the differences in the vulva's shapes between younger and older animals. In younger animals, the vulva is smaller, with considerably less skin and thus less susceptible to the environment temperature fluctuations. On the other hand, in older animals the vulva is longer, with more folded skin and more susceptible to the cold environment (figure 20). This way, during the night, the vulva in older animals will show a bigger drop in temperature, while in the gilts its temperature will remain steadier. Additionally, along the day the VST will increase faster in younger animals than in the older ones, as the blood irrigation in the latter's vulva is less efficient given their considerably higher amount of folded skin.

As no significant difference in GST between gilts and sows was found, we can assume the increased VST in gilts was not related to a general increase in the body temperature, but only on that specific area.

**Figure 20:** Comparison between the vulva's shape of a gilt (left) and a sow (right). The sow's vulva is considerably bigger, with more folded skin. Original image.



The aim of this study was to find a significant change in the vulvar temperature related to an event in the estrous cycle, possibly the ovulation. In order to analyze the temperature records and compare the results from different animals, these records must have the same reference point in the estrous cycle. Since there was a significant variation in the OE among the animals, with estrus starting between day 3 and day 6, their temperature records were compared relative to an event easily identifiable, in this case the OE. To analyze the variation of VST and GST along the estrous cycle, the records were grouped in 24-hours intervals and the variation between groups tested using ANOVA, minimizing this way the influence of the daily variation already discussed.

The average VST was significantly higher before than during estrus ($p < 0.001$, annex 9), with an average value of 32.2 ± 1.9 $^{o}$C and 31.0 ± 2.8 $^{o}$C, respectively before and during estrus. The VST stayed relatively steady before estrus ($p = 0.86$), with an average value of 32.3 ± 1.8 $^{o}$C, starting to decrease close to the OE ($p < 0.001$, annex 10). At day 1 of estrus the average VST was 31.7 ± 2.4 $^{o}$C, at day 2 31.0 ± 2.4 $^{o}$C and at day 3 29.6 ± 3.1 $^{o}$C (table 6 and figure 21). The variation found in the VST appears to be related with the reddening and swelling of the vulva during the proestrus, typical signs of the upcoming estrus. These signs appear 2 to 5 days before SE and begin to subside 24 to 36 hours prior to OE (Worwood, 2007). As these signs are due to an increased local blood flow, it is expected the local temperature to show a

similar increase, in accordance with our findings. With the OE, these symptoms start to decline in intensity and so it does the local temperature, leading to a higher VST during the proestrus than during the estrous period. Curiously, in a similar study, Scolari (2010) revealed opposite results, stating that both sows and gilts "displayed significantly increased temperature during estrus as opposed to the non-estrous period". Since we did not had access to the data from this study, it's difficult to find an explanation for the discrepancy in these results. Nevertheless, the already mentioned physiological events occurring during this period support our findings, which make us believe in the consistency of our results.

The GST was in average 2.32 ± 1.9 $^{o}$C lower than the VST and did not show any significant variation along the trial ($p = 0.47$, annex 11), so it can be assumed the variation detected in the VST was not due to a change in the whole body temperature, but only in the vulvar area (table 6 and figure 22).

**Table 6:** Vulvar skin temperature (VST), gluteal skin temperature (GST) and vulvar-gluteal temperature (VGT) relative to onset of estrus (OE) in all animals (n=34). The cells in gray correspond to the detected change-points for the VGT. Temperature reported in Celsius degrees.

| Hours to OE | VST | GST | VGT |
|---|---|---|---|
| **-72** | 31.3 ± 2.2 | 28.2 ± 2.3 | 3.1 ± 2.6 |
| **-66** | 32.5 ± 1.8 | 30.0 ± 2.3 | 2.5 ± 1.8 |
| **-60** | 33.1 ± 1.7 | 30.6 ± 2.6 | 2.5 ± 1.7 |
| **-54** | 32.3 ± 1.4 | 28.9 ± 2.5 | 3.4 ± 2.4 |
| **-48** | 31.7 ± 1.5 | 28.7 ± 2.4 | 2.9 ± 2.2 |
| **-42** | 33.1 ± 1.3 | 30.6 ± 1.3 | 2.5 ± 1.3 |
| **-36** | 32.8 ± 1.7 | 29.9 ± 2.4 | 2.9 ± 1.8 |
| **-30** | 31.9 ± 2.1 | 28.9 ± 2.2 | 3.0 ± 1.4 |
| **-24** | 31.3 ± 2.0 | 28.2 ± 2.4 | 3.0 ± 1.8 |
| **-18** | 33.2 ± 1.7 | 30.8 ± 1.7 | 2.4 ± 1.4 |
| **-12** | 32.7 ± 1.8 | 30.3 ± 2.3 | 2.3 ± 1.5 |
| **-6** | 31.8 ± 2.0 | 29.5 ± 2.0 | 2.3 ± 1.4 |
| **0** | 31.7 ± 2.3 | 29.0 ± 1.8 | 2.6 ± 1.7 |

**Table 6 (continuation):** Vulvar skin temperature (VST), gluteal skin temperature (GST) and vulvar-gluteal temperature (VGT) relative to onset of estrus (OE) in all animals (n=34). The cells in red correspond to the detected change-points for the VGT. Temperature reported in Celsius degrees.

| Hours to OE | VST | GST | VGT |
|---|---|---|---|
| **+6** | 32.3 ± 1.9 | 31.0 ± 1.9 | 1.9 ± 1.3 |
| **+12** | 31.9 ± 2.0 | 30.6 ± 2.0 | 1.3 ± 1.8 |
| **+18** | 30.4 ± 2.7 | 28.7 ± 2.7 | 1.7 ± 1.8 |
| **+24** | 31.1 ± 1.8 | 29.3 ± 2.0 | 1.8 ± 1.7 |
| **+30** | 32.5 ± 1.4 | 31.0 ± 1.5 | 1.6 ± 0.9 |
| **+36** | 31.3 ± 2.3 | 29.8 ± 2.4 | 1.5 ± 1.3 |
| **+42** | 29.3 ± 2.7 | 27.4 ± 2.6 | 2.0 ± 1.9 |
| **+48** | 29.4 ± 2.9 | 27.2 ± 3.4 | 2.2 ± 2.1 |

**Figure 21:** Variation in vulvar skin temperature (VST) and 4-points average. The blue shaded area represents the standard deviation (SD) of VST and the red gradient the standing estrus. The blue, solid line represents the average VST from all animals (n=34), while the red, dashed line represents the 4-points average method, where for each hour the two previous measurements, the one obtained at that same hour and the next is considered in order to stabilize their daily variation. A significant decrease in VST is detected starting close to onset of estrus (OE).

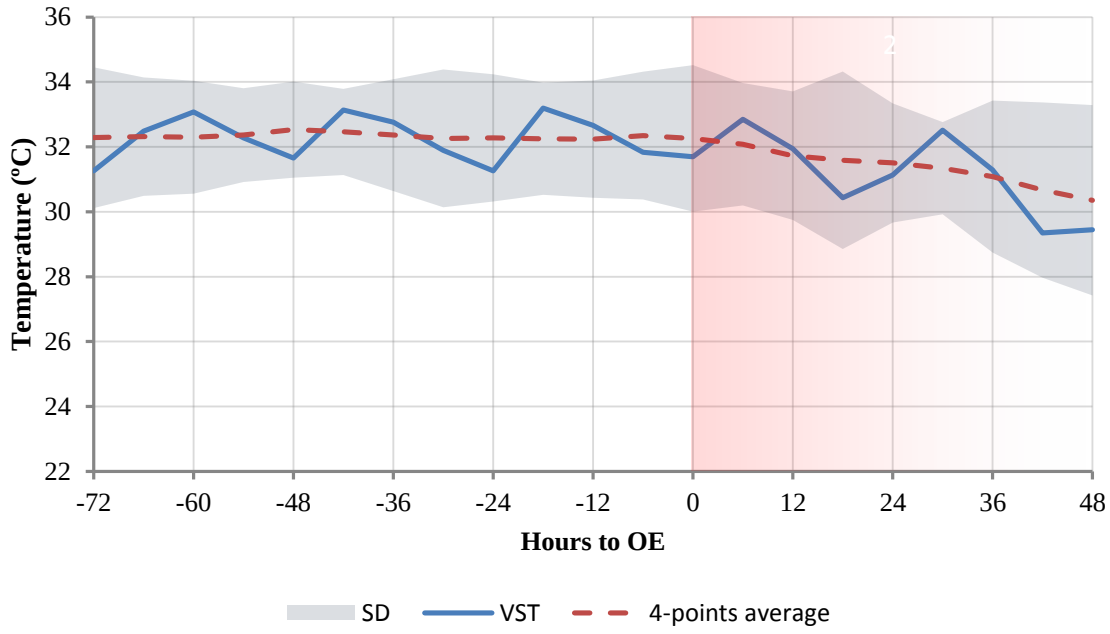
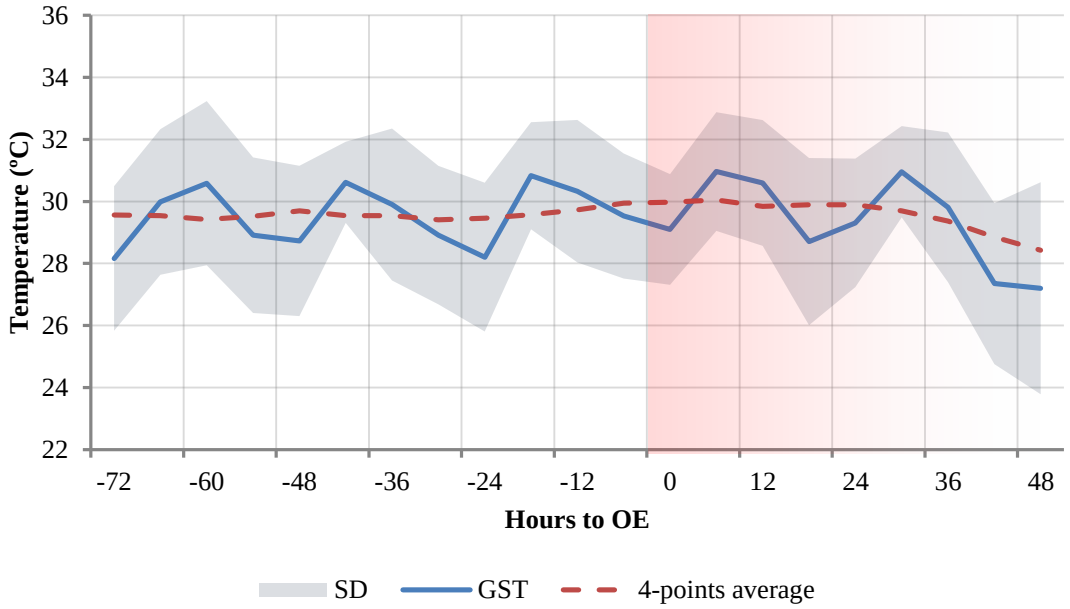


