# INVESTIGATION OF SKIN TEMPERATURE DIFFERENTIALS IN RELATION TO ESTRUS AND OVULATION IN SOWS USING A THERMAL INFRARED SCANNING TECHNIQUE

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in VMS-Veterinary Clinical Medicine
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

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ABSTRACT

Accurate estrus detection is an essential component of a successful artificial insemination program in modern swine operations. It is necessary to establish efficacious means of estrus detection and to optimize reproductive performance in the herd. Measurement of physical and physiological traits such as body temperature, vaginal electrical resistance and vulva reddening have been investigated as methods to aid in estrus detection in swine. The relationship between vulvar skin temperature and ovulation has not been previously investigated. Therefore, the objective of this study was to assess changes in vulvar skin temperatures that occur during the periovulatory period using digital infrared thermography (IRT), which has already been successfully used as a therapeutic and diagnostic tool in various fields and species in veterinary medicine. The experimental group consisted of a total of 25 gilts and 27 multiparous sows, and a control group consisted of 30 sows at 60 days of gestation. All Yorkshire-Landrace females were housed individually in a temperature and humidity controlled environment. IRT vulvar skin temperatures were measured twice daily (8 am and 4 pm) using the infrared digital thermocamera (FLUKE IR FlexCam® Thermal Imager, Fluke Corporation, Everett, WA). Estrus detection was performed twice daily with the aid of an adult boar. Once standing estrus was observed, transrectal real time ultrasound was performed twice daily (8 am and 4 pm) in order to monitor follicle development and determine the time of ovulation. Ovaries were visualized using an Aloka 500V ultrasonics machine (Aloka Inc., Tokyo, Japan) fitted with a transrectal 7.5 MHz linear transducer which was fitted into a rigid, fixed-angle PVC adapter. Average vulvar skin temperatures (VST) and hours were reported (mean ± SEM) and compared using a MANOVA and Tukey-Kramer tests using SAS. Significant differences were reported at $P \leq 0.05$. Evidence of ovulation, with the disappearing of the dominant follicle was detected at approximately 38 ± 9 hours after onset of estrus in gilts, and 43 ± 12 hours in sows. Temperature was collected at the same time during all the days of the experiment. The mean VST of sows during estrus was significantly higher ($p \leq 0.05$) than gilts, although collected at the same time. During estrus, the mean VST of gilts reached a peak of 35.6 ± 1.6 °C at 32 h prior to ovulation and then decreased significantly to 33.9 ± 1.7 °C 8 h prior to ovulation. This marked change in mean VST was detected between 36 and 12 h prior to ovulation. There was a similar trend in sows with a peak VST of 36.1 ±1.3 °C at 24 h prior to ovulation and then dropping to 34.6 ±1.6 °C 12 h prior to ovulation. There was no significant difference ($p \geq 0.05$) between VST in gilts and sows at the

time of ovulation. This study demonstrated that vulvar skin temperatures of sows and gilts measured by digital infrared thermography change significantly during the periovulatory period. Additionally, there are distinct times that VST rises and then falls precipitously in sows compared to gilts. The potential to use digital infrared thermography as a predictor for ovulation in swine appears to be a promising tool. Further studies involving predictor models and hormonal assays need to be performed.

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CHAPTER 1

# INTRODUCTION

Accurate estrus detection is an essential component of a successful breeding program in modern swine operations. It is labor intensive, time consuming and an economically important aspect of the production system. Over the last 20-30 years swine production systems have changed, there are fewer farms, with these farms holding larger numbers of breeding females. Along with this restructuring in the swine industry, the application of techniques to improve reproductive efficiency and controlled breeding has occurred [Rasbech 1984]. Some of the assisted reproductive techniques now commonly utilized on modern swine operations include artificial insemination (AI), hormonal induction of estrus in gilts, estrus synchronization of sows and gilts, and B mode ultrasonography for pregnancy diagnosis [Almond and Dial 1987; Crabo et al. 1992; Pressing 1992; Britt 1996]. Even with the addition of all of these technologies accurate and efficient determination of ovulation, and consequently the best time to AI cannot be known until after the female has gone out of estrus.

Sows ovulate approximately 36 h after the onset of estrus (two-thirds of the way through the estrus period) and can range from 10 to 58 h [Soede et al. 1995]. With this large variation, it is difficult for breeding managers to know precisely when to inseminate. Prediction of ovulation is still a challenge in swine reproduction. Researchers such as Soede et al. (1997) have utilized various new technologies such as ultrasonography to predict ovulation, but this method is invasive and not practical for routine use in swine operations, however it has a great potential for investigating basic questions involved in research. For optimal fertility, insemination should occur within 24 h of ovulation [Soede et al. 1995; Nissen et al. 1996].

Measurement of vaginal and core body temperature changes have been studied in cattle [Junge et al., 1984; Osawa et al., 2004] as well as pigs [Junge et al., 1984; Soede et al., 1997] to determine the relationship between these values and ovulation. Additionally, investigations of skin temperature differentials in relation to estrus have been studied in dairy cattle using a thermal infrared scanning technique [Hurnik et al. 1985]. Henne (1991) investigated body temperature of gilts during the estrus period using temperature probes anchored inside the female's vagina. It was determined that although there was a large variability of the temperature values at the onset and during estrus, they noticed a drop in temperature 2 days before standing heat with a gradual increase and the highest temperature on day 2 of standing heat, which coincides with the time near ovulation. Soede et al. (1997) examined the intravaginal temperature of sows during days 4 and 10 after estrus synchronization with altrenogest and found that changes in intravaginal temperatures of sows could not be related to the timing of ovulation.

Behavioral and physical signs of estrus are commonly used by the detector (boar and man) to determine the best time to inseminate female pigs [Rotjkittikhun et al. 1992]. Vulvar reddening and swelling during estrus has been related to an increase in estrogen levels, which stimulates blood flow to the reproductive tract and associated genital organs [Rotjkittikhun et al. 1992]. Vulvar reddening is a secondary sign of heat and should be used in conjunction with other signs to confirm heat. This increase in blood flow increases the skin temperature of the vulva during estrus. Currently, there have been no studies performed in swine using vulvar skin temperature as an indicator of estrus or ovulation.

The current study was designed to investigate the relationship between vulvar skin temperature (VST) and time of ovulation in swine using a highly sensitive and accurate infrared thermography (IRT) digital camera.

Infrared thermography is a noninvasive technique through which temperatures are monitored and recorded, thereby allowing visualization of heat flow. The noninvasive and high resolution characteristics of the thermographic systems make them valuable diagnostic as well as therapeutic aids. Modern thermal imaging systems comprise technically advanced thermal cameras coupled to computers with sophisticated software solutions. Thermography can be applied as a diagnostic tool by detecting changes of blood flow and thermal modeling of various body regions; hence it's widely used in oncology, allergic diseases, angiology, plastic surgery, rheumatology, reproductive problems and elsewhere. Thermography is a safe, accurate and, most importantly, a non-invasive diagnostic method in clinical medicine. It has proven to be highly sensitive and accurate during studies performed in human medicine for diagnosis of breast cancer [Keyserlingk et al., 1998; Parisky et al., 2003] among other medical applications. It has also been widely used in veterinary medicine for detection of reproductive issues in bulls [Purohit, 1985; Kastelic et al. 1996, 1997a, 1997b; Gabor et al. 1998], to accesses animal welfare [Stewart et al., 2005], diagnose foot and leg problems in horses and aid in diagnose and treatment of lameness [Strömberg, 1975; Weil et al. 1998; Eddy et al., 2001] among others.

The objective of the current investigation was to explore the possibility of utilizing thermal infrared scanning of the vulva in pigs to detect measureable temperature changes related to ovulation and so to introduce a new aid for estrus detection in swine that will allow AI time in a greater synchrony with ovulation.

# CHAPTER 2

# LITERATURE REVIEW

## A. Statement of the Problem

With the continued expansion of the pork industry, greater emphasis is placed on maintaining consistent productivity from the sow herd. Approximately 50% of sows are replaced annually due to reproductive problems [Baltussen et al., 1998]. In order to decrease nonproductive sow days, it is essential to identify sows in estrus and breed the sow at the appropriate time. This will ensure high conception and pregnancy rates resulting in an increase in the number of piglets produced per sow per year. The identification of physiological changes associated with estrus provides accurate information about the most appropriate time for artificial insemination (AI), i.e. insemination near ovulation. Estrus detection is one of the most important aspects of pig husbandry and the one most important factor in an AI program. It is essential to establish efficacious means of estrus detection. Less than ideal estrus detection is major limiting factor for optimal reproductive performance. Currently, the most common method for estrus detection in pigs is the Lordosis /Back Pressure Test, which is a natural response as the female is preparing herself to be mounted by the boar for breeding [Almond G.W., 2007]. This process requires trained staff and takes up a great amount of effort and is time consuming, accounting for 30% of total farm labor [Perez et al., 1986]. The cost of estrus detection weights heavily when evaluating the herd's productivity. Assuming 20 sows can be checked in one hour, it was estimated a cost of $0.40 per sow per day for estrus detection, plus $3.93 for insemination labor [Flowers et al., 1993]. According to a report from Minitube and

another from Dr. John Mabry from Iowa Pork Industry Center, each of a sow's nonproductive day is equivalent to $2.00, which in relation to a reproductive cycle can add up to $38.00.

Even with good estrus detection methods, it may still be difficult to attain maximum fertility results since there is a wide range between timing of ovulation in relation to onset of estrus. According to Soede et al. (1997), pigs ovulate 10 to 58 hours after the onset of estrus. Weitze et al. (1994) proposed a much wider range of 24 to 96 hours. Best fertility is attained mating within 24 h prior to ovulation [Nissen et al., 1997]. The ideal time for insemination is 8 to 12 h prior to ovulation [Soede et al 1995]. The availability of these time periods provide a breeding window, however, the large variety in hours makes it even more critical for accurate estrus detection with the goal of mating as close to ovulation as possible. The great question is being able to predict when the pig will ovulate.

To try to overcome this issue and ensure properly timed mating, multiple insemination programs have become a standard procedure on the modern swine farm. Double inseminations increase farrowing rates by 8 to 12% and litter sizes by 0.2 pigs [Crabo and Dial, 1992]. These numbers remain high as long as AI is performed during the fertile period of estrus. Fertility and litter size decline when the last of multiple inseminations is performed during late estrus or metestrus due to a post breeding inflammation [Rozeboom et al. (1996)]. Hence, it is critical to be able to time breeding as close to ovulation as possible.

As a way to counteract all the less than ideal situations faced when trying to achieve optimal reproductive performance, new, practical and effective tools to aid in estrus detection are warranted in the modern swine industry. Various alternatives for improving detection of animals in estrus have been described. Automated estrus detection methods are performed through measurement of physical activity by accelerometer, infrared sensor, automation of visit to the

boar pen. Measurement of physical and physiological traits such as body temperature, vaginal electrical resistance and vulva reddening have also been investigated as methods to aid in estrus detection in swine. However, for the most part, these methods may be expensive, require well trained personnel and may not be practical in a field setting or were demonstrated to be inaccurate.

The economic reality of swine breeding facilities is the need for simple, fast and inexpensive tools to help improve fertility by allowing mating at the appropriate time during estrus.

## B. Anatomy and Physiology

### 1. Anatomy of Reproductive Organs

*Ovaries*

The ovaries are the primary organs of reproduction in the female. Not only do they release oocytes at the time of ovulation, but they also produce estrogen and progesterone, which are essential hormones in the porcine estrous cycle [Frandson 2003]. Oxytocin, relaxin, inhibin and activin are also produced by the ovaries at various stages of the female's reproductive cycle, although in lesser quantities than progesterone and estrogen, but just as important. [Senger, 1999].

