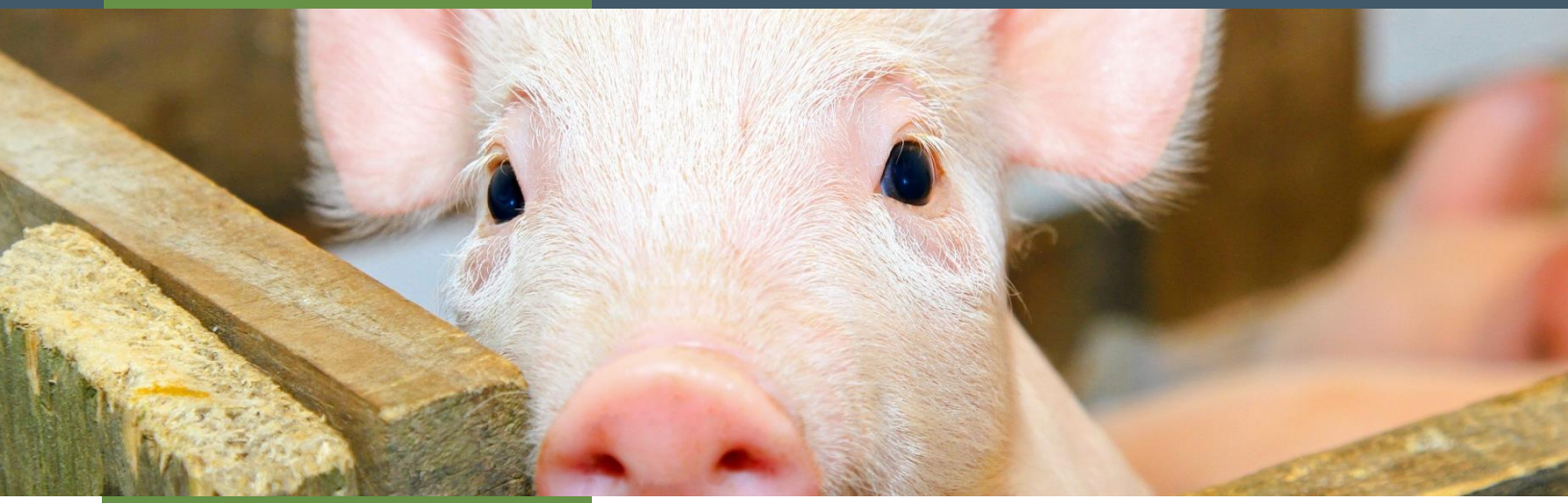
Validation of the Period for Insemination Indicated by PigWatch®, relative to Sow Ovulation

May 2016

Final Report



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RO-MAIN The vanguard of pig reproduction

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Summary

Sow estrus detection, together with effective targeting of the right time for insemination, are the key components for efficient breeding of sows. This stage of herd management has a significant impact on herd profitability.

Variability in the length and intensity of estrus period in sows makes it difficult to determine the ovulation time and, therefore the ideal time to conduct insemination. According to scientific studies, the ideal time is about 24 hours before ovulation. Since ovulation occurs two thirds of the way through the estrus period and lasts from 24 to 60 hours, inseminating sows at the ideal moment in their reproductive cycle is quite a challenge for the producer. For this reason, many producers inseminate sows twice or three times during the estrus period, which results in increased workload and higher spending on the purchase of doses of semen. Other producers find it hard to pinpoint the ideal time, which leads to reduced fertility and smaller litter sizes, and a subsequent falloff in herd productivity.

The PigWatch® system was developed to predict the best time to inseminate each sow. Prediction algorithm is working on the compilation and analysis of sow behavioural data from weaning until the end of the estrus cycle. During development of PigWatch®, reproductive performances were used as an indicator that the technology was working properly. Research tells us that the decision to inseminate should correspond to a period close to the sow's ovulation. Purpose of this project, was to estimate the correlation between the predicted best time to inseminate, as proposed by PigWatch®, and the real ovulation period of each sow, as determined by the temporal variation of two reproductive hormones, progesterone and estradiol.

There were 122 sows involved over the course of the project and all inseminations were performed in accordance with the requests for insemination proposed by PigWatch®. The time of the AI request for each sow was compared to the presumed time of ovulation, which was determined by hormonal profile (temporal variation of the hormones, progesterone and estradiol). Since the PigWatch® system uses sow behavioural data for its analysis, a blood sampling method was developed to carry out enough samples to accurately judge the time of ovulation, while causing minimal behavioural disruption to sows. Pregnancy testing took place 35 days after breeding and the total number of piglets born per sow and per litter was recorded as an estimator of sows' performances.