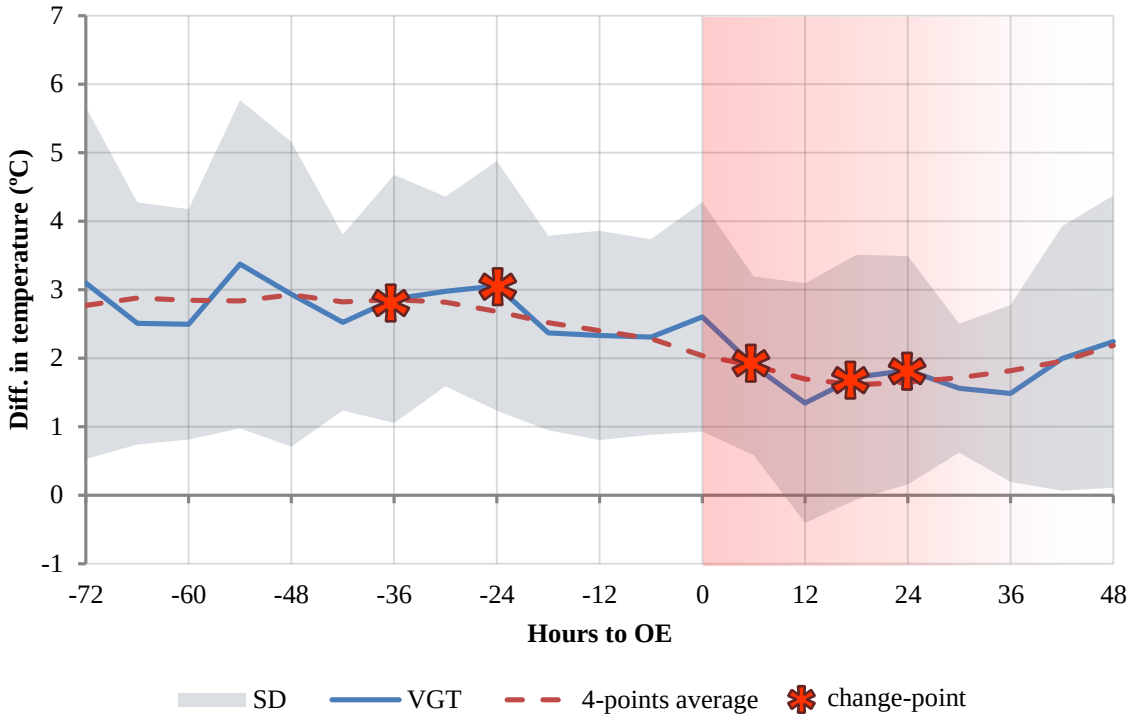
**Figure 22:** Variation in gluteal skin temperature (GST) and 4-points average. The blue shaded area represents the standard deviation (SD) of GST and the red gradient the standing estrus. The blue, solid line represents the average GST from all animals (n=34), while the red, dashed line represents the 4-points average method, where for each hour the two previous measurements, the one obtained at that same hour and the next is considered in order to stabilize their daily variation. No statistically significant variation in GST was found during the trial.




For each individual measurement, the difference between the vulvar and gluteal temperature was obtained (VGT). With this approach it was possible to minimize the influence of the daily variation in our results, since VST and GST were both affected by the circadian rhythm and, thus, analyze the results from every 6 hours, getting a more precise interpretation of the temperature fluctuations (table 6).

The VGT remained constant ($p = 0.88$) before estrus, starting to decrease significantly ($p < 0.001$) 36 to 24 hours prior to OE, reaching the lowest value 12 to 18 hours after OE, after which stabilizes, increasing very slightly but not significantly ($p = 0.20$, figure 23). 5 change-points (Cp) were detected when analyzing this VGT, at -36, -24, +6, +18 and +24 hours relative to OE.

**Figure 23:** Variation in vulvar-gluteal temperature (VGT) and 4-points average from all animals. The blue shaded area represents the standard deviation (SD) of VGT and the red gradient the standing estrus (SE). The VGT started decreasing 24 to 36 hours prior to OE and stabilized 12 to 28 hours after OE. 5 change-points were detected.




This analysis, however, raises an issue that should be consider: as we are taking into account the records of all animals, with different DE and consequently different times of ovulation in relation to OE and assuming the temperature variation is related to ovulation, a considerable variation in the temperature profiles is expected, since the time of ovulation will vary largely between these animals. This is proved by the high number of Cp detected, suggesting the existence of different temperature profiles among the animals. Thus, it was necessary to compare the records of animals synchronized in terms of estrous cycle and, thus, with similar temperature profiles. This was achieved by comparing animals with the same WEI and, consequently, with similar estimated time of ovulation.

This way, two sub-groups of animals were created, regarding animals with simultaneous OE: group D4, composed by 11 animals with OE at day 4, 0600 h, and group D5, composed by 7 animals, starting estrus at day 5, 0600 h. The group results are reported in table 7. For the individual records off all animals, see annex 12.6.

**Table 7:** Vulvar-gluteal temperature (VGT) related to onset of estrus (OE) in groups D4 and D5. The cells in gray correspond to the detected change-points for the VGT. The maximum and minimum temperature peaks are tagged with (M) and (m), respectively. Temperature reported in Celsius degrees.

| Hours to OE | D4 | D5 |
|---|---|---|
| **-90** | | 2.8 ± 1.3 |
| **-84** | | 2.0 ± 0.7 |
| **-78** | | 2.9 ± 1.5 |
| **-72** | | 3.8 ± 3.0 |
| **-66** | 3.0 ± 2.0 | 2.9 ± 2.0 |
| **-60** | 1.1 ± 1.2 | 3.8 ± 1.6 |
| **-54** | 3.4 ± 1.5 | 3.8 ± 2.3 |
| **-48** | 2.7 ± 1.6 | 5.3 ± 2.4 (M) |
| **-42** | 1.9 ± 1.0 | 3.4 ± 1.0 |
| **-36** | 2.0 ± 1.2 | 3.4 ± 2.1 |
| **-30** | 3.5 ± 1.0 | 2.4 ± 1.6 |
| **-24** | 3.8 ± 1.9 (M) | 3.5 ± 2.3 |
| **-18** | 2.0 ± 1.1 | 2.6 ± 2.0 |
| **-12** | 2.0 ± 1.0 | 1.5 ± 1.4 |
| **-6** | 2.5 ±1.2 | 1.4 ± 1.2 |
| **0** | 2.6 ± 1.3 | 2.5 ± 1.1 |
| **+6** | 2.7 ± 1.2 | 1.1 ± 0.9 (m) |
| **+12** | 0.5 ± 1.5 (m) | 1.4 ± 1.0 |
| **+18** | 1.6 ± 1.4 | 2.3 ± 1.9 |
| **+24** | 2.1 ± 1.7 | 1.9 ± 1.3 |
| **+30** | 1.7 ± 0.8 | 1.6 ± 1.1 |
| **+36** | 0.9 ± 1.0 | 1.3 ± 1.3 |
| **+42** | 1.7 ± 1.2 | 2.2 ± 2.4 |
| **+48** | 2.7 ± 1.3 | 3.0 ± 3.0 |
| **+54** | 1.4 ± 1.0 | 3.2 ± 2.6 |
| **+60** | 0.8 ± 1.4 | |
| **+66** | 2.6 ± 2.0 | |
| **+72** | 2.8 ± 1.9 | |
| **+78** | 1.8 ± 1.6 | |

In group D4, one Cp in the trend of the VGT was found 6 hours after OE. This Cp resulted from a change in the trend of the VGT, which increased during the proestrus ($p = 0.003$) reaching a maximum average value of $3.8 \pm 1.9$ $^{o}$C 24 hours before OE. After this point, the VGT started decreasing markedly ($p = 0.002$) until reaching a minimum value ($0.5 \pm 1.5$ $^{o}$C) 12 hours after OE, after which starts increasing again ($p = 0.04$, figure 24).