Graafian follicles are made up of several cell layers, being them the theca and granulosa cells. Both kids of cells are involved in the production of estrogen and after ovulation, granulosa cells are the principal progesterone producing cells in the corpus luteum [Bearden & Fuquay, 1997].

Follicle stimulating hormone is released from the anterior pituitary and acts on receptors of the ovary to convert testosterone to estrogen in the granulosa cells. FSH act synergistically to promote granulosa cell proliferation and thus follicular growth [Peluso, 1983]. Once growth of these follicles begins, they will continue to grow until it either ovulates or deteriorates by a process called atresia [Peluso, 1983]. Continuous development and subsequent atresia maintains a proliferating pool of follicles 1-6 mm in diameter [Foxcroft et. al., 1985], which has been estimated to contain approximately 50 follicles per animal during the luteal and early follicular phase of the porcine estrous cycle [Anderson, 1993].

*Oviducts / Uterine Tubes*

Essential reproductive processes take place within the sow's oviducts such as pick-up of the newly ovulated ova, transport of the gametes to the fertilization site and, the transport of the developing embryos to the uterus [Martinez, 1983].

The pig's oviduct shows a definite spontaneous motility throughout the estrous cycle, which is associated with gamete transport to the fertilization site and ova descent to the uterus [Martinez, 1983].

*Vulva and Vestibule*

The external part of the female's reproductive tract is the vulva, consisting of major and minor labia, which meet dorsally and ventrally to form the dorsal and ventral commissures [Frandson, 2003]. The ventral commissure is pendulous and where the clitoris is found. The high estrogen levels associated with the onset of estrus generally results in a continuous state of erection of the clitoris [Senger, 1999] and also promote rapid epithelium growth leading to swelling and increased turgidity of the vulva [Safranski, 2007].

*2. Physiology of the Estrous Cycle*

The domestic sow is a non seasonal polyestrous animal with the approximate length of the estrous cycle being 21 days [Senger, 1999; Safranski, 2007]. Initiation of ovarian activity during puberty is established by activation of the hypothalamic-pituitary-adrenal axis, leading to an increase in the normal gonadotropin secretion and in consequence, activation of the estrous cycle [Turner et al., 2002]. The estrous cycle can be divided into two distinct phases according to the dominant ovarian structure, being either follicular or luteal phase (Figure 2.1). The follicular phase incorporates proestrus and estrus and it is the time from regression of the corpora lutea (with decline in progesterone levels) to ovulation, when the primary ovarian structures, the dominant follicles are growing and producing estradiol [Senger, 1999]. The luteal phase incorporates metestrus and diestrus and it is the time from ovulation until corpora lutea regression. It is a longer phase than the follicular and the dominant structure is the corpora lutea secreting progesterone [Senger, 1999].

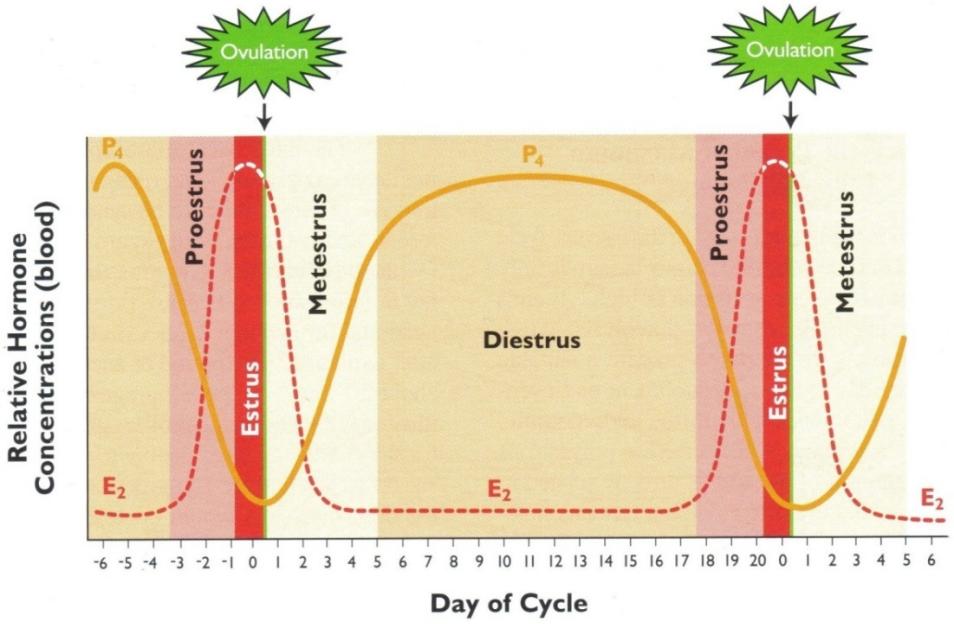


Figure 2.1. Hormonal changes of the Estrous Cycle (Adapted from Senger, 1999)

*Proestrus*

Proestrus is marked by the occurrence of rapid follicular growth. It begins with the regression of the corpus luteum and consequent drop in progesterone and extends to the start of the next estrus [Safranski, 2007]. The drop in progesterone caused by lysis of the corpora lutea by prostaglandin $F_{2\alpha}$ at the end of diestrus removes the hypothalamus from its negative feedback inhibition. The hypothalamus reaches a threshold level of estrogen in the absence of progesterone, leading to a release of large quantities of GnRH which stimulate the anterior lobe of the pituitary to secrete FSH and LH [Senger, 1999]. GnRH release is essential for tonic FSH and LH release, which leads to follicle growth and is crucial in establishing proper pulsatile LH pattern release, which is necessary for final follicle growth and ovulation [Driancourt et al., 1995].

A cohort of small and medium follicles present on the surface of the ovary is recruited for further growth under FSH stimulation and estradiol production. This recruitment pool has about 50 follicles, 1-6 mm in size [Knox 2005]. Within the recruitment pool, follicles will go through selection, being the ones that will mature to ovulate. As follicles enter the selection phase, inhibin and estradiol are produced by the growing follicle and hence, inhibit FSH secretion from the anterior lobe of the pituitary. At this time FSH and LH roles begin to shift, considering that at selection FSH drops to its lowest point and LH increases [Senger, 1999]. Most follicles at selection are approximately 5 mm in size. Smaller sized follicles fail to respond to the hormonal stimuli and go into atresia [Ryan et al., 1994; Kemp, 1998]. Late during proestrus the effects of estrogen will make behavioral symptoms of approaching estrus more prominent and detectable [Safranski, 2007]. Pulsatile LH is also essential for follicle development, as evidenced by Esbenshade (1987) and is critical for growth of follicles >2mm in size [Driancourt et. al., 1985].

*Estrus*

Estrus is characterized by visible behavioral symptoms of female receptivity to the male and standing for mating. The duration of estrus of sows can vary from 24 to 96 hours [Weitze et al., 1994; Soede et al., 1995) and this duration of estrus is influenced by factors such as parity, season, stress, boar effects, and weaning-to-estrus interval [Weitze et al., 1994; Kemp et al., 1996].

During estrus, follicles are found at different stages of development. The ones that escaped atresia, become dominant, at the time of selection are mature enough to respond to the LH surge and will determine the ovulation rate [Hunter et al., 1990]. The preovulatory surge of LH occurs during early estrus and is dependent on adequate numbers of large follicles present in the ovaries, since these are needed for the production of threshold estrogen concentrations, which initiates the physical expression of estrus and induction of the LH surge [Knox, 2005]. They continue to produce increasing amounts of estradiol and inhibin, which is produced by the antral follicle and selectively inhibits the release of FSH from the anterior pituitary. The increasing level of estradiol reaches a peak in serum between days 18 and 20 of the estrous cycle and prompts and stimulates (through a positive feedback mechanism on the hypothalamus) the preovulatory surge of LH from the pituitary causing final growth and maturation and ovulation [Britt et al., 1985; Senger, 1999]. The release of inhibin likely modulates the release of FSH during estrus, thereby preventing overstimulation of the ovaries [Safranski, 2007].

According to different authors, ovulation occurs approximately between 10 and 58 h [Soede et al., 1995] or between 24 and 96 h [Weitze, K.F et al., 1994] after onset of estrus in sows and lasts about 2 hours as determined by transrectal real-time ultrasound evaluation [Soede et al., 1992]. Follicles are selected for ovulation as they shift their dependence on FSH to LH,

meaning, there's a decrease in FSH receptors during the first 3 days of estrus and at the same time an increase in LH receptors [Lucy 2001]. The decrease in FSH concentrations at this time is also associated with follicular atresia [Guthrie et al., 2001].

Ovulation is triggered by a surge of LH/hCG which stimulates a cascade of proteolytic enzymes (plasminogen activator (PA), plasmin, collagenase and matrix metalloproteinase 1 (MMP-1)). The physical process of ovulation (Figure 2.2) occurs as the colloid osmotic pressure of the follicular fluid increases, permitting water to enter the follicle and as active collagenase causes a digestion of the collagen in the follicle wall, and plasmin, as well as possibly other proteolytic enzymes may cause a further dissociation of the follicular wall mainly at the apical region of the follicle forming the stigma and consequent rupture of the follicle [LeMaire, 1989; Tsafriri, 1995, Kilen & Schwartz, 1998]. Increased levels of LH/hCG also increase prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and histamine which influence smooth muscle by causing contractions and causing vascular changes such as increased permeability and vasoconstriction and release of enzymes, all leading to ovulation [LeMaire, 1989, Tsafriri, 1995; Senger, 1999].

*Metestrus*

Metestrus is the period of transition between estrogen dominance to progesterone dominance. The granulosa cells give rise to the luteal cells and corpus luteum formation [Arthur, 1996]. LH is a luteotropin hormone, since it has the dominant controlling influence on formation and function of the corpus luteum. After the LH surge, estradiol production is reduced and the dominant hormone from this point on is progesterone. LH binds to the membrane receptors of granulosa cells and at ovulation initiates reactions within these cells that result in luteinization and production of progesterone. LH may maintain the function of the corpus luteum by increasing the blood flow through this luteal structure [Peluso et al., 1983].

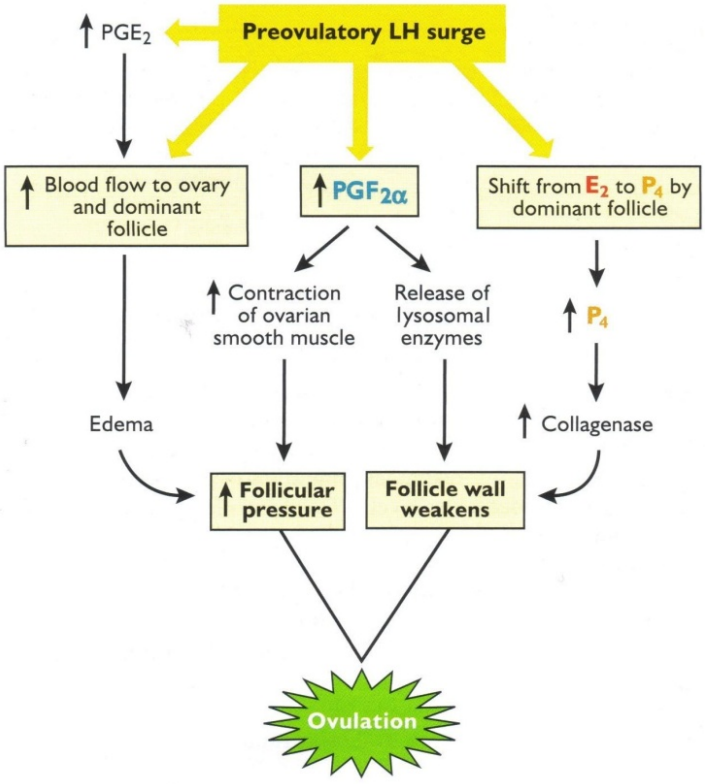


Figure 2.2. Ovulation Events (Adapted from Senger 1999)

*Diestrus*

Diestrus is the longest stage of the estrous cycle and is characterized as the period in the cycle when the corpus luteum is fully functional and progesterone levels are high. It is also called the luteal phase. For the sow it extends from about day 4 through day 13-15 [Bearden & Fuquay, 1997; Senger, 1999].