The results show that 95% of AI requests made by the PigWatch® system came in the period 32 hours before, and 8 hours after ovulation. Each sow was inseminated 1.16 times on average, and the average fertility rate was 95.1% for an average of 15.26 total piglets born.

In conclusion, the PigWatch® system, which works according to an analysis of the sow's behaviour, can predict the right time to inseminate and its use also reduces the number of semen doses used per sow, while good reproductive performances.

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Glossary

Estradiol, or more precisely, 17β-estradiol, is a steroid and estrogen sex hormone, and the primary female sex hormone[1].

Fertility rate (%): Indicator of sow reproduction that estimates the proportion of sows pregnant 35 days after insemination. This indicator is usually calculated on a group of sows inseminated in a given period (ex: week, month);

$$\text{Calculation} = \frac{\text{Number of sows considered pregnant at 35 days}}{\text{Number of sows bred during the same period 35 days before}}$$

Farrowing rate (%): Indicator of sow reproduction that estimates the proportion of sows that farrowed 115 days after insemination. This indicator is usually calculated on a group of sows inseminated in a given period (ex: week, month);

$$\text{Calculation} = \frac{\text{Number of sows that farrowed 115 days after insemination}}{\text{Number of sows bred during the same period 115 days before}}$$

Follicular phase or proestrus (4 – 6 days): The follicular phase of the reproductive cycle describes the growth period of one or more follicles until ovulation (Figure 400). The follicular phase of the reproductive cycle follows the luteal phase or lactation. In non-pregnant sows, this phase begins four to six days before ovulation. In lactating sows, the follicular phase begins at weaning.

Follicle stimulating hormone (FSH) is a pituitary hormone operating in conjunction with luteinizing hormone (LH). FSH is involved in the maturation of ovarian follicles in females and sperm development in the tubules of the testes of males [2].

Gonadotropin-releasing hormone (GnRH) is produced in the cells of the hypothalamus. GnRH stimulates the synthesis and secretion of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) by the anterior pituitary gland[3].

Half-life (t1/2) is the time required for the amount of something to fall to half its initial value[4].

A hormone is any member of a class of signaling molecules produced by glands in multicellular organisms that are transported by the circulatory system to target distant organs to regulate physiology and behaviour[5].

Luteinizing hormone (LH) is produced by the anterior pituitary gland as a result of stimulation by GnRH and is one of two gonadotropic hormones concerned with the regulation of the gonads (sex glands)[6].

[1] https://en.wikipedia.org/wiki/Estradiol
[2] Adapted from: http://www.britannica.com/science/follicle-stimulating-hormone
[3] Adapted from: http://www.britannica.com/science/gonadotropin-releasing-hormone
[4] https://en.wikipedia.org/wiki/Half-life
[5] https://en.wikipedia.org/wiki/Hormone
[6] Adapted from: http://www.britannica.com/science/luteinizing-hormone

Luteal phase (13 – 15 days): The luteal phase of the reproductive cycle corresponds to the period from ovulation to the functional regression of the corpus luteum. Among the non-pregnant sows, this phase begins after ovulation and ends with the onset of the next follicular phase, i.e. approximately 16 days (21 days for a cycle - five days for the follicular phase). In pregnant/gestating sows, this phase lasts from fertilization until the time of farrowing, i.e. approximately 114 days.

Estrus period (standing estrus): The estrus period, or standing estrus of the sow is the period of acceptance of the male and shows itself by different characteristic signs, such as a red and swollen vulva, mucous discharge, loss of appetite, characteristic growl, nervousness, seeking the boar and standing rigid and characteristic erect ears when pressure is applied to the back.

Ovulation period: Duration between first and last ovulation. In sows ovulation period varies between one and three hours.

Progesterone is a steroid hormone that is secreted mainly by the corpus luteum cells of the ovaries and is involved both in the gestation and embryogenesis, and the estrus cycle of various mammal species[7].

[7] Adapted from : https://fr.wikipedia.org/wiki/Progest%C3%A9rone

Abbreviations

FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
LH	Luteinizing Hormone
HCG	Human Chorionic Gonadotropin
SIS	Sow Insemination System

1 Introduction

1.1 Background and key issue

In order to have optimal reproductive performances, sow insemination has to happen within a relatively short time in the sow's estrus period. Studies have shown that the ideal time is approximately twenty-four hours before ovulation. For the producer this ideal time is entirely theoretical because he does not know when ovulation will occur. The estrus period, is characterized by responsiveness to the boar. It usually lasts between 40 and 60 hours but it can be as short as 24 hours, or even extend up to 96 hours. In short, managers of farrowing operations find it challenging to inseminate the majority of sows at the right moment in their estrus period.