In group D5, one Cp was found 24 hours before the OE. The VGT increased until 48 hours before OE ($p = 0.017$), reaching $5.3 \pm 2.4$ $^{o}$C and then starts decreasing ($p = 0.002$), reaching the lowest value 6 hours after OE ($1.1 \pm 0.9$ $^{o}$C). After this negative peak, the VGT stabilizes and starts increasing slightly, but not significantly ($p = 0.47$, figure 25).

**Figure 24:** Variation in vulvar-gluteal temperature (VGT) and 4-points average in group D4 (n=11). The blue shaded area represents the standard deviation (SD) of VGT and the red gradient the standing estrus. A change point is detected 6h after OE, with a maximum (M) temperature peak obtained 24 prior to OE and a minimum (m) temperature peak 12h after OE.

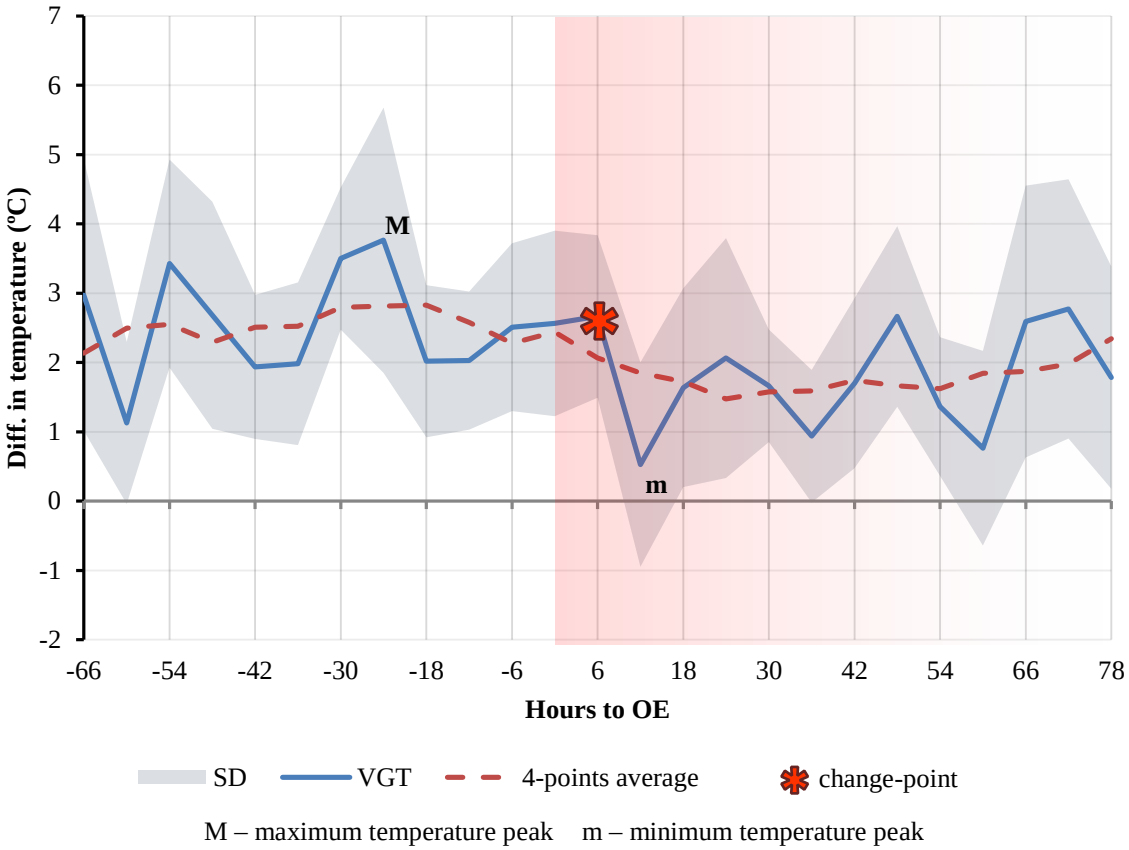
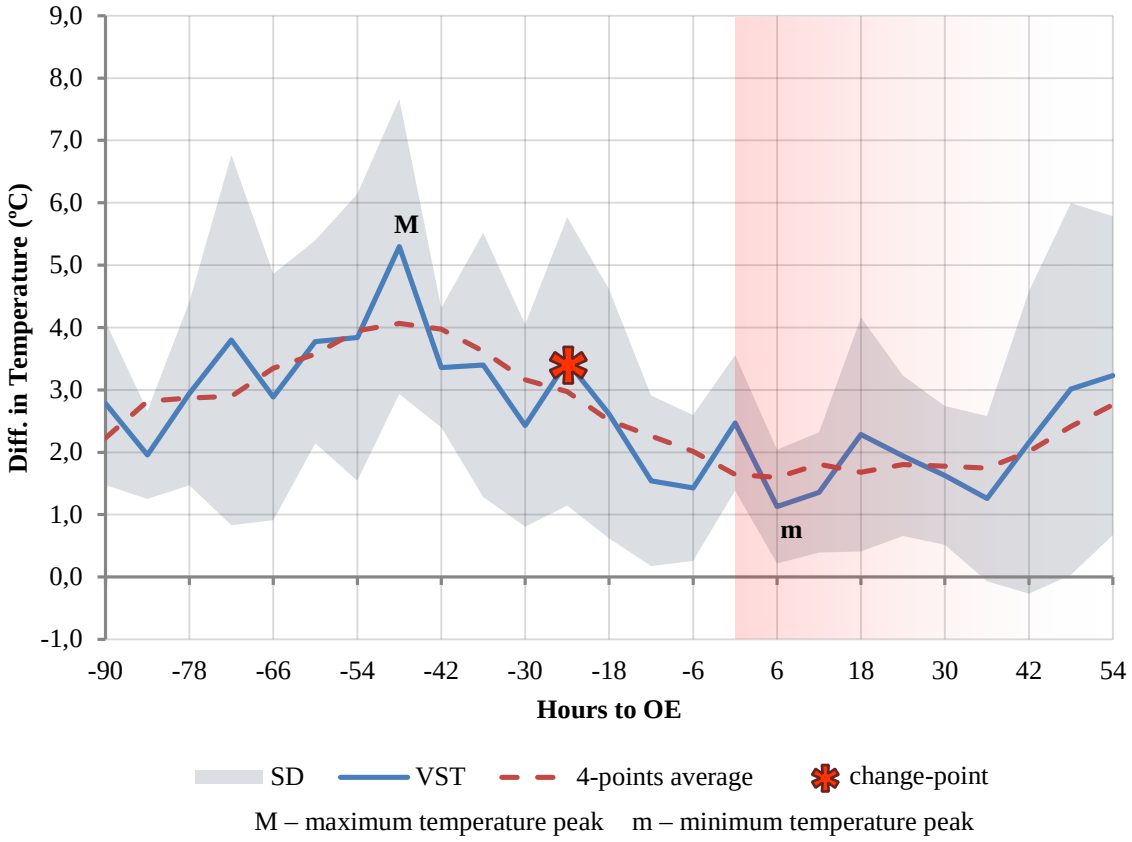


**Figure 25:** Variation in vulvar-gluteal temperature (VGT) and 4-points average in group D5 (n=7). The blue shaded area represents the standard deviation (SD) of VGT and the red gradient the standing estrus. A change point is detected 24h before OE, with a maximum (M) temperature peak obtained 48h prior to OE and a minimum temperature peak (m) 6h after OE.