After ovulation, the follicles undergo dramatic structural changes as they develop into the corpus luteum, producing progesterone [Kilen & Schwartz, 1998]. Progesterone has a dominant role in regulating the estrous cycle. It is evident in serum between 2 and 4 days after estrus and reaches its maximum at around days 12-15 of the cycle [Whittemore 1993]. It stimulates maximal secretion by the endometrial glands which undergo hyperplasia and hypertrophy, quiescence of endometrial musculature and tightening of the cervix [Arthur, 1996; Senger,

1999]. In the mammary gland, progesterone promotes final alveolar development prior to parturition, thereby allowing initiation of lactation [Senger, 1999]. All these changes are based on the principle of ensuring the development of a proper uterine environment for implantation of the conceptus [Foxcroft, 1993].

During diestrus with the corpus luteum functional, high concentrations of progesterone inhibit release of FSH and LH through its negative feedback control of the hypothalamus and anterior pituitary; progesterone also inhibits behavioral estrus, which also occurs during pregnancy [Senger, 1999]. Although progesterone causes endometrial quiescence, in swine, the spacing of pre-implantation embryos is dependent on myometrial contraction and even though steroid secretion by the embryo itself may regulate spacing, there's also an increased sensitivity of the myometrium to the embryonic signals dependent on maternal steroid changes [Foxcroft, 1993].

C. Estrus Expression

Estrus is defined as the period when the female is receptive and will stand for mating with males. Elevated estradiol along with low progesterone induces profound behavioral changes in the female. In addition to standing for mating, there are other behavioral as well as physiological signs of estrus.

High levels of estrogens have been associated with the behavioral signs of estrus and are of paramount importance. Estrogen affects all the components of estrus, including receptivity, proceptivity and all other sexual behavior signs [Edqvist & Stabenfeldt, 1993; Bearden & Fuquay, 1997]. Estrogen targets primarily the reproductive tract mucosal epithelium which responds dramatically to its effects leading to expression of estrus behavior. The major effect of

estrogen over the reproductive tract is the increase of blood flow to the reproductive organs, causing genital swelling, and change in tissue electrical conductivity, increased mucosal secretion, initiation of uterine gland growth and elevated myometrial tone and hyperemia which in turn, allows for leukocyte delivery to the submucosa serving as humoral protection, phagocytizing all foreign material after copulation [Senger, 1999].

The swelling (edema) of the vulva and cervix is the most marking sign of estrus, brought about by the increased blood flow that increases the local capillary pressure and causes lymph to buildup in the external genitalia, which is in fact the swelling [Foxcroft, 1993; Senger, 1999]. In pigs, cervical distension ensures an effective "lock" for the penis and so, ensures effective insemination into the body of the uterus [Foxcroft, 1993]. The amount of cervical and vaginal serous-mucous secretion also increases and changes in fluidity, color, conductivity, pH and crystallization pattern [Zink et al., 1984, Foxcroft, 1993]. This mucus serves as lubrication during copulation, promotes sperm penetration of the utero-cervical junction and a barrier to contamination of uterus by flushing out foreign material introduced during copulation [Foxcroft, 1993; Senger, 1999]. In the uterus, estradiol causes increased tone (due to myometrial contractions) and motility of the muscularis in all regions of the reproductive tract which partially responsible for sperm transport and there's also stimulation for the development and growth of the uterine glands [Senger, 1999]. The oviduct's epithelium increases its secretory rate under estrogen influence. Also, the cilia within the oviduct increase its motility facilitating gamete and fluid transport [Senger, 1999].

*Behavior Associated with Reproduction*

In females, sexual behavior is divided in proceptive and receptive behavior [Beach, 1976]. Proceptive behavior has been defined by Beach as 'various reactions by the female towards the male which constitute her assumption of initiative in establishing or maintaining sexual interaction' and receptive behavior is defined as 'female responses necessary and sufficient for the male's success in achieving intra-vaginal (or intra-cervical) ejaculation'. During estrus the sow shows receptive behavior and it is the only time when she permits mating [Zink et al., 1984].

The female expressing estrus seeks out the male either by smell, sound or sight and will approach him for the purpose of head-to-head contact and will stay close to the boar for longer periods of time as estrus approaches. This is characteristic of proceptive behavior [Zink et al., 1984]. The courtship behavior in pigs can include sniffing, head-to-head contact, nosing, chin-resting, mounting and copulation [Fabre-Nys, 1993; Gordon, 1997]. Vocalization, jaw champing and frothing at the mouth is usually evident from both parties, but especially from the male [Whittmore, 1993]. Other gradual changes associated with estrus in the sow are increased restlessness, reduced appetite, mounting other animals, and male-like sexual behavior [Gordon, 1997].

The mating posture of the estrus female is common in most domestic species and it's limited to an active immobilization, eventually accompanied by deviation of the tail. The mating stance of the sow is clear and long lasting [Fabre-Nys, 1993]. The so-called "standing response" is when the sow stands immobile, arches her back and pricks the ears illustrated in Figure 2.3 and 2.4. This standing response is particularly stimulated by olfactory and tactile stimuli by the boar [Zink et al., 1984]. A pheromone identified as 5α-androsterone, produced by the boar testis

and salivary glands is one of the responsible components for the female's sexual receptivity and response to allow for mounting by the boar [Fabre-Nys, 1993].

Figure 2.3. Sow showing sings of behavior estrus in standing position with arched back.

Figure 2.4. Sow showing signs of behavior estrus with erect ears.

D. Estrus Synchronization

A reduction in reproductive performance affects a herd when fewer sows return to estrus after weaning, when there's an increase in the time to return to estrus and when there's a higher rate in return to estrus after mating, all of these collectively lead to lower farrowing rates. Non-productive days (NPDs) accumulate for females that are not pregnant or lactating and include the period between entry of gilt into the "gilt pool" and her first service, and the interval between weaning and mating in sows. To enhance reproductive efficiency in the breeding herd, NPDs must be minimized.

The possible reason for an increase in NPDs may be explained by pathologically suppressed gonadotropin secretion and ovarian inactivity; hence, exogenous gonadotropins are frequently used to stimulate ovarian activity [Van de Wiel et al., 1981].

Estrus synchronization programs are able to facilitate the reintegration of the sows into a breeding schedule employing fixed-time artificial insemination.

1. Weaning

Weaning age has an important influence on litters/mated female/year. But to maximize overall sow herd performance, weaning age must be set at an appropriate level. Reducing lactation length will decrease subsequent fertility of the female by extending the wean-to-estrus interval past the ideal interval of 3 to 5 days, reducing conception rate and decreasing subsequent litter size [Estienne et al., 1998]. Therefore, to maximize throughput in an operation, weaning age should be set to a degree where it least affects sow reproductive performance. In most herds, the greatest impact on reproduction is observed in lactation periods of less than 17 days [Estienne et al., 1998].

The sow remains in anestrus during lactation because the suckling influence of the litter suppresses ovarian and pituitary hormonal activity, and stimulates the release of prolactin. Once suckling is reduced or the litter is weaned, prolactin levels gradually decline, and increases in blood levels of LH and estradiol stimulate estrus. This hormonal suppression provides the sow time for uterine involution [Senger, 1999; Almond, 2007], during which, the uterus must undergo a rapid loss of length and weight during the first two to three weeks of lactation. Impaired rates of uterine involution and wean-to-estrus interval, and subsequent embryonic deaths, have been linked to lactation lengths of less than 19 days [Kaeoket, 2008]. Similar results have been demonstrated by Fernández et al. (2005) where they concluded that the wean-to-estrus intervals are shortest for sows weaned between three and four weeks after farrowing. Sows weaned at less than 10 days of lactation demonstrate a much longer wean-to-estrus interval and in contrast, a greater percentage of sows that lactate for more than 20 days return to estrus by Day 7. This is all linked to enough time for uterine involution [Kaeoket, 2008].

2. Altrenogest

Progestin Altrenogest (Matrix; Intervet America Inc., Millsboro, DE) is an orally active progestagen which inhibits gonadotropin release, imitating the biological activity of progesterone. It does not prevent luteolysis, but it blocks the onset of estrus even after luteolysis [Horsley et al., 2005].

A. Synchronization of Gilts using Altrenogest

Swine operations maintain a pool of replacement gilts to ensure that a sufficient number of animals will be available to meet their breeding target. It is ideal if the replacement gilts exhibit estrus all at a predicted time, allowing this way a more efficient use of gestation and farrowing facilities and so, it is important to synchronize these gilts [Bates et al., 1991].

In order to synchronize estrus in gilts, Altrenogest is administered orally as a top-dressing on food at a dose of 15-20 mg/day/gilt for 14-18 consecutive days. Estrus can be expected 5 to 7 days after the last day of Altrenogest treatment [Bates et al., 1991; Kaeoket, 2008].

In gilts it has been demonstrated by Stevenson and Davis (1982) that a 14-day-treatment with altrenogest is long enough for corpora lutea to regress and for the females to come into estrus synchronously, although an 18-day-treatment resulted in a better synchronization effect.

B. Synchronization of Weaned Sows using Altrenogest

Sows, especially first parity ones may have an extended wean-to-estrus interval and an increase in embryonic loss mainly due to the lactational catabolism following farrowing [Koutsotheodoros et al., 1998]. Altrenogest can be used to synchronize early weaned sows without affecting the reproductive performance since it extends the wean-to-estrus interval and hence, allowing extra time for the female to recover from the lactational catabolism [Stevenson et al., 1982; Santos et al., 2004; Patterson et al., 2008]. It improves the percentage of sows in estrus within 7 days after weaning, increases ovulation rate, embryonic survival and subsequent litter size (Kemp et al. 2006).

3. P.G.600

The reproductive efficiency within a swine herd can be evaluated based on the proportion of sows that return to estrus within 7 days after weaning [Estienne et al., 2001]. To prevent a late return to estrus after weaning, a combination of gonadotropins can be administered. The exogenous gonadotropins, pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), act on the ovary to induce follicle growth and subsequent ovulation [Kirkwood et al., 1998] and these gonadotropins are commercially available in a product called P.G. 600 (Intervet America Inc., Millsboro, DE). P.G. 600 (400 IU of PMSG and 200 IU of

hCG), a non-prescription drug used to stimulate the onset of heat and ovulation in prepubertal gilts and weaned sows and thus can decrease non-productive sow days (NPD) [Estienne, 2001].

Administration of P.G.600 to sows on the day of weaning stimulates ovarian follicle development and results in a greater expression of estrus with a shorter wean-to-estrus interval (Knox et al., 2001). Administration of P.G. 600 to sows at weaning induces follicle growth, leading to an increase in estrogen, an LH surge, and improves return to estrus in multiparous sows and primiparous sows and also reduces the wean-to-estrus interval in both primiparous and multiparous sows [Bates et al., 1991; Estienne et al., 1998; Knox et al., 2001].

The average time of ovulation is not normally affected by treatment with P.G. 600 and sows will typically ovulate 45 hours after estrus [Knox et al., 2001].

4. Altrenogest + PG 600

A combination of the orally active progestin altrenogest and P.G. 600 given 24 h after the last feeding of altrenogest successfully synchronized estrus in cycling gilts [Estienne et al., 2001; Estienne et al., 2002]. Administration of P.G. 600 24 hours after withdrawal of altrenogest increases ovulation rate compared with gilts treated with altrenogest alone (Estienne et al. 2001). However, pregnancy rate and litter size at day 30 were not affected by P.G. 600 treatment (Horsley et al. 2005).

E. Breeding Management of the Sow

1. Types of Mating Systems

In order to have successful mating it is necessary the coordination of insemination with ovulation. There are two types of matings that can be applied in swine productions, natural

service and artificial insemination [Christenson et al., 2004]. Natural mating can be performed in one of two systems, group mating or hand mating system [Almond, 2007].

A. Group or Pen Mating

Pen mating is not a common practice currently in production. It functions as a group of females is kept on pasture outside lots or in relatively large confinement pens and consists of putting a number of boars in with a proportionate number of sows or gilts. Each female is then bred unknowingly as she comes into estrus. In group breeding, there are many variations, but there are three general methods: continuous breeding, boar rotation breeding, and sow rotation breeding [Almond, 2007]. In continuous breeding, all herd boars have continuous access to the sows and gilts, which are kept in the pen for about 70 to 80 days or until they show advanced signs of pregnancy [Almond, 2007]. In the boar rotation breeding, females are confined in certain lots and each day an appropriate number of boars are placed in the pen, left for 24 hours and then altered with another set of boars. After about 25 days these females are moved to gestation pens [Almond, 2007]. In sow rotation breeding, a group of females is placed in a lot for about 25 days and then they are moved to the gestation pens [Almond, 2007]. Pen mating relies on boars accurately identifying and breeding sows in estrus. It is also important to have enough boar power, requiring adequate number of boars with a common boar-to-sow ratio of 1:8 [Anderson, 1993].

B. Hand Mating

In the hand mating system once a day heat checks are performed by moving groups of gilts or sows to a centralized pen with a mature teaser boar. As females are detected in estrus, they are exposed to the breeding boar. The teaser may be moved through several pens with females [Almond, 2007]. For sows, estrus detection starts 3 days after weaning and females

remain with the boar for at least 5 minutes, ideally for 15 minutes [Almond, 2007]. Sows are mated once daily at 24-hour intervals for as long as they stand [Almond, 2007]. In this system, fewer boars are required, there's greater genetic merit and uniformity of progeny and breeding dates are accurately know in comparison to the pen mating system [Almond, 2007].

2. Artificial Insemination

Artificial insemination (AI) is the most commonly used mating system in the United States. It presents several advantages over the previous systems discussed, such as the ability to assess semen quality before use and hence, identification of infertile and subfertile boars, leading to a higher degree of conception rates [Anderson, 1993].

Most swine breeding programs adopt the strategy of administering two to three individual inseminations, every 12-24 hours apart after the detection of estrus. Performing multiple inseminations can be critical due to the relatively short viability of oocytes and spermatozoa in the female reproductive tract and also because time of ovulation in the sow is highly variable, making it quite unpredictable [Flowers, 1998]. Fertilization results are highly dependent on the time of insemination relative to ovulation; however, the moment of ovulation may vary between 35 to 45 hours after the onset of behavior estrus [Soede, 1997], hence, the need for multiple AI.

The procedure for AI is described by Gordon (1997) as introducing the insemination catheter through the lips of the vulva into the vagina and directing it upwards and forwards towards the spiral configuration of the cervix. Once in contact with the cervix, the catheter is twisted in an anticlockwise direction to lock it into the cervix. The semen container is then attached to the catheter and pressure gently applied over a period of several minutes to run the semen into the uterus.

3. Estrus Detection

Pen mating and hand-mating systems rely on the boar for estrus detection. In order to achieve success in an AI program, it is necessary that animal managers be able to recognize the behavioral signs of estrus and be aware of the factors which contribute to normal estrual behavior. Since females will sometimes cycle and ovulate without manifestation of estrus, it is also important that managers know how to recognize the physiological signs of approaching ovulation [Christenson, 2004].

Estrus detection is the most important feature in the breeding herd and so, improper detection of females in estrus leads to poor timing of AI which impairs reproductive performance [Rozeboom et al., 1996]. If mating if performed more than 24 hours before or after ovulation, it will result in reduced fertilization rate and consequently reduced farrowing rate and litter size [Kemp, 1996].

Estrus detection should be performed twice daily, which eliminates most cases of false estrus, since sows that are not truly in estrus will rarely stand for two consecutive detection periods [Altmann, 1941]. Accurate estrus detection is greatly dependable on the presence of a mature boar and an experienced stockperson. Sows and gilts should have daily contact with the boar and be taken to the boar rather than the boar to them, which will facilitate the full expression of estrus [Senger, 1999]. However, a key point in detecting estrus quickly and efficiently is to not allow the female to receive boar stimuli such as contact, sight, sound or smell for one or two hours before the time for estrus detection as this will reduce the effectiveness of detecting females in estrus [Christenson, 2004]. In addition, the manner in which boar is exposed to the females can influence the accurate detection of the standing reflex; because it involves muscle contractions and maintenance of the standing reflex requires a considerable amount of

energy. As a result, females in estrus exhibiting the standing reflex can become fatigued or tired. If this happens, then she usually cannot resume a standing reflex for several hours [Christenson, 2004]. Consequently, boar exposure during estrous detection should be restricted to small groups of sows, either 5 to 10 crates or 1 to 2 pens. These small groups should be examined carefully and receptive females identified [Gordon, 1997].

Females expressing estrus will show typical signs such as lordosis, swelling and reddening of the vulva, vocalization and boar-seeking behavior, "ear popping" or pricking of the ears, and standing for back pressure [Safranski, 2007].

A. Male presence

The male seeking behavior of the sow is in terms stimulated by the boar's pheromones [Kirkwood et al., 1981]. Pheromones produced by boars are the most potent and effective inducer of the standing reflex in receptive females. Two of the steroids produced by the boar testis, 3α-androstenol and 5α-androstenone are believed to be concentrated in the submaxillary gland of the mature boar and secreted into the saliva, where they act as pheromones [Kirkwood et al., 1981].

If sows are housed in crates, running a boar in front of sows while a person applies back pressure is a common and effective method of estrous detection. Sows in crates that are in estrus will move forward and assume the standing reflex as the boar moves in front of the crate. Sows in crates that actually try and move away from back pressure, even though they may exhibit other positive signs, probably are not in true estrus. If back pressure is applied and the sow is not in estrus, then the animal will attempt to escape the back pressure. Females housed in pens displaying behavior estrus will sometimes attempt to follow the movement of a boar as he passes

in front of their pen. This is due to the fact that sexually receptive females seek out males more so than males finding sows that are in heat [Gordon, 1997].

This responsiveness to boar stimuli, used for the detection of estrus, increases in the period immediately preceding ovulation as the female prepares to allow mounting [Langendijk et al., 2000].

B. Lordosis / Back Pressure Test

Lordosis, or mating posture assumed by the receptive female, triggers significant sexual arousal behavior on the part of the male. It is a highly specific female motor response associated with the willingness to mate [Senger, 1999]. This is a natural response as she is preparing herself to be mounted by the boar for breeding [Gordon, 1997].

This behavioral characteristic which is often used as the basis of estrus detection is the animal's response to the "riding-test". When provided with the appropriate stimuli, receptive females will initiate isometric contractions of most of their skeletal muscles. This results in the female remaining rigid or "locked up" in anticipation of being mounted by a boar for breeding. Often the ears of sows and gilts will become erect during the standing reflex. This is commonly called the "ear popping" or pricking of the ears response [Gordon, 1997]. This response provides some convenience when performing AI in that sows can be inseminated without restraint if pressure is maintained on the rump [Flowers et al., 1993].

Measurement of Vaginal Electrical Resistance

Changes in the electrical conductivity of vaginal mucus has been used to try to predict with some accuracy the optimum time to inseminate sows with fresh semen [Zink et al., 1984], however, a study performed by Stokhof et al. (1996) showed that although vaginal conductivity

increased slightly during estrus, it varied considerably between sows and no relationship was found between vaginal conductivity and ovulation time as determined by ultrasonography.

Vulvar Changes

Vulvar reddening and swelling are related to the rise in circulating estrogens during the follicular phase, which stimulates blood flow in the genital organs which is more noticeable in gilts than sows [Langendijk et al., 2000]. There's a significant increase in the density of estrogen receptors in the pig uterine and cervical tissue during the late follicular phase and is highest during estrus. Following the rise of the preovulatory LH surge, plasma estradiol concentrations start to drop and reach basal levels during the day before ovulation and so, a concomitant decrease in related vulvar reddening might be expected [Van de Wiel et al., 1981]. Similar results were obtained in a study conducted by Langendijk et al. (2000), where vulvar reddening took place at 21 hours before ovulation, indicating a relationship between the decline in circulating estrogens before ovulation and vulvar reddening. The same study also showed that the range in the interval between vulvar reddening and ovulation appears to be related to the duration of vulvar reddening, since sows with a shorter duration of vulvar reddening will ovulate within a 75 hour range from the end of reddening and sows with a longer duration of vulvar reddening will ovulate in about 30 hours.

F. New Technologies for Estrus Detection

1. Non-automated Methods

*Ultrasonography*

The introduction of the transrectal untrasonography has made it possible to relate the timing of estrus to ovulation as well as hormonal changes around ovulation in sows. Ultrasonography can greatly aid in the understanding of follicular dynamics and time of estrus and ovulation in sows.

Although it has become increasingly applied on farms as a pregnancy diagnosis method, ultrasonographic examinations of the ovaries have only been applied in experimental studied guided mostly toward determining the time of ovulation.

Ultrasound exams can be performed when sows begin showing the first signs of estrus and it can be used to monitor follicle development until ovulation [Soede et al., 1998]. This monitoring can be performed using sequential examinations of the ovaries to follow changes and a reduction in the number of follicles present in the ovaries [Knox et al., 1999].

2. Automated Estrus Detection Methods

As females approach estrus, they begin to show an increase in physical activity and change in a series of body functions. Based on this information, several heat detection methods have been developed, and specially automated methods for group housed post-weaning sows where it can be difficult to identify and access the individual sow in estrus. Automated methods for estrus detection are often designed to facilitate management in large swine operations using the so-called principle of "management by exception", which means that the breeder can focus the attention on the sows to be checked for estrus and make more adequate management decisions, considering that formal estrus detection method takes about 30% of the total labor input [Perez et al. (1986)].

Cornou (2006) reviewed methods for automated estrus detection in group-housed sows which can be based on sow's activity measurements, using accelerometers, infrared sensors, and sow's activity near the boar pen and body temperature measurements.

*Accelerometer*

The first sign that females are coming into heat is an increase in activity and vocalization [Cornou, 2006], hence, there's an increase in both exploratory and motor activity at this time [Signoret, 1970].

When housed in crates, sows commonly move backward and forward or from side to side within the crate and often attempt to nibble or nose females in adjacent crates. When housed in pens, characteristic activities include sniffing; nuzzling the rear and fore flanks; and attempting to mount or ride other females. It is important to remember that sows attempting to mount or those actually riding other females are not in heat, but rather either just coming in or going out of their period of sexual receptivity [Signoret, 1970].

An accelerometer is a device for measuring actions induced by movement or acceleration and it can detect magnitude and direction of the acceleration. A study conducted by Bresser (1993) on the efficacy of use of accelerometers for the detection of estrus in group-housed sows suggested that this method could reduce in 10 to 15% the number of sow check-ups when compared to no automated system. Another study conducted by Geers et al. (1995) reported physical activity to be 10 times higher the day before estrus.

*Visit to the Boar Pen*

It is an accepted fact that females in general will seek the male during their receptive period mainly due to the effects of reproductive hormones, mainly estrogen. Beach (1976) has proposed that during estrus, the female will show periods of attractivity, proceptivity, and

receptivity, meaning it will seek for the male, initiates, establishes and maintain sexual interaction with the male and finally show positive responses towards the male leading to copulation.

Using these proven facts certain automated estrus detection methods have been developed such as the use of parameters on sow visits to a boar, in which the boar is housed in a separate pen from the sows, but can still have nose contact with them. Experiments performed by Houwers et al. (1988) Blair et al. (1994) experiments had a similar conclusion which was that sows in diestrus showed little interest in the boar and increased boar visitation was highly correlated with observed estrus.