Contrary to what was expected, the VGT showed a regular variation pattern similar to the one found in VST and GST. We assumed the circadian rhythm influences in the same proportion the VST and GST, making these two variables change proportionally along the day; as the VGT results from the difference between VST and GST, we did not expect to find this variation pattern. Looking deeply into this variation in Group D4, the VGT was found to be significantly higher at 0600 h than at 1800 h (2.7 ± 1.8 $^{o}$C vs 1.23 ± 1.3 $^{o}$C, $p < 0.001$). Thus, it was clear the variation in VST and GST was not proportional along the day, leading to the fluctuations observed in the VGT. When analyzing the VST and GST in these animals, the drop in temperature from 1800 h to 0600 h was found to be much more marked in the GST, decreasing in average 11%, from 31.3 ± 1.6 $^{o}$C to 28.2 ± 2.7 $^{o}$C, while the VST only decreased in average 6.2%, from 32.8 ± 1.8 $^{o}$C to 30.9 ± 1.9 $^{o}$C.

Given the evidences collected, it is clear the regular variation pattern found in the VGT is due to a more marked decrease in the GST than in the VST during the night. From our experience, this might be related to the change in posture and the difference in terms of physical activity observed between day and night. While the VST is minimally affected by posture and physical activity, the same can not be said regarding the GST; indeed, during the day the animals are standing up and exercising their leg muscles, but while sleeping the lack of exercise and their more relaxed posture lead to a greater drop in temperature at night, when compared to the variation observed in the VST.

## 5.4. Ovulation diagnosis with ultrasonography

The ovary US had a dual purpose in this study. First of all, test the applicability of US for ovulation diagnosis in sows and gilts and compare the two available techniques described in the literature: the transabdominal (TAU) and the transrectal (TRU) approach. Secondly, and only if the first goal was successfully achieved, use the data collected to establish a possible relationship between the VGT variations and the time of ovulation.

The echographies were performed with the support of 3 professors from the National Veterinary School of Toulouse, two of them from the Reproduction Department, with a wide experience in bovine's reproductive ultrasonography. Due to a tight schedule, these exams were performed not during all trial, but only for 2 days (day 5 and 6), when we expected to have a higher percentage of ovulations occurring. 20 animals were tested (14 sows and 6 gilts), in a total of 44 exams ultrasound scans.

Comparing the transrectal and transabdominal technique, the first proved to be considerably more precise and informative than the second. In 75% of the exams using transrectal technique it was possible to visualize the ovaries, being this value considerably lower (57%) when the transabdominal approach was used.

When performing the transabdominal technique (figure 26.1), the ovaries were found immediate cranial to the urinary bladder, between the loops of the uterine horns. The visualization of ovaries was considerably easier on the right side, due to the presence of the cecum on the left side. This makes the observation of both ovaries particularly difficult and usually only the right ovary can be visualized. As said before, the body condition also has influence in the image quality and in the success of diagnosis, being this technique more easily applied in slimmer animals. Wagner-Rietschel (1991) also found it easier to perform in

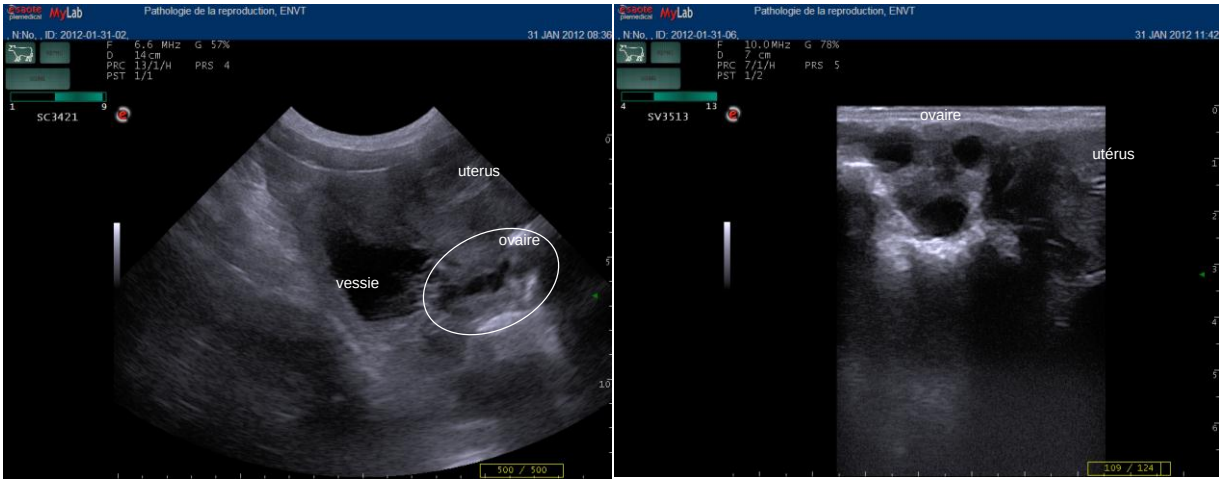
primiparous sows than in sows with more than four litters but we did not found a significant difference in our study. Usually it was not possible to detect CH or CL, due to their similar echogenicity with the ovary's interstitial tissue. Thus, the identification of ovaries using this technique is generally only possible before ovulation, with the presence of follicles. Some authors (Waberski et al., 2000; Kauffold et al., 2004) claim that this technique is more suitable to use under field conditions as it takes less time to achieve a diagnosis when compared to TRU, although from our experience this is largely dependent on the movements of the animal. In fact, if the animal is not very cooperative, the difficulty to get a clear image is significantly higher and it might take us even more time compared to the transrectal technique.

TRU is the gold standard for ovulation diagnosis in pigs (Boulot, 2010). With this technique, the ovaries were usually found slightly anterior and just lateral to the bladder. It did not stress considerably the animals and, according to Soede and Kemp (1993), has no apparent effect on fertilization when performed close to ovulation, which was not evaluated in our study. It required the introduction of the researcher's hand together with the ultrasound probe, so hand size becomes a limiting factor. In gilts it was possible to introduce only the fingers with the probe, while in older sows it was possible to introduce the forearm. With this technique it was considerably easier to find the ovaries (75% of the exams, against 57% when using TAU) and visualize both, which was usually not possible with the transabdominal technique. It was significantly easier to identify CH and, that way, identify the ovaries after ovulation. The follicles were visualized with a much better resolution (figure 26.2) since the probe was closer to the ovaries, which allowed us to use higher frequencies; additionally, a more reliable follicle count could be performed, which was particularly difficult with the other technique. It was minimally affected when the animal moves or lies down due to pelvic and rectal retention of the probe. The presence of feces between the probe and the intestinal mucosa might lead to appearance of dark areas in the image; when this happened, the probe had to be removed, cleaned and inserted again. To prevent this artifact, the feces were removed before the exam, which was easier in older sows but not always possible in gilts, and the probe inserted with a slight pressure downward, in order to pass below any feces that remained in the rectum.

**Figure 26:** Ovary ultrasonography of a gilt.

**Figure 26.1 (left):** Visualization of one ovary (ovaire), bladder (vessie) and uterus using transabdominal ultrasonography.

**Figure 26.2 (right):** The ovary of the same animal visualized 3 hours later using transrectal ultrasonography. Note the improved resolution of follicles and their bigger size.




The results from the ultrasonography exams are summarized in table 8. In 3 animals (15%) the echographies were not conclusive because the ovaries were not visualized and so no diagnostic could be established. In these 3 animals, only TAU was used, which might explain the failure in locating those structures. Several factors might turn difficult the visualization of the ovaries when using the transabdominal technique, such as movements from the animal, the amount of fat (observation is usually easier in slimmer animals than in fatter ones) or the presence of intestinal loops between the probe and the ovaries (Martinat-Botté et al., 1998; Kauffold et al., 2004). Regarding this last point, TAU should be performed preferentially on the right side, since in the left side the cecum frequently hinders the finding of those structures (Waberski et al., 2000).

In 7 animals (35%) ovulation was suspected to have already occurred before the first exam. Although the time of ovulation could not be established, it was assumed that it had already occurred based usually in 2 points: first, several consecutive exams performed with no evidence of follicles; second, visualization of corpora hemorrhagica (CH). Still, this second point could not be confirmed in all animals as CH have a similar echogenicity of the ovary's interstitial tissue, making its observation particularly difficult when using TAU, being usually only detectable with TRU (Waberski et al., 2000).

**Table 8:** Descriptive of the ovarian ultrasonography exams.

| | |
|---|---|
| **Number of exams performed** | 44 |
| **with transabdominal ultrasonography** | 34 |
| **with transrectal ultrasonography** | 10 |
| **Number of tested animals** | 20 |
| **of which sows** | 14 |
| **of which gilts** | 6 |
| **% of tested animals** | 55% |
| **Number of animals with inconclusive results** | 3 animals (15% of tested population) |
| **Number of animals with conclusive results** | 17 animals (85% of tested population) |
| **Ovulation had already occurred** | 7 animals (35% of tested population) |
| **Ovulation did not occurred during the course of the exams** | 6 animals (30% of tested population) |
| **Diagnosis of ovulation established** | 4 animals (20% of tested population) |

In 6 animals (30%) all the exams performed during the trial revealed the presence of follicles and it was assumed the ovulation did not occur during that period. Animals might be erroneously classified in this group if a differential diagnosis of follicles is not correctly made. These differential diagnosis include structures like vessels and ovarian cysts that might be misinterpreted as follicles due to their similar shape and echogenicity. In the presence of vessels, it is useful to change the orientation of the transducer. When the cross-section is closer to longitudinal, the vessels are seen as tubular structures, clearly different in shape from follicles (Martinat-Botté et al., 1998). Ovarian cysts, on the other hand, are very similar to follicles and their distinction might be harder. The classification is usually made based on

their size, as periovulatory follicles are generally under 8-10 mm, while above 11 mm these findings are classified as cysts (Knox & Althouse, 1999; Waberski et al., 2000).