Visit to the boar pen can be considered a very useful tool for estrus detection, offering a high degree of sensitivity and specificity; however this system's negative aspect is the fact that it monitors animals individually, therefore it can miss animals in estrus at the same time [Cornou, 2006].

*Body Temperature Measurement*

Several researches have demonstrated a deviation in body temperature around the time of estrus in the many different species of animals; however there are many confound differences among these researches. Junge and Holtz (1984) measured intravaginal temperature of sows and cows and diagnosed a defined drop of temperature which was at its lowest two days before the female was observed in standing estrus and a high point 2 days after standing estrus, which coincides with the period close to ovulation. On the other hand, a study performed by Henne (1991) on rectal temperature, measured twice daily during estrus in synchronized gilts and found a large variability between animals; at onset of estrus the temperature rose in 30% of the gilts, did not change in 20% of the gilts, and declined in 50% of the gilts.

Other researches support the fact of body temperature oscillations during the estrous cycle, which may be due to corpus luteum regression as supposed by Kyle et al. (1998) or to estrogen-induced changes in peripheral vasodilatation as shown by Czaja and Butera in an experiment with guinea pigs (1986). However due to so many conflicting parameters as to whether body and/or vaginal temperature is related to the time of estrus and if those parameters are accurate and reliable, body temperature measurement is not currently used as a way of estrus detection is swine operations.

*Infrared sensor*

Not much research has been published in regards to estrus detection in sows using infrared sensor other than a study conducted by Freson et al. (1998) where the sow's body activity was monitored continuously by an infrared sensor mounted above the shoulder area. The research was based on the principle that the sensor became electrically charged when the temperature of the sow changed. This change in temperature was generated by a change in position. All this information was transmitted to a computer where it was stored for further analyses. The work described the advantages of this method as the ability to monitor estrus without touching the sows; fixed installation on a limited number of stalls and no calibration required. The author also stated that 86% of the sows were correctly classified when using the mean daily activity as the selection parameter, with 79% sensitivity and 68% specificity.

## G. Infrared Thermography

Infrared thermography (IRT) is the process of acquisition and analysis of thermal information from non-contact thermal imaging devices. It is a modern, noninvasive and safe technique to determine the thermal profile of a certain object or being.

Considering all materials and beings generate heat radiation in the infrared part of the light spectrum depending on their temperature and emissivity, the infrared camera captures this radiation and transforms it into images which can then be evaluated [Knízková I. et al., 2007]. The temperature of a being has electromagnetic radiation which is characterized by its wavelength and intensity [Schaefer et al., 2003]. The wavelength of an object depends on its surface temperature: the higher the temperature, the shorter the wavelength at which most of the radiation is emitted [Schaefer et al., 2003]. The wavelengths are in the infrared range of the electromagnetic spectrum, consequently measuring radiation emitted by such objects is generally called *infrared thermography* [Schaefer et al., 2003].

The function of the emissivity is (ε) is the single most important attribute necessary for thermal measurement [Knízková I. et al., 2007]. It is the ratio of the actual amount of electromagnetic radiation emitted by an object to the amount emitted by an ideal blackbody at the same temperature, since a blackbody is a perfect absorber and emitter of radiation.

An object or being will absorb some of the radiation and convert it into heat, reflected some at its surface and radiation will also pass right through an object or being. The infrared camera picks up the total radiation from a surface. The data obtained by an infrared camera is computer-processed and shown in the form of temperature maps that provide for a detailed analysis of the temperature field [Knízková I. et al., 2007].

The use of digital infrared thermography (IRT) has been thoroughly investigated as a diagnostic tool in human medicine and has been growing in the field of veterinary medicine, primarily for an aid in diagnostic purposes to detect areas of inflammation. Variations in skin temperature result from changes in tissue perfusion and blood flow, especially in pathologic scenarios, and so, this information is used to assess the area [Harper, 2000]. A major advantage

of the method is the fact that it measures heat emissions and does not require a direct physical contact with the surface monitored, thus allowing remote measurement of temperature distribution [Speakmen, 1998].

There are some limitations and factors that need to be considered when using IRT especially in veterinary medicine. Thermograms must be collected out of direct sunlight and wind drafts, since the effect of weather conditions, circadian and ultradian rhythms, time of feeding, milking, laying and other physiological animal behavior are also factors along with hair coats and skin cleanliness that will greatly affect the final temperature measurement [Knízková I. et al., 2007].

1. Applications of IRT in livestock

*Bovine*

IRT has been widely researched in cattle among other species. The IRT method has value as a diagnostic tool for assessing udder function and can be considered a useful method for indirect and noninvasive evaluation of the condition of teats and udders as indicated by Kunc et al. (2007) who concluded that IRT shows potential as an early detection method for mastitis. Nikkah et al. (2003) observed hooves of dairy cows. Images of hooves were taken using IRT to determine temperature of the coronary band, and that of a control area above the coronary band. The authors recommend IRT as tool for monitoring hoof health.

In the reproductive field, Hellebrand and colleagues (2003) concluded that the external pudendum temperature follows the core body temperature, and thus IRT can be utilized for estrus climax determination. Also, a study performed by Osawa et al. (2004) had the goal to use thermography to detect vulva surface temperature changes during estrus and concluded that it can indeed improve estrus detection rate in cows with silent or normal estrus, since temperature

increased significantly between three days before and the day of ovulation. However that study did not have a substantial amount of animals and results may be deceiving.

In bulls, it has been used to diagnose testicular inflammation and degenerative disease, since the compromised testicle will show temperature 2.5 degrees to 3 degrees (C) above that in the contralateral side [Purohit, 1985]. And many pathologic and physiologic aspects of testicular function related to temperature have been explained by researches with IRT as shown by Kastelic et al. (1996, 1997a, 1997b) and Gabor et al. (1998).

*Equine*

In equine medicine, IRT has been mostly widely used to diagnose foot and leg problems in horses and aid in diagnose and treatment of lameness [Strömberg, 1975; Weil et al. 1998; Eddy et al., 2001]. However, Bowers et al. conducted a study to investigate the use of thermography in order to be able to diagnose pregnancy status in mares and concluded that it can be performed and used as a trusted noncontact method for mid- to late-gestation pregnancy diagnosis in mares.

*Swine*

As with other species, in swine, IRT has been used in a number of ways. It has been used to detect osteoarthrosis tarsi deformans (OATD), in the tarsus of Swedish Landrace boars [Sabec and Lazar, 1990] and to detect febrile responses in pigs following intranasal inoculation with *Actinobacillus pleuropneumonia* [Loughmiller et al., 2001]. Infrared thermography has also shown to be a useful tool in meat quality control in slaughter plants as shown by Gariepy et al. (1989) who observed a correlation between incidence of meat quality defects and increasing skin

surface temperature of pigs prior to stunning and concluded that IRT can be a practical and rapid method of detecting which pigs will yield a significant proportion of meat quality defects. Schaefer et al. (1989) studied the relationship between stress sensitivity and meat quality in pigs were able to diagnose a higher drip loss and percentage of pale, soft and exudative meat (PSE) may be expected in pigs with a lower superficial temperature.

*Animal Welfare*

IRT has shown to be a reliable tool to measure stress in animals, being noninvasive and accurate and therefore helping to assess animal welfare [Stewart et al., 2005]. Using IRT, Schwartzkopfgenswein and Stookey (1985), were able to detect a prolonged inflammatory response observed in hot-iron animals in comparison with animals branded with freeze brand, indicating more discomfort associated with hot iron branding.

Adamec et al. (1997) studied the possibility of reducing heat stress on fattening pigs during the summer period by means of water evaporative cooling. The authors concluded that evaporative cooling decreased heat stress on pigs, and improved growth and feed conversion.

# CHAPTER 3

# MATERIAL AND METHODS

## A. Animals and Housing

The study was conducted using 25 nulliparous and 27 pluriparous crossbred (Yorkshire x Landrace) females housed individually in crates size 24”x 67”. The boars used for heat detection were 1.5-2 years of age, housed pens size 5’x8’ located in breeding barn. All animals were kept in a temperature and humidity controlled environment recorded daily. This study was performed in accordance with the regulations of the Institutional Animal Care and Use Committee.

## B. Experiment Group

### *Synchronized gilts*

Gilts were synchronized using an altrenogest solution, a synthetic progestagen (Matrix®, Intervet/Schering-Plough Animal Health, Millsboro, DE), 15MG (6.8ML) fed to each female daily for 14 days and administered PMSG and HCG (PG600®, Intervet/Schering-Plough Animal Health, Millsboro, DE) on the last day of altrenogest treatment. The day of PG 600 treatment was considered as day 0 of the experiment.

### *Weaned Sows*

The day of weaning was considered as day 0 of the experiment.

### *Controls*

The control group consisted of 30 sows per breeding replicate (3 replicates=90 total). These sows were at least 50 days of gestation, diagnosed pregnant by ultrasonography. These sows were imaged with the infrared thermocamera (FLUKE IR FlexCam® Thermal Imager, model

Ti55, Fluke Corporation, Everett, WA) in order to establish a normal vulvar skin temperature for animals not undergoing hormonal changes associated with the estrous cycle.

C. Detection of estrus

Weaned Sows were exposed to the boar for heat detection starting 2 days post-weaning (Day 2). Gilts were exposed to the boar for heat detection starting 2 days after the end of a 14 days treatment with Altrenogest for estrus synchronization (Day 2).

From Day 2 onwards, estrus detection was performed twice daily (every 8 hrs) after the thermal images were obtained. The females were taken to a pen next to the boar's pen and allowed about 5 minutes to acclimate to the new environment. Onset of estrus was confirmed by behavioral subtle signs such as swelling of the vulva, vocalization, boar seeking behavior, standing reflex, lordosis (chronic flexing of the back muscles) and ear pricking.

D. Thermal imaging

From Day 2 onwards, vulvar skin temperature was measured using the infrared digital thermocamera (FLUKE IR FlexCam® Thermal Imager, model Ti55, Fluke Corporation, Everett, WA) before onset of signs of estrus in order to establish a baseline temperature for the female and digital thermal imaging continued through estrus and until the female was detected as having ovulated and not showing any more signs of estrus. The digital thermal imaging was performed with the females in gestation crates before moving them to the boar for estrus detection. Images were acquired while the females were being fed, facilitating the procedure by having them up and standing still.

*Scanning Procedure*

During the scanning procedure the sows/gilts remained in their crates, and each animal was monitored from a fixed distance of 2 feet, measured from the posterior end of the female. The monitoring itself focused on the gluteal region of the sow's/gilt's body, which included the anal and vulvar areas (Figure 3.1). A black spot was marked on each gluteal region of the pig (Figure 3.1A) with the aim of taking measurements at the same location during each imaging event. Cleaning of this posterior area was not performed unless the area was excessively wet and/or dirty as not to disturb the skin temperature.

The digital infrared thermal images were downloaded into the computer and visualized using the SmartView™ Version 1.7 image analysis software (Fluke Thermography, Plymouth, MN). This software allows the user to display the temperature at any given point on the image. Additionally, specific areas such as the vulva can be outlined and the average, minimum and maximum temperature values calculated (Figure 3.1B). When analyzing the infrared images, average temperatures of the gluteal areas were also recorded.

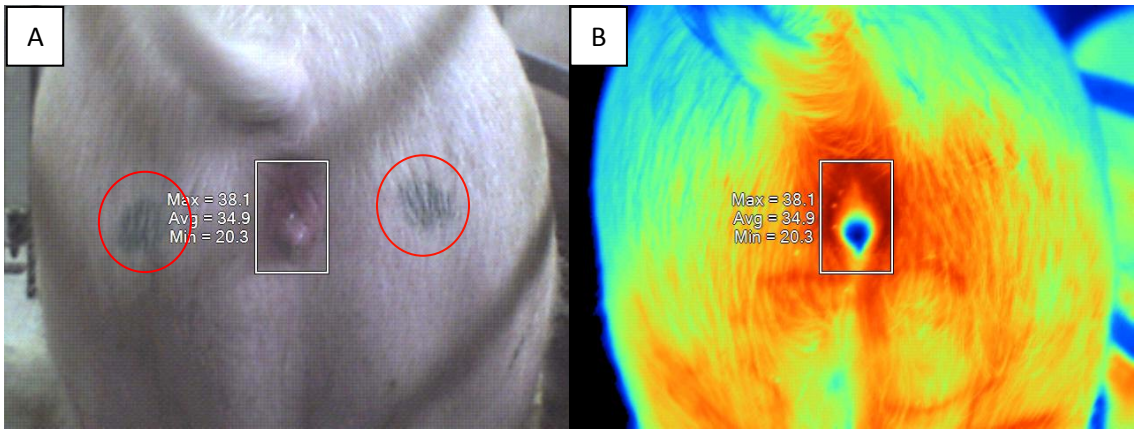


Figure 3.1: Digital infrared thermal image of a sow's posterior area with the vulva selected Panel A: White-light image. Black spots on the gluteal region (outlined by red circles) were used as guides. Panel B: Infrared thermal image.

E. Real-time Ultrasound

Once standing estrus was observed, transrectal real time ultrasonography (RTU) was performed twice daily at 8-hour intervals (8 am and 4 pm) to determine the occurrence of ovulation. Ovaries were visualized using an Aloka 500V ultrasonics machine (Aloka Co, Tokyo, Japan) fitted with a transrectal 7.5 MHz linear transducer which was fitted into a rigid, fixed-angle PVC adapter (Knox 1999). After ovulation was diagnosed, one more ultrasound exam was performed in order to confirm the diagnosis.

Ovulation was the most important parameter for the experiment since the objective of the project was to determine if there was a relationship with the vulvar skin temperature. The female was classified as either ovulated or not ovulated. Ovulation was determined to be complete when there were fewer than four follicles ≥ 6.5 mm in diameter remaining on the ovaries and noticeably fewer large follicles relative to previous observations.

During transrectal RTU examinations, the sows remained in the standing position and the probe, well lubricated with Lubrivet® gel, was introduced into the rectum with the transducer turned over, then it was rotated 180◦ in complete contact with the rectal mucosa. To find the genitourinary structures the probe's transducer was turned down so that the bladder and the uterine horns could be observed in longitudinal section. Then, the transducer was rotated 45–90◦ clockwise and counterclockwise to locate, respectively, the left and right ovary as explained by Ginther and Kot (1994).

F. Statistical Analysis

All mean values for hours and temperatures are reported as mean ± standard error (SE). Vulvar temperatures of gilts and sows during the non-estrus and estrus period were compared

using an ANOVA and Student's t test using SAS (SAS Institute Inc., Cary, NC) to test the significance of putative temperature spikes. The average temperature for each measurement time interval was compared to the average temperature of the following time interval (8 or 16 h intervals). A significant value reported at $p<0.05$.

# CHAPTER 4

# RESULTS

Temperature data collected at the same time each day was submitted to statistical analysis in order to identify significant vulvar temperature fluctuations.

In sows, the mean wean to estrus interval (WEI) was 86 ± 2.3 hours (mean ± SEM). Gilts showed estrus at 75.5 ± 2 hours after last day of altrenogest and PG600®. Average duration of estrus for gilts was 46 ± 1.2 hours and for sows was 50 ± 1.7 hours. Ovulation took place at average 38 ± 9 hours after onset of estrus in gilts, and 43 ± 12 hours in sows.

Average vulvar temperature of gilts during estrus was significantly higher ($p= 0.01$) than during the non-estrus period (Figure 4.1). Similarly, sows also displayed significantly increased temperature during estrus ($p< 0.001$) as opposed to the non-estrus period (Figure 4.2). When comparing gilts and sows, the latter displayed significantly higher vulvar temperatures ($p=0.03$) during the estrus period than gilts, but in contrast, there was no significant difference ($p>0.05$) between vulvar temperatures in gilts and sows during the non-estrus period. There was no significant difference between the gilts and sows' vulvar temperatures at the time of ovulation ($p>0.26$).

During estrus, there is an increase in average vulvar temperature that occurs at distinct times as ovulation approaches both in gilts and sows. After a "peak" in temperature is reached, there is a significant decrease in temperature that occurs. The most marked increase in temperature was present at 36 hours before ovulation for gilts and at 24 hours before ovulation for sows. The frequency at which animals reached peak temperature is illustrated in Figures 4.3 for gilts and 4.4 for sows.

Gluteal area temperature was measured, analyzed, and compared with vulvar temperature. The temperature difference (TD) between the vulvar and gluteal area during estrus is illustrated in Figure 4.5 for gilts and 4.6 for sows. The difference in degrees of temperature followed the same trend as the vulvar temperatures. The gluteal area temperatures remained

relatively steady during the measurement periods. This means that the surface temperature of the animal did not change significantly during the non-estrus and estrus periods, only vulvar temperature changed. TD increased at 36 hours before ovulation in gilts and at 24 hours in sows, then decreased at 12 hours prior to ovulation and had a small increase at ovulation in gilts, but the rise in TD at ovulation did not occur in sows.

Pairwise comparisons were performed between the time periods during estrus. There was a significant decrease in temperature from 36 hours before ovulation to 12 hours before ovulation in gilts (p=.002) and also between 36 hours before ovulation and time of ovulation (p=.033) (Figure 4.7). In sows, it was possible to observe a distinct decrease in degrees of temperature between 24 and 12 hours before ovulation (p=0.000) and between 24 hours before ovulation and time of ovulation (p=0.000) in sows (Figures 4.8).

In gilts, there was a 0.02% decrease in temperature from 36 to 24 hour and a 0.04% decrease from 36 to 12 hours before ovulation. Then there was a 0.02 % increase in temperature from 12 hours before to ovulation. Figure 4.9.

Temperature in sows showed 0.04% increase from 60 to 48 hours before ovulation, maintained a relatively stable level and then a significant decrease from 24 to 12 prior to ovulation of 0.05%. (Figure 4.10)

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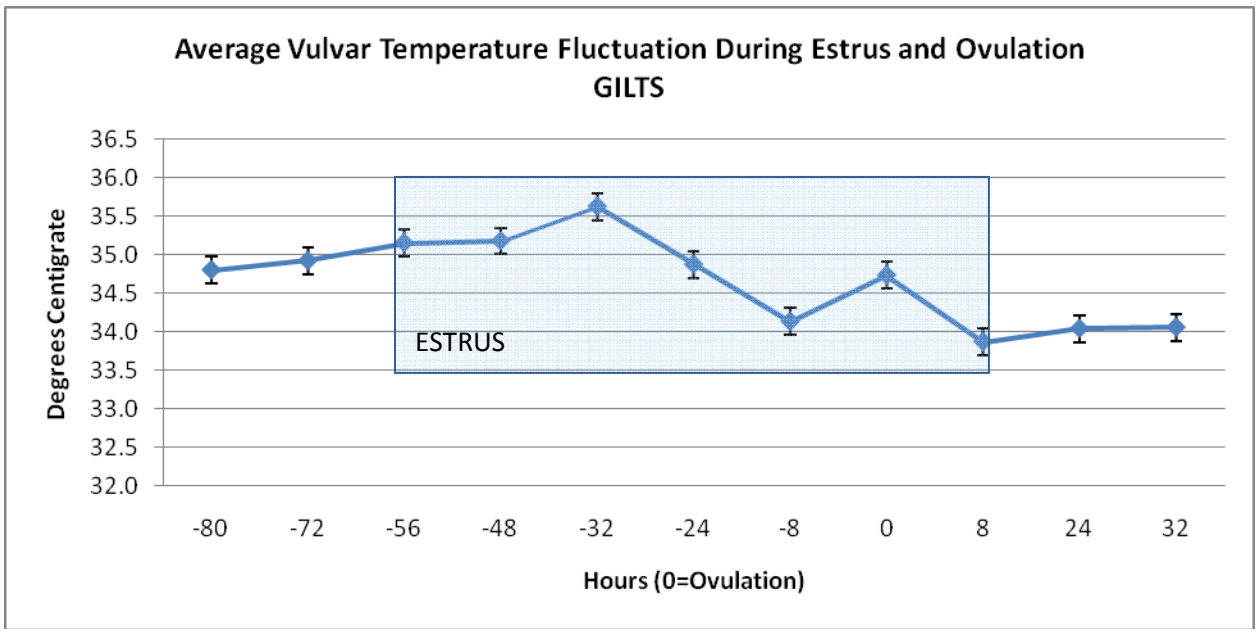


Figure 4.1. Illustration of the vulvar temperatures of gilts before and during estrus

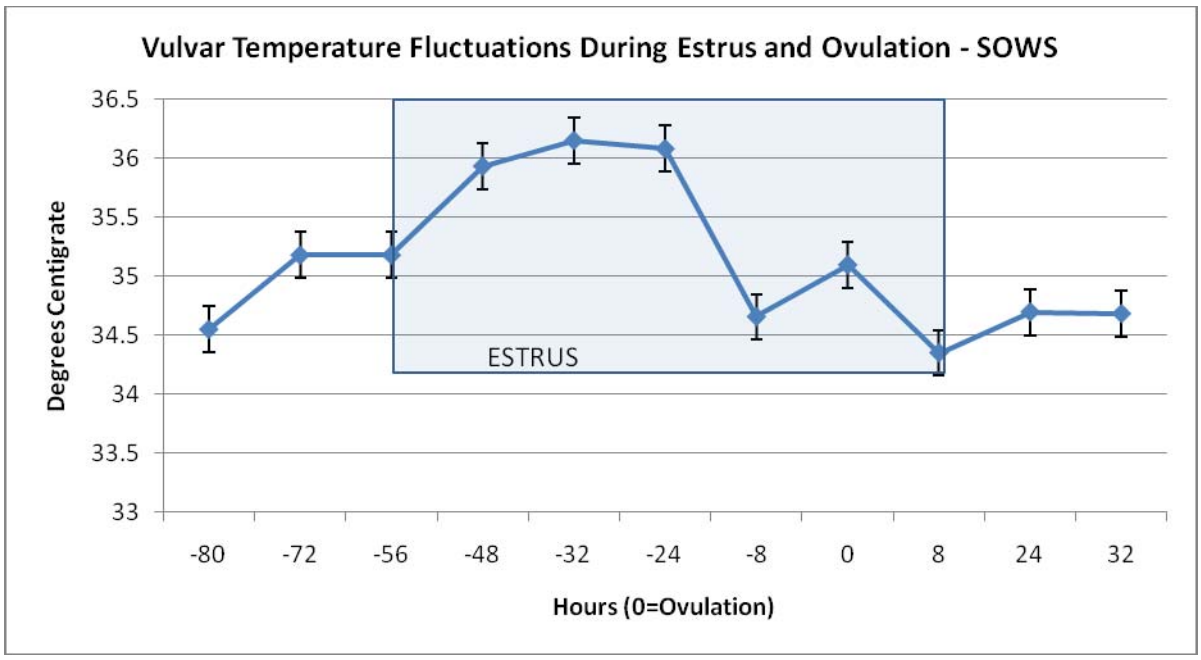


Figure 4.2. Illustration of the vulvar temperature of sows before and during estrus.