Only in 4 animals it was possible to establish a suspected diagnosis of ovulation, which corresponds to 20% of the followed animals. This low efficacy can be explained by several factors. First, due to the tight schedule of the members of this team and the unavailability of the equipment itself during such a long period, it was impossible to perform the echography during the 7 days of the trial. With this narrow 2-days-window, it was impossible to match the time of the exams and the occurrence of ovulation in all animals. Second, although this team was experienced in bovine's reproductive US, it was the first time we performed it to visualize the ovaries in swine. As stated by Wagner-Rietschel (1991, cited by Waberski et al., 2000), the success of the diagnosis is closely related to the experience of the veterinarian. During a 16 week period, an inexperienced veterinarian performed over 3000 exams with an increase in the success rate, from 68 to 93% at the end of this period. Thus, the success rate and the level of trust in these results would certainly be higher if the exams were performed for a longer period.

According to the diagnosis established in these 4 animals, ovulation took place in average 18 ± 9.5 hours after onset of estrus. These results are distinct from the ones found in other studies, such as Soede et al., (1995) that reported a value of 35 ± 8 h, or Nissen et al., (1997), 42 ± 13 h. First, it is clear that the interpretation of the results from such a small number of animals has a limited value. Nevertheless, when looking for the data of each animal individually, some conclusions can be taken. In one of the animals (ID 1103), the ovulation was diagnosed 6 hours right after OE and in another one (ID 1062) 12 hours after OE, while the estrus had an approximate length of 48 hours in both cases (table 9). As already widely debated in the literature, ovulation occurs fairly constantly at the beginning of the last third of estrus (Soede et al., 1995; Nissen et al., 1997; Waberski et al., 2000). Although some variation is always expected from farm to farm and even between animals from the same farm, the work of Kemp & Soede (1996) found that with a WEI of 5 days (as the 2 animals we are considering here), only 16% ovulated in the first 24 hours of estrus. That said, it can be assumed our echography results are questionable and of a limited value.

**Table 9:** Comparison between the expected time of ovulation (based on the principle that occurs on average 70% the way through estrus [Soede and Kemp, 1997]) and our hypothetical diagnosis of ovulation (based on our findings from the ovaries ultrasonography). The results from ultrasonography clearly differ from what was expected.

| Animal ID | Onset of Estrus | End of estrus | Durantion of estrus | Expected time of ovulation (relative to OE) | Diagnosis of ovulation (relative to OE) |
|---|---|---|---|---|---|
| **1062** | Day 5, 0600h | Day 7, 0600h | 48 h | + 34 h | + 12 h |
| **1103** | Day 5, 1200h | Day 7, 1200h | 48 h | + 34 h | + 6 h |
| **1697** | Day 5, 0600h | Day 7, 0600h | 48 h | + 34 h | + 24 h |
| **2353** | Day 4, 1200h | Day 7, 0600h | 66 h | + 46 h | + 30 h |

In order to understand the failure in diagnosing the ovulation, it is important to understand what might have gone wrong. The method used for this diagnosis relies in the absence, and not on the evidence, of a structure, in this case the follicles. When the follicles are no longer visualized, the diagnosis of ovulation is established. This obviously has its flaws, as sometimes it turns to be particularly difficult to visualize these structures, which does not necessarily means the ovulation already occurred. This is particularly important when performing TAU and several factors that can negatively affect the observation were already discussed before. This way, we can assume that this method, as an ovulation detection tool, have a decent specificity (if the follicles are found, the animal clearly did not ovulate, assuming a good DD was made) but a somewhat lower sensitivity, largely dependent on the experience of the operator (as the follicles might be there but do not be visualized). Comparing our results with the ones from other authors, the ovulation occurred considerably sooner and this way it is possible that in same animals we missed the follicles, leading to a false and precocious diagnosis of ovulation.

Although ovary ultrasonography has been widely tested under experimental conditions as a tool to detect ovulation, it is important to note that this technique cannot predict when the ovulation will occur, which would be of the utmost importance in pig's production so the AI

could be performed during those 24 hours prior to ovulation (Soede et al., 1995; Nissen et al., 1996). In fact, several investigations performed in sows and gilts could not establish a relationship between the sonographic images of follicles and the time of ovulation (Soede et al., 1998; Waberski et al., 2000). As the ovulation approaches the follicles get bigger, but at ovulation their sizes might range from 8 to 10 mm, so the size itself cannot be used to predict its occurrence. It has been described a change in the shape of the follicles prior to ovulation, from a round to a more oval shape (Waberski et al., 2000), but this change is of difficult interpretation and thus cannot be considered a reliable indicator.

## 5.5. Alternative methods to estimate occurrence of ovulation

As the results from the ovaries ultrasonography were not always conclusive and only in a small number of animals not representative of the entire population a diagnosis of ovulation was established (even with some reservations about those results), a different method was required to estimate the time of ovulation. According to Waberski et al. (2000), "at present, the most valuable estimation of the oncoming time of ovulation is the observation of the interval from weaning to the onset of estrus". This is especially useful when we want to predict a future ovulation and do not know in advance the DE. Soede and Kemp (1997), by their turn, stated that "at present, the duration of estrus is the best 'predictor' of ovulation time, (…) since ovulation takes place on average 70% of the way through estrus." In order to estimate the time of ovulation in our study, both methods were considered and their results compared.

In 1996, Soede and Kemp studied the relationship between WEI - DE and the interval from OE to ovulation in 201 multiparous sows. The estrus detection was performed every 8 hours in presence of a mature boar and ovulation diagnosis was made using TRU every 4 hours. As TRU is currently, as already mentioned, the gold standard for ovulation detection in sows and the exams were performed in really narrow intervals, the ovulation results are expected to have a great precision.

Regarding the DE, this study reported an average value of 53 ± 10.8 hours for a 4 days WEI and 49 ± 13.2 hours for a 5 days WEI. These results are very close to ours (53.7 ± 8.1 hours and 45.6 ± 4.8 hours for a 4 and 5 days WEI, respectively) and so the ovulation profile is also expected to be very similar.

For a WEI of 4 days, according to the aforementioned study, ovulation was found to take place in average 37 ± 10.8 hours after OE. Based on this, the maximum and minimum peaks found in the VGT in Group D4 occurred, respectively, 61 ± 10.8 and 25 ± 10.8 hours before eOv. For a WEI of 5 days, ovulation took place 34 ± 6.6 hours after OE and so our maximum and minimum peaks in Group D5 occurred 82 ± 6.6 and 28 ± 6.6 hours before eOv. These results are summarized in table 10.

**Table 10:** Occurrence of vulvar-gluteal temperature maximum (M) and minimum (m) peaks in groups D4 and D5 and its relation with the expected time of ovulation. The determination of ovulation is based on the study performed by Soede and Kemp (1996). Special attention should be paid to the proximity of minimum peaks in both groups, relative to expected ovulation.

| Sample Group | Onset of Estrus | M (relative to OE) | m (relative to OE) | Expected time of ovulation (relative to OE) | M (relative to expected ovulation) | m (relative to expected ovulation) |
|---|---|---|---|---|---|---|
| **D4** | Day 4 0600h | - 24 h | + 12 h | + 37 ± 10.8 h | - 61 ± 10.8 h | - 25 ± 10.8 h |
| **D5** | Day 5 0600h | - 48 h | + 6 h | + 34 ± 6.6 h | - 82 ± 6.6 h | - 28 ± 6.6 h |

When considering the estimation method of ovulation suggested by Soede and Kemp (1997), 70% of the way through estrus would correspond, in our study, at ovulation occurring 37.6 ± 8.1 hours after OE on Group D4 (against 37 ± 10.8 hours from the previous method) and 31.9 ± 4.8 hours on Group D5 (against 34 ± 6.6 hours from the previous method). As both approaches lead to very similar results it was possible to validate our results and assume, this way, the occurrence of ovulation at those times.