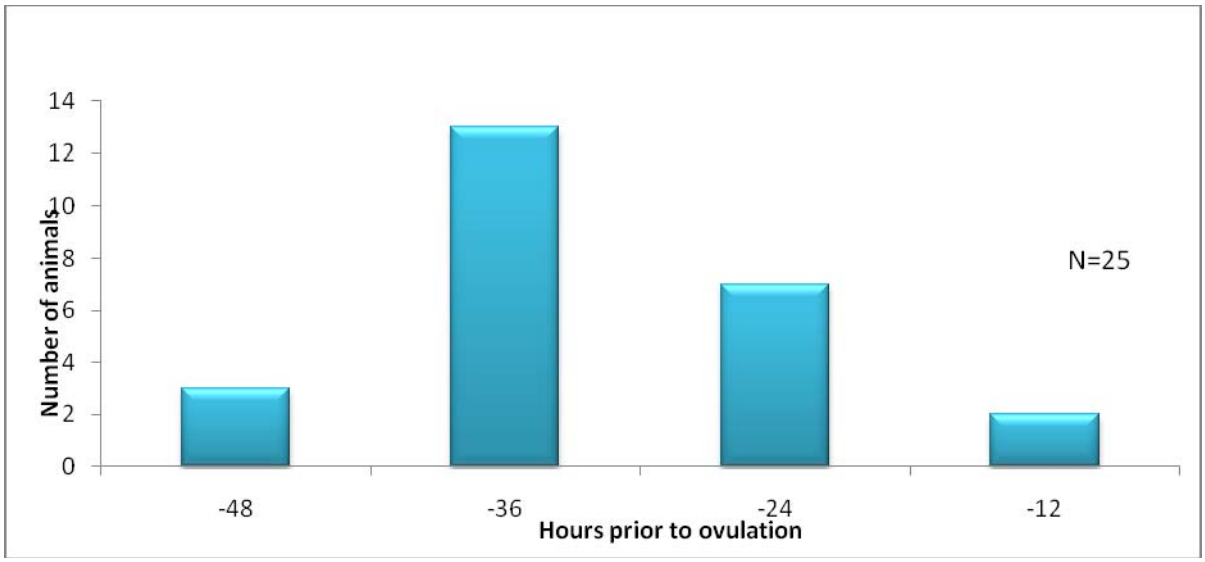


Figure 4.3. Frequency of animals with increase in vulvar temperatures at different times before ovulation - Gilts

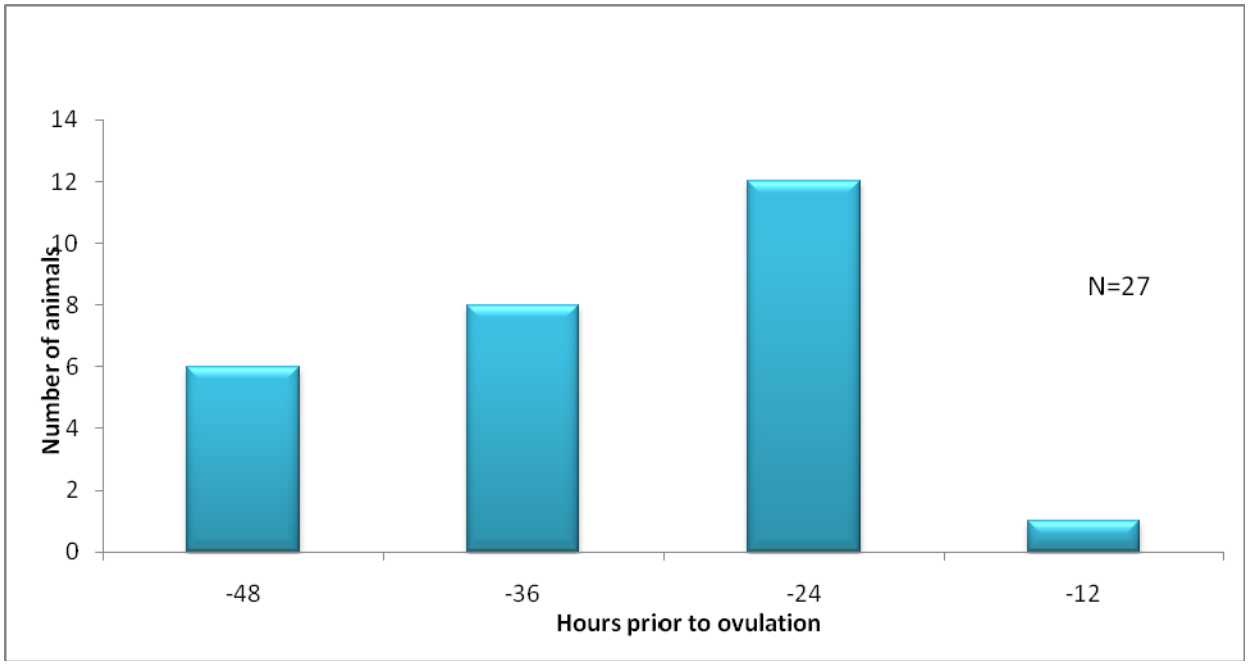


Figure 4.4. Frequency of animals with increase in vulvar temperatures at different times before ovulation - Sows

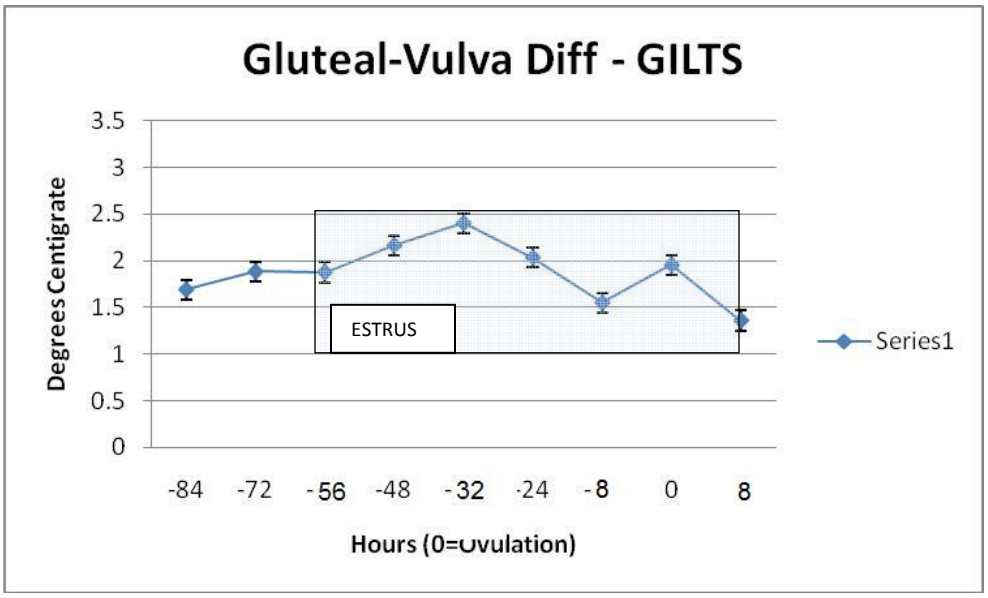


Figure 4.5. Difference between vulvar and gluteal temperatures during estrus and ovulation in Gilts

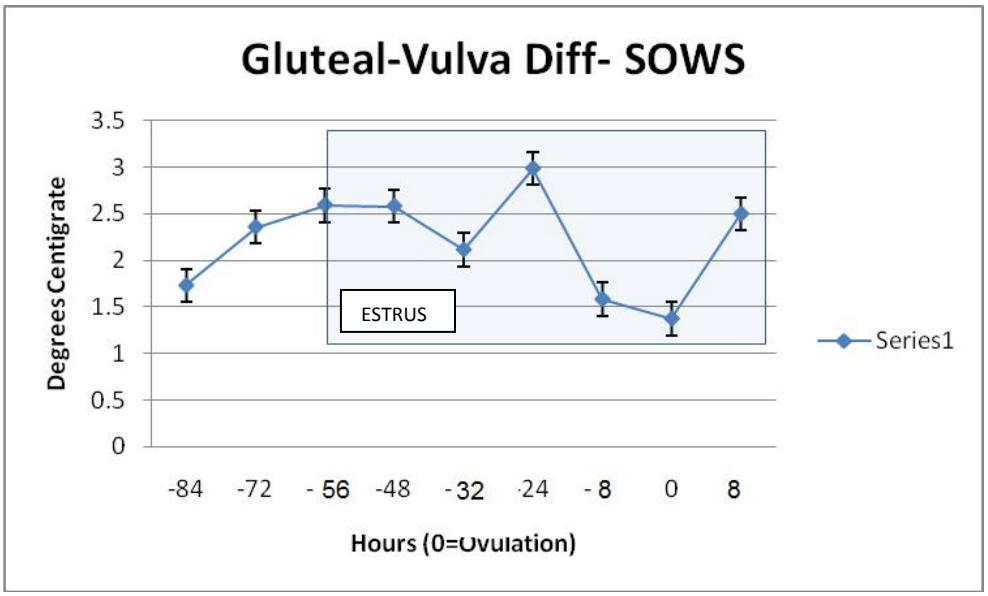


Figure 4.6. Difference between vulvar and gluteal temperatures during estrus and ovulation in Sows

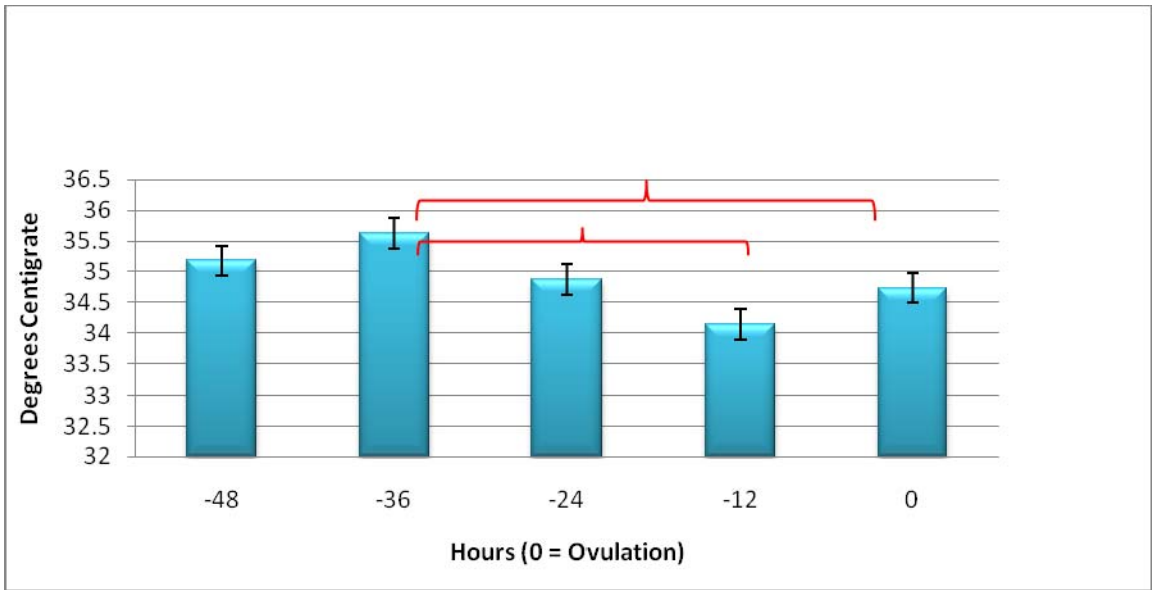


Figure 4.7. Comparison between average temperatures during estrus through ovulation in gilts. The brackets show where the significant differences are found.

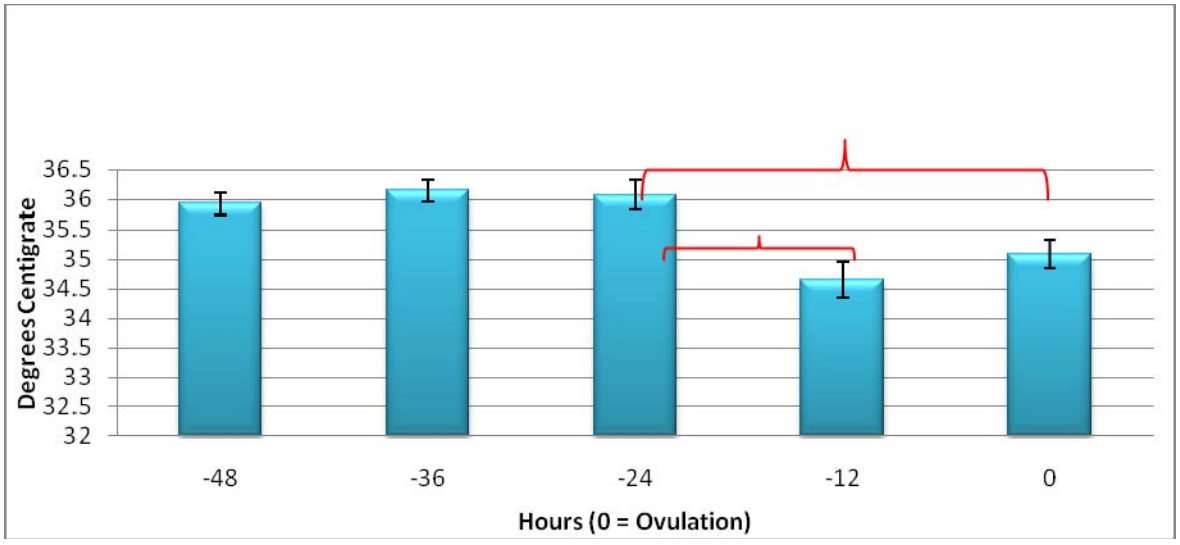


Figure 4.8. Comparison between average temperatures during estrus through ovulation in sows. The brackets show where the significant differences are found.

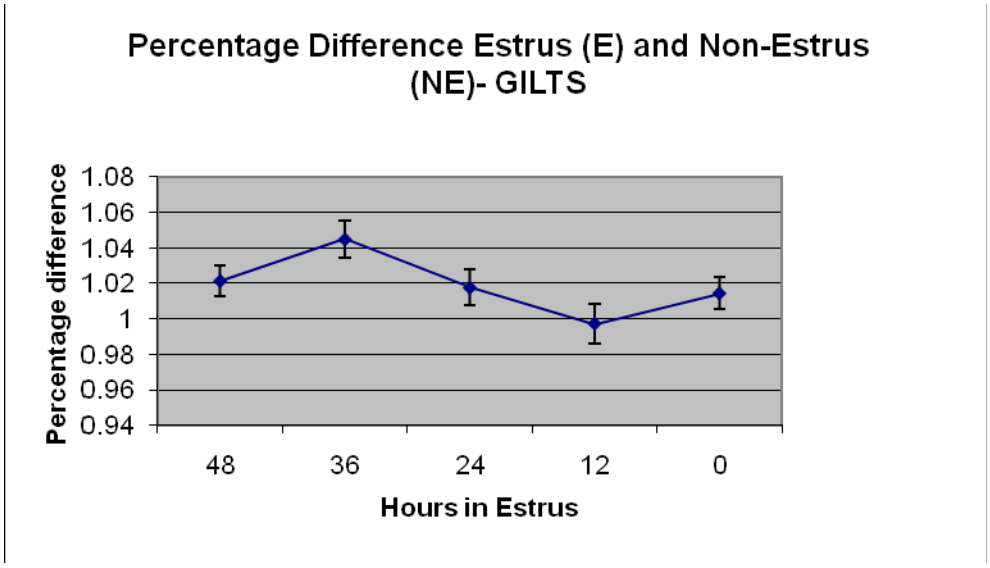


Figure 4.9. Percentage temperature difference between estrous and non-estrous gilts

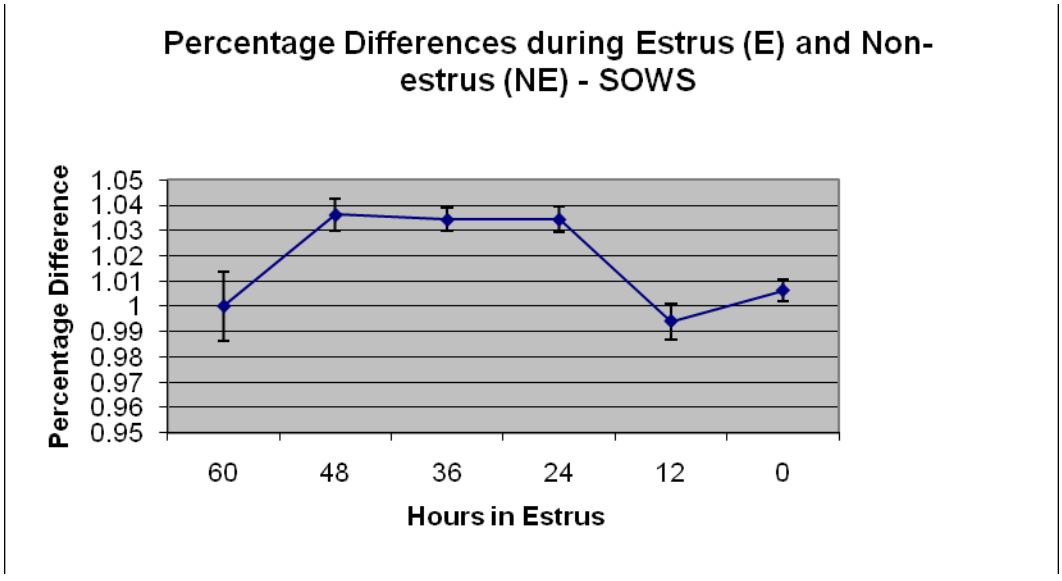


Figure 4.10 Percentage temperature differences between estrous and non-estrous sows

# CHAPTER 5

# DISCUSSION

In this study, thermographic imaging was able to determine a rise in temperature several hours prior to ovulation, which can be useful in predicting time of ovulation when used along with conventional methods such as use of the boar for estrus detection. No studies have previously been performed to investigate the use of Infrared thermography as an aid to estrus detection and ovulation predictor.

Only one study has been performed in attempt to correlate vaginal temperature and ovulation in sows. Soede et al. (1997) related that vaginal temperature is not related to the time of ovulation in sows, and although the temperature showed a daily rhythm, being higher in early morning than in the evening, there was no overall consistent change in vaginal temperature from 96 hours before to 48 hours after ovulation. In that study, there was a relatively low number of animals used (n=10) and they used a different technique for temperature measurement, which was temperature transmitters which assessed temperature every 12 hours and RTU was performed every 2 hours to determine ovulation. The results from our experiment agrees with the fact that no rise in vulvar surface temperature occurred to a significant degree at time of ovulation, however, 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.

Other researches attempted to relate body temperature to estrus, such as Junge-Wentrup and Holtz (1984), who, using temperature probes anchored deep inside the sow's vagina concluded that temperature dropped 2 days before onset of estrus and rose reaching a maximum 2 days after standing estrus, time near ovulation, and so, could be a useful tool for prediction of ovulation, result which agrees with the present study. Henne (1991) was unsuccessful in trying to correlate rectal temperate to estrus, finding a great variation in temperature between animals during estrus.

The advantage of the present study is that pigs were maintained at an enclosed environment, free of drastic temperature, light and humidity changes and also free from wind and sunlight, allowing for nearly accurate results in temperature readings.

We decided to analyze multiparous and primiparous animals separately due to their reproductive physiology differences. All animals displayed normal estrus behavior as determined by exposure to the boar and back pressure test.

There was a clear trend in temperature and a distinct difference between sows and gilts that could be observed from the results, which was a rise in temperature at 36 hours in gilts and 24 hours in sows prior to ovulation. This rise was always followed by a decrease in temperature reaching a low threshold at 12 hours before ovulation, when it began to rise again.

It is not clear why surface vulvar temperature presented such fluctuation. Unfortunately hormone assays were not performed, which could have aided in explaining temperature fluctuation, since it is likely linked to hormonal changes during the cycle. Two possible reasons for the vulvar temperature increase are LH peak prior to ovulation and a rise in Estradiol (E2) levels due to follicle development.

In cattle, a rise in vaginal temperature coincides with the timing of the LH-peak [Mosher et al., 1990; Fisher et al., 2008], which occurs at 21-27 hours before ovulation [Rajamahendran et al., 1989; Mosher et al., 1990; Rajamahendran and Taylor, 1991]. Clapper (1990) also determined a rise in temperature along with the LH peak in cattle and concluded that temperature is a good predictor of LH surge and hence, a better predictor of ovulation, since it occurs after a rather consistent interval after the LH surge. In sows, the LH-peak occurs at about 30 hours before ovulation [Soede et al.,1994; Mburu et al., 1995] or 6.5 ± 0.7 hours after the onset of estrus [Knox et al., 2003]. Since in the present study temperature was only measured at every 12 hours, we noticed the rise in temperature either at 36 or 24 hour prior to ovulation which could coincide with the timing for LH peak and a rise in temperature could be expected at around 30 hours before ovulation.

A more acceptable explanation for the rise in vulvar temperature is the pre-ovulatory increase in estrogen levels. A similar study performed by Osawa et al. (2004) in cattle determined that infrared thermography may be used as a screening tool for estrus detection and ovulation. They measured Estradiol-17b (E2) and found that both the temperature and E2 level increased between 3 days before and the day of ovulation in cows.

There's no literature available about the correlation of increase of estrogen during the follicular phase with temperature in pigs. Endocrine changes prior to estrus are characterized by increases in serum LH and (FSH) due to increased concentrations of GnRH in the hypothalamus.

The increased gonadotropins stimulate follicular growth, which results in increased estrogen levels [Stevenson et al., 1981; Cox and Britt, 1982]. Highest levels of estrogen occur one day before or the first day of estrus [Knox et al., 2003].

The approaching estrual period is characterized by rising plasma estrogens and it has been well demonstrated and documented by various experiments that estrogen causes vasodilation and increases blood flow to through the uterus and associated structures of the reproductive tract in the different species [Ford SP, 1982; Bell et al., 1995; Naderali et al., 1999; Naderali et al., 2001; Sprague et al., 2009]. Inspection of the female's genitalia and adjacent parts reveals obvious cyclic changes which are undoubtedly of vascular in origin. Abrams et al. (1972) performed studies in sheep that imply an increased heat production associated with biochemical responses related to increased estrogen levels. In that sense, generalizing from the previous works presented, a rise in vaginal thermal condition may be expected during periods when estrogen is known to be present in high concentrations. If this occurs, one might be able to use this principle, based on estrogen-induced rise of vaginal and vulvar blood flow rate along with increased temperature of the area to predict ovulation.

Based on our results and the timing at which rise of temperature occurred, we assume that it is due to the vascular changes such as increase in blood flow that occur in the vaginal and vulvar tissues triggered by elevated levels of estrogen. However, it is not clear the reason for vulvar temperature decrease 12 hours prior to ovulation.

# CHAPTER 6

# CONCLUSION

The results obtained from this study indicate that there is a clear vulvar temperature fluctuation during estrus in sow and gilts; however, although there are evidences as for these fluctuations being related to hormonal changes, it is not possible at this time to confidently determine such because hormones were not assessed in the present study and hence, further investigation using hormonal essays is necessary in order to determine a definite relation between temperature changes and hormones in the sow and gilt.

The temperature fluctuations can be a good predictor of ovulation and so, it appears at present that the usefulness of infrared scanning for routine prediction of ovulation in pigs is a promising tool for when used along with regular estrus detection by the boar. Further investigation is warrant in order to establish an adequate protocol for routine farm use in the absence of a boar. The technique however has immense potential as a research tool for the study of skin temperature patterns and help well understand temperature fluctuations that occur during the pig's estrous cycle.

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