## 5.6. Relationship between temperature variations and ovulation

The proximity in the occurrence of the minimum peaks observed in Group D4 and D5 related to eOv (-25 ± 10.8 h and -28 ± 6.6 h, respectively), suggests that this negative peak might be somewhat related to the occurrence of ovulation. However, the occurrence of the maximum

peaks changed significantly in group D4 and D5 (-61 ± 10.8 h and -82 ± 6.6 h before eOv, respectively). Curiously, in both groups the maximum peak corresponds to the measurement performed in day 3, 0600 h. At the time of this measurement the RT was particularly low, having decreased 4.8 $^{o}$C in the previous 12 hours and reaching one of the lowest values ever recorded (table 5). As mentioned before, a low RT leads to a greater decrease in the GST compared to the VST and, consequently, an abnormal increase in the VGT. Thus, one can assume that, although the estrous cycles of these two groups were delayed approximately 24 hours, the simultaneous occurrence of these maximum peaks reveal the influence of an external variable, in this case the RT.

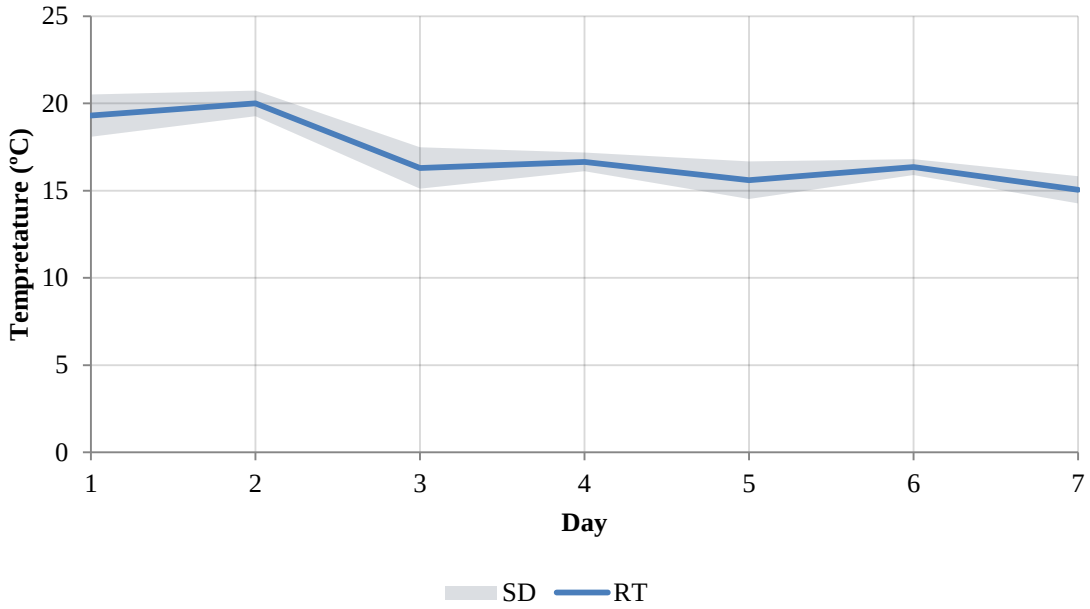
The variation observed in the VGT during the estrous cycle reflects the changes in the VST since the GST, as previously mentioned, did not change significantly along the trial. Although it can be assumed these temperature variations are related to an increased blood flow in the vulvar area during proestrus, the underlying mechanisms promoting these changes are still not absolutely clear. In cattle, similar studies found the sudden increase in VST coincides with the LH-peak (Clapper, Ottobre & Zartman, 1990; Fisher, Morton, Dempsey, Henshall & Hill, 2008), which occurs in average 9.1 ± 6.8 hours after OE (Saumande & Humblot, 2005). In pigs, the LH surge occurs 6.5 ± 2.3 hours after OE (Knox, Vatzias, Naber & Zimmerman, 2003). Since in our study the VGT maximum peak was found 24 to 48 hours prior to OE, no relationship can be established between the LH peak and the VST in pigs.

When considering the estradiol levels, a closer relationship is found. As ovulation gets closer, the developing pre-ovulatory follicles produce increasing amounts of estradiol, culminating in a peak 24 to 48 hours prior to OE (Guthrie, Henricks & Handlin, 1972), which will induce a plateau in the swelling and reddening of the vulva 24 to 36 hours prior to OE (Worwood, 2007) responsible for the VGT maximum peak obtained precisely 24 and 48 hours before OE in group D4 and D5. These findings are supported by the study performed by Stelletta et al. (2012), who monitored the VST of 9 mares using infrared thermography (IRT) during the follicular and luteal phase. They found this temperature was significantly higher during the follicular phase and assumed that this was probably due to the influence of the estrogen produced by the follicles, which induced a marked hyperemia in the vulvar region.

A curious fact is that when considering the VST alone, this progressive increase was not clear in our study, but only when the VGT was analyzed. This was probably due to the progressive decrease in the RT during the course of the trial, which masked the increase in the VST (figure 27). When considering the VGT, the influence of RT in the daily average temperature

was partially annulled and the increasing trend became clear. This illustrates the importance of performing our analysis not only with raw temperatures but also relatively to a control area, in this case the gluteal region.

**Figure 27:** Variation in the daily average room temperature (RT) during the course of the trial. The shaded area represents one standard deviation (SD). The decreasing trend is evident, with an average of 19.3 ± 1.2 ºC at Day 1 and 15 ± 0.8 ºC at Day 7.




Therefore, it seems clear that the estradiol levels play an important role in the vulvar changes observed. Several experiments described the vasodilatation properties of estrogen and the subsequent rise in blood flow (Cockell & Poston, 1997; Naderali, Walker, Doyle & Williams, 1999; Smolders et al., 2002). These effects are particularly evident in the genital organs due to an exponential increase in the density of estrogen receptors, as shown by the experiments of Stanchev, Rodriguez-Martinez, Edqvist and Eriksson (1990), who reported an increase in estradiol receptors in the sow's uterine and cervical tissue during the proestrus, reaching a maximum concentration right before standing estrus. Ozasa and Gould (1982) found an increase in the levels of estrogen receptors in the chimpanzee perineal skin during the late follicular phase, reaching values 3 to 4 times higher than the ones observed during the early follicular and luteal phases.

Consequently, it can be assumed the increasing levels of estradiol produced by the pre-ovulatory follicles followed by the increasing levels of estrogen receptors in the genital organs during the proestrus lead to a significant vasodilatation and increased blood flow, which by its turn will be detected by thermography based on the increased local temperature.

Previously, other authors tried to establish a relationship between body temperature and ovulation in pigs, but their results are often discordant. In 2010, Scolari performed a similar study in which vulvar and gluteal temperatures were measured twice daily and ovulation detected using TRU. The same pattern in the VGT was found but with some differences related to the time of occurrence. In that study, the VGT increased progressively, reaching a peak 32 to 24 hours prior to ovulation, respectively in gilts and sows, and then started to decrease reaching a minimum value 12 hours before ovulation in gilts and at time of ovulation in sows. The main problem lies in the followed protocol, as these measurements were performed only twice daily at 0800 h and 1600 h, leading to two asymmetric windows of measurements: 8 hours between the 0800 h and 1600 h and 16 hours between 1600 h and 0800 h. Although the precision of these events are questionable in terms of time of occurrence, the significant temperature variation observed cannot be ignored, which made Scolari conclude "the decrease in temperature from 36 and 24 hours prior to ovulation found in our study was statistically significant and so, this technique could prove to be an aid in predicting time of ovulation."

Junge-Wentrup and Holtz (1984, cited by Scolari, 2010) attempted to establish a relationship between vaginal temperature and estrus using temperature probes anchored deep inside the sow's vagina. They observed a temperature drop 2 days before OE followed by a subsequent increase, reaching a maximum 2 days after standing estrus, close to the ovulation. Soede et al. (1997), by their turn, measured the vaginal temperature of 10 sows from 4 days before to 2 days after ovulation, finding no changes in temperature that could be related to ovulation.

From our findings, the significant variation found in the VGT reflects the changes in the VST due to the variations in the estradiol blood levels. Since a close relationship can be established between the estradiol levels, the LH peak and the time of ovulation (Soede et al., 1994), we believe the variation observed in the VGT can be indirectly related to the occurrence of ovulation. Although a significant difference was found in the occurrence of the maximum peaks between the two tested groups due to the influence of RT, the proximity of the results concerning the minimum peak makes us believe that this technique may have some potential as an ovulation diagnosis tool.

# 6. Conclusions

The sows' reproductive performance is one of the key factors to the success of a pig's herd. An accurate prediction of ovulation is of the utmost importance, but the available methods to achieve it are of limited applicability in the farms' daily routine. In this project we measured the VST of sows before and during estrus and tried to establish a relationship between its variations and the time of ovulation.

The evolution of VST was inferred based on the temperature differentials between the vulvar and the gluteal area, showing an increasing trend during the proestrus, during which a maximum peak is achieved; after this peak, it started decreasing markedly, reaching the lowest value approximately 25 to 28 hours before expected time of ovulation.

Although the trial showed promising results, some points worth our attention must be raised. The RT had a direct influence in the results, a fact particularly obvious when analyzing the maximum peaks in group D4 and D5. Thus, although the progressive increase in the VGT can be explained by physiological events, the extreme temperature fluctuations registered in the room had some influence in the time at which these VGT peaks were recorded. The advanced ventilation system of the building was not capable of counteract the extreme temperatures recorded outside, an important factor that should be kept in mind in future similar trials. The simple fact that the animals have to sleep and, just by doing that, will induce a greater variation in the VGT, demonstrate the difficulties in controlling the influence of external variables in the temperature records. Room temperature, incidence of sunlight, feces, stance, level of physical activity and circadian rhythm can thus induce a considerable variation in the temperature measurements obtained by IRT, which makes it necessary to have all these variables controlled when using this technology.

We had to limit our work to one batch and so the number of tested animals was another limiting factor. Comparisons between gilts and sows (in terms of temperature evolution, time of occurrence of temperature peaks or the magnitude of those variations) were desirable and could reveal interesting results, but given the small number of gilts in this trial, the reliability of their results would be extremely low. The analysis performed in group D5, composed only by 7 animals, could also benefit from a bigger sample size.

As a relationship between temperature variations and estradiol blood levels is suggested, it would be useful to perform regular hormonal assays in each animal, namely for FSH, LH,

progesterone and estrogen, in order to confirm this correlation and establish a cause-effect association. Additionally, these measurements could work also as a complementary tool in the ovulation diagnosis.

Finally, the main obstacle we faced performing this trial was the detection of ovulation. Although the echographies had the dual purpose of, first, compare the transrectal and transabdominal technique and secondly, detect the occurrence of ovulation, this second point was not successfully achieved and so the ovulation had to be estimated based on the literature recommendations. From our experience, the success in ovulation diagnosis using ovary echography relies mandatorily in an intensive previous training period and only the transrectal technique possesses the required sensitivity and specificity to accurately detect its occurrence.

Based on our findings, we conclude IRT may have some applicability to predict ovulation in sows under experimental conditions. However, several drawbacks may hinder the use of this technique under field conditions: First, the price of the equipment: at the time of this project, the infrared camera used in this project was being commercialized around $5.500 in United States. Second, this technique has proved to be extremely time-consuming and laborious: the measurements have to be performed at regular intervals, which imply measurements outside the regular working time, and the entire process of capture, analysis and insertion in the system of these records takes a considerable time. For example, to perform these measurements in 36 animals, I was in the farm between 0530 h to 0030 h and required approximately a total of 7 hours each day just to take the measurements, analyze the thermograms and introduce the data in my database. One can conclude the time it would be required to use this technique in a batch composed by 100 or 200 females. Finally, the animals have to be perfectly clean, since the feces strongly influence the temperature readings, something hard to achieve under farm conditions.

In further studies, the effective correlation between these temperature changes and the hormonal fluctuations should be evaluated using hormonal assays, in order to establish a cause-effect relationship, and the ovulation accurately diagnosed. Although, from a theoretical point of view, it was possible to assume a relationship between temperature variations, estradiol blood levels and ovulation, further studies should evaluate the individual variability in the occurrence of these events and, thus, assess the precision of this technique for ovulation prediction.

# 7. References

N.B.: This section also includes the references supporting the complementary annexes.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# Annex

N.B.: Only the annexes regarding the statistical analysis are available in this printed version. The remaining annexes can be found in the CD-ROM available in annex 12.

## **Annex 1:** One-way ANOVA for the repeatability test.

Test of Homogeneity of Variances (also considering animal 1126)

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 5,924 | 9 | 39 | ,000 |

Robust Tests of Equality of Means (also considering animal 1126)

| | Statistic[a] | df1 | df2 | Sig. |
|---|---|---|---|---|
| Welch | 20,305 | 9 | 15,614 | ,000 |

a. Asymptotically F distributed.

**One-way ANOVA with the remaining 4 animals**

Test of Homogeneity of Variances

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| ,638 | 7 | 32 | ,721 |

Robust Tests of Equality of Means

| | Statistic[a] | df1 | df2 | Sig. |
|---|---|---|---|---|
| Welch | 26,581 | 7 | 13,703 | ,000 |

a. Asymptotically F distributed.

Descriptives

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| 1 | 5 | 34,500 | ,3742 | ,1673 | 34,035 | 34,965 | 34,2 | 35,0 |
| 2 | 5 | 33,040 | ,3130 | ,1400 | 32,651 | 33,429 | 32,7 | 33,5 |
| 3 | 5 | 32,840 | ,3130 | ,1400 | 32,451 | 33,229 | 32,5 | 33,1 |
| 4 | 5 | 33,640 | ,4219 | ,1887 | 33,116 | 34,164 | 33,3 | 34,1 |
| 5 | 5 | 33,140 | ,3847 | ,1720 | 32,662 | 33,618 | 32,8 | 33,6 |
| 6 | 5 | 31,760 | ,3209 | ,1435 | 31,362 | 32,158 | 31,3 | 32,1 |
| 7 | 5 | 32,320 | ,3564 | ,1594 | 31,878 | 32,762 | 31,9 | 32,7 |
| 8 | 5 | 31,660 | ,3647 | ,1631 | 31,207 | 32,113 | 31,3 | 32,1 |
| Total | 40 | 32,863 | ,9588 | ,1516 | 32,556 | 33,169 | 31,3 | 35,0 |

**ANOVA**

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 31,754 | 7 | 4,536 | 35,405 | ,000 |
| Within Groups | 4,100 | 32 | ,128 | | |
| Total | 35,854 | 39 | | | |

**Annex 2:** Correlation tests and linear regression analysis between weaning-to-estrus interval (WEI) and duration of estrus (DE).

## Correlations

| | | WEI | DE |
|---|---|---|---|
| WEI | Pearson Correlation | 1 | -,593** |
| | Sig. (2-tailed) | | ,001 |
| | N | 29 | 29 |
| DE | Pearson Correlation | -,593** | 1 |
| | Sig. (2-tailed) | ,001 | |
| | N | 29 | 29 |

**. Correlation is significant at the 0.01 level (2-tailed).

## Linear Regression

Model Summary

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,593[a] | ,352 | ,328 | 7,159 |

a. Predictors: (Constant), WEI

Coefficients[a]

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 84,130 | 8,353 | | 10,072 | ,000 |
| | WEI | -7,696 | 2,010 | -,593 | -3,829 | ,001 |

a. Dependent Variable: DE

**Annex 3:** Student's t-test comparing the vulvar skin temperature (VST) of the measurements obtained at 0600 h and 1800 h

Paired Samples Statistics

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | 1800 h | 32,470 | 238 | 1,9885 | ,1289 |
| | 0600 h | 29,899 | 238 | 2,6252 | ,1702 |

Paired Samples Test

| | Paired Differences | | | |
|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |
| | | | | Lower |
| 1800 h - 0600 h | 2,5706 | 2,5124 | ,1629 | 2,2498 |

Paired Samples Test

| | Paired Differences | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|
| | 95% Confidence Interval of the Difference | | | | |
| | Upper | | | | |
| 1800 h - 0600 h | 2,8914 | | 15,784 | 237 | ,000 |

**Annex 4:** Student's t-test comparing the gluteal skin temperature (GST) of the measurements obtained at 0600 h and 1800 h.

Paired Samples Statistics

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | 1800 h | 30,708 | 238 | 1,9524 | ,1266 |
| | 0600 h | 27,059 | 238 | 2,6396 | ,1711 |

Paired Samples Test

| | Paired Differences | | | |
|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |
| | | | | Lower |
| 1800 h - 0600 h | 3,6487 | 2,4774 | ,1606 | 3,3324 |

Paired Samples Test

| | Paired Differences | t | df | Sig. (2-tailed) |
|---|---|---|---|---|
| | 95% Confidence Interval of the Difference | | | |
| | Upper | | | |
| 1800 h – 0600 h | 3,9651 | 22,721 | 237 | ,000 |

**Annex 5:** Correlation tests and linear regression analysis between vulvar skin temperature (VST) and room temperature (RT).

## Correlations

| | | VST | RT |
|---|---|---|---|
| VST | Pearson Correlation | 1 | ,605** |
| | Sig. (2-tailed) | | ,001 |
| | N | 28 | 28 |
| RT | Pearson Correlation | ,605** | 1 |
| | Sig. (2-tailed) | ,001 | |
| | N | 28 | 28 |

**. Correlation is significant at the 0.01 level (2-tailed).

## Linear Regression

Model Summary

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,605[a] | ,366 | ,342 | 1,36433 |

a. Predictors: (Constant), RT

Coefficients[a]

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 22,758 | 2,261 | | 10,065 | ,000 |
| | RT | ,511 | ,132 | ,605 | 3,876 | ,001 |

a. Dependent Variable: VST

**Annex 6:** Correlation tests and linear regression analysis between gluteal skin temperature (GST) and room temperature (RT).

## Correlations

| | | GST | RT |
|---|---|---|---|
| GST | Pearson Correlation | 1 | ,496** |
| | Sig. (2-tailed) | | ,007 |
| | N | 28 | 28 |
| RT | Pearson Correlation | ,496** | 1 |
| | Sig. (2-tailed) | ,007 | |
| | N | 28 | 28 |

**. Correlation is significant at the 0.01 level (2-tailed).

## Linear Regression

Model Summary

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,496[a] | ,246 | ,217 | 1,751 |

a. Predictors: (Constant), RT

Coefficients[a]

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 20,757 | 2,903 | | 7,150 | ,000 |
| | RT | ,493 | ,169 | ,496 | 2,913 | ,007 |

a. Dependent Variable: GST

**Annex 7:** Student's t-test comparing the vulvar skin temperature (VST) of gilts and sows, before and during estrus.

## T-test Gilts vs Sows, before estrus

Group Statistics

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Before_E | Gilts | 78 | 32,8654 | 1,78361 | ,20195 |
| | Sows | 78 | 31,9051 | 1,94081 | ,21975 |

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | |
|---|---|---|---|---|---|
| | | F | Sig. | t | df |
| Before_E | Equal variances assumed | ,195 | ,660 | 3,217 | 154 |
| | Equal variances not assumed | | | 3,217 | 152,914 |

Independent Samples Test

| | | t-test for Equality of Means | | |
|---|---|---|---|---|
| | | Sig. (2-tailed) | Mean Difference | Std. Error Difference |
| Before_E | Equal variances assumed | ,002 | ,96026 | ,29846 |
| | Equal variances not assumed | ,002 | ,96026 | ,29846 |

Independent Samples Test

| | | t-test for Equality of Means | |
|---|---|---|---|
| | | 95% Confidence Interval of the Difference | |
| | | Lower | Upper |
| Before_E | Equal variances assumed | ,37066 | 1,54985 |
| | Equal variances not assumed | ,37062 | 1,54989 |

## T-test Gilts vs Sows, during estrus

Group Statistics

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| During_E | Gilts | 46 | 31,7022 | 2,13827 | ,31527 |
| | Sows | 46 | 30,4174 | 2,50229 | ,36894 |

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | |
|---|---|---|---|---|---|
| | | F | Sig. | t | df |
| During_E | Equal variances assumed | 1,329 | ,252 | 2,647 | 90 |
| | Equal variances not assumed | | | 2,647 | 87,864 |

Independent Samples Test

| | | t-test for Equality of Means | | |
|---|---|---|---|---|
| | | Sig. (2-tailed) | Mean Difference | Std. Error Difference |
| During_E | Equal variances assumed | ,010 | 1,28478 | ,48530 |
| | Equal variances not assumed | ,010 | 1,28478 | ,48530 |

Independent Samples Test

| | | t-test for Equality of Means | |
|---|---|---|---|
| | | 95% Confidence Interval of the Difference | |
| | | Lower | Upper |
| During_E | Equal variances assumed | ,32065 | 2,24891 |
| | Equal variances not assumed | ,32033 | 2,24923 |

**Annex 8:** Student's t-test comparing the gluteal skin temperature (GST) of gilts and sows.

Group Statistics

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| GST | Gilts | 124 | 29,6218 | 2,89194 | ,25970 |
| | Sows | 124 | 29,2556 | 2,65294 | ,23824 |

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | |
|---|---|---|---|---|---|
| | | F | Sig. | t | df |
| GST | Equal variances assumed | ,188 | ,665 | 1,039 | 246 |
| | Equal variances not assumed | | | 1,039 | 244,192 |

Independent Samples Test

| | | t-test for Equality of Means | | |
|---|---|---|---|---|
| | | Sig. (2-tailed) | Mean Difference | Std. Error Difference |
| GST | Equal variances assumed | ,300 | ,36613 | ,35243 |
| | Equal variances not assumed | ,300 | ,36613 | ,35243 |

Independent Samples Test

| | | t-test for Equality of Means | |
|---|---|---|---|
| | | 95% Confidence Interval of the Difference | |
| | | Lower | Upper |
| GST | Equal variances assumed | -,32803 | 1,06029 |
| | Equal variances not assumed | -,32806 | 1,06031 |

**Annex 9:** Student's t-test comparing the vulvar skin temperature (VST) before and during estrus in all animals.

Group Statistics

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| VST | Before_E | 479 | 32,2397 | 1,93039 | ,08820 |
| | During_E | 356 | 30,9559 | 2,78140 | ,14741 |

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | |
|---|---|---|---|---|---|
| | | F | Sig. | t | df |
| VST | Equal variances assumed | 37,118 | ,000 | 7,869 | 833 |
| | Equal variances not assumed | | | 7,473 | 597,777 |

| | | t-test for Equality of Means | | | |
|---|---|---|---|---|---|
| | | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference |
| | | | | | Lower |
| VST | Equal variances assumed | ,000 | 1,28377 | ,16314 | ,96355 |
| | Equal variances not assumed | ,000 | 1,28377 | ,17179 | ,94639 |

| | | t-test for Equality of Means |
|---|---|---|
| | | 95% Confidence Interval of the Difference |
| | | Upper |
| VST | Equal variances assumed | 1,60398 |
| | Equal variances not assumed | 1,62114 |

**Annex 10:** One-way ANOVA to infer the evolution of vulvar skin temperature (VST) of all animals along the trial. Temperature records are grouped in 24 hours groups, relative to OE

Test of Homogeneity of Variances

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 2,721 | 4 | 662 | ,029 |

Descriptives

| Hours to OE | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| -72 to -54 | 124 | 32,2976 | 1,91882 | ,17231 | 31,9565 | 32,6387 |
| -48 to -30 | 136 | 32,3618 | 1,80362 | ,15466 | 32,0559 | 32,6676 |
| -24 to -6 | 136 | 32,2390 | 2,01873 | ,17310 | 31,8966 | 32,5813 |
| 0 to +18 | 136 | 31,7294 | 2,40891 | ,20656 | 31,3209 | 32,1379 |
| +24 to +42 | 135 | 31,0852 | 2,41527 | ,20787 | 30,6740 | 31,4963 |
| Total | 667 | 31,9375 | 2,17967 | ,08440 | 31,7718 | 32,1032 |

Descriptives

| Hours to OE | Minimum | Maximum |
|---|---|---|
| -72 to -54 | 26,50 | 35,60 |
| -48 to -30 | 25,90 | 36,20 |
| -24 to -6 | 26,50 | 36,30 |
| 0 to +18 | 24,20 | 36,30 |
| +24 to +42 | 24,30 | 35,10 |
| Total | 24,20 | 36,30 |

ANOVA

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 156,876 | 4 | 39,219 | 8,633 | ,000 |
| Within Groups | 3007,267 | 662 | 4,543 | | |
| Total | 3164,143 | 666 | | | |

**Annex 11:** One-way ANOVA to infer the evolution of gluteal skin temperature (GST) of all animals along the trial. Temperature records are grouped in 24 hours groups, relative to OE

Test of Homogeneity of Variances

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1,602 | 4 | 662 | ,172 |

Descriptives

| Hours to OE | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| -72 to -54 | 124 | 29,4194 | 2,64642 | ,23766 | 28,9489 | 29,8898 |
| -48 to -30 | 136 | 29,5390 | 2,29286 | ,19661 | 29,1501 | 29,9278 |
| -24 to -6 | 136 | 29,7228 | 2,35227 | ,20171 | 29,3239 | 30,1217 |
| 0 to +18 | 136 | 29,8397 | 2,34604 | ,20117 | 29,4419 | 30,2376 |
| +24 to +42 | 135 | 29,3711 | 2,54144 | ,21873 | 28,9385 | 29,8037 |
| Total | 667 | 29,5816 | 2,43471 | ,09427 | 29,3965 | 29,7667 |

Descriptives

| Hours to OE | Minimum | Maximum |
|---|---|---|
| -72 to -54 | 20,60 | 34,60 |
| -48 to -30 | 23,00 | 33,70 |
| -24 to -6 | 23,50 | 35,10 |
| 0 to +18 | 21,90 | 35,00 |
| +24 to +42 | 22,90 | 34,30 |
| Total | 20,60 | 35,10 |

ANOVA

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 21,264 | 4 | 5,316 | ,896 | ,466 |
| Within Groups | 3926,659 | 662 | 5,932 | | |
| Total | 3947,923 | 666 | | | |

## **Annex 12:** CD-ROM containing the complementary annexes.

Files also available at http://tinyurl.com/fmv-annex

- Annex 12.1. Ultrasonography: Physical principles
- Annex 12.2. Emissivity of different materials
- Annex 12.3. Specifications of the thermal camera Fluke TiR1.
- Annex 12.4. Artificial insemination protocol used in the farm.
- Annex 12.5. Daily variation in plasma melatonin concentrations in the goose.
- Annex 12.6. Database with the individual records of all animals.