To ensure fertilization of each sow, researchers often recommend doing two inseminations per estrus. Producers adapt these recommendations and develop various procedures that often require two (see recommendations, CIPQ, n.d.) and sometimes three or four inseminations (Poilvet, 2003) per estrus period. Generally, a first insemination is performed at the first behavioral symptoms associated with estrus, followed by another insemination every following 24-hour period up to estrus cessation. This method gives good results, provided that good estrus detection has taken place. For this reason, it requires the presence of skilled employees as well as the use of multiple doses of semen.

The PigWatch® system is a technological innovation developed jointly by the Quebec company, Conception Ro-Main, and its Italian partner, LPS Electronics. The goal of this technology is to predict the best time to inseminate weaned sows, based on real-time analysis of sow behaviour in their stall. Product development has been achieved mainly through measurement of sow behaviour before and during estrus, the development of electronic modules and of mathematical algorithms to analyze behavioural data and predict the time for insemination. Before this project, the correlation between PigWatch® prediction, based on analysis of sow behaviour, and the optimal time for insemination, as determined by sow ovulation, was not known. This project's prime objective was to determine whether the PigWatch® system with the beta version of the new prediction algorithm (Sow Insemination System version 5 - Beta SIS5) issues AI requests at the right time in relation to the sow's ovulation.

1.2 Project Objectives

1.2.1 Aim

Ascertain if the PigWatch® (SIS5 Beta algorithm) system issues AI requests at the right time with respect to ovulation in sows, as determined by two physiological measurement methods: 1) determination of ovulation by ultrasound; 2) determination of the time of ovulation by analyzing the temporal variation of reproductive hormones during the estrus period.

1.2.2 Specific Objectives

- Determine time of ovulation for each sow scientifically, by blood sampling and measuring the serum concentration of the hormones, estradiol and progesterone.
- Determine the time of ovulation for each sow physiologically, using transrectal or transabdominal ultrasound techniques.
- Compare the time when PigWatch® indicates that AI needs to be done, versus ovulation identified by hormonal profile and ultrasonography.

- Determine whether the period identified in the PigWatch® AI request matches the ideal physiological stage for insemination and establish the concordance rate.
- Measure the sow's fertility rate, post-insemination (35 days).
- Calculate PigWatch® system's potential for technical and economic improvement, using different scenarios for a typical swine operation.
- Improve the accuracy of the PigWatch® tool by optimizing its algorithm, if need be.

2 Description of the PigWatch® system

PigWatch® is a computerized AI management system designed to predict the best time to inseminate recently weaned sows. The PigWatch® system consists of an infrared motion sensor installed on the top of every sow's stall, plus a data analysis module and a module to interface with the producer (see Figure 1).

Motion sensors allow constant and non-intrusive monitoring of sow behavior by assessing their real-time level of activity in their stalls. Three infrared sensors observe the sow's behaviour continuously. The monitoring unit is installed directly above each sow and each case. The display system consists of lights; it indicates the sow's exact status (in estrus or not), if it has already been inseminated and whether or not to do the AI at that precise moment.

The PigWatch® system is a computer that analyses the real-time behavioural data of each sow. Algorithm at the heart of the system performs complex calculations to determine and then indicate the exact time to do the insemination. The PigWatch® user interface provides the information as graphs detailing the progression of each sow's during estrus (see Figure 2).

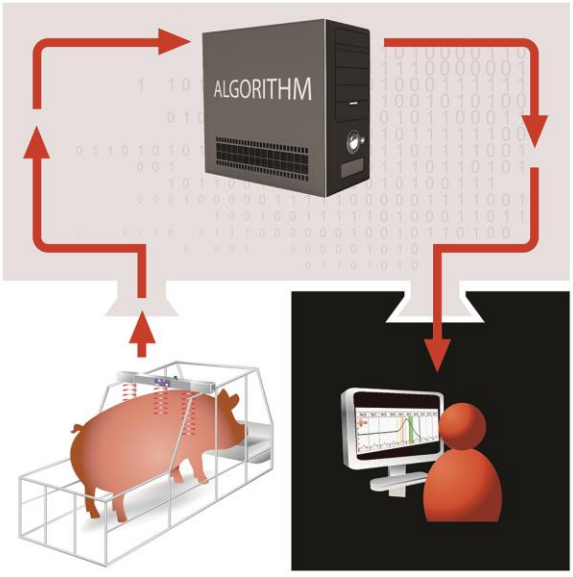

Figure 1 Illustration showing the three components of PigWatch® system: 1) the motion sensor module installed on the sow's stall 2) the analysis and data processing module 3) the module that interfaces with the producer.

Figure 2 Illustration displaying the information communicated to the producer. The green area shows the ideal time for insemination.

The PigWatch® algorithm was optimized to predict the ideal time for insemination of sows, while taking into account the presence of farm workers in the barn. The aim of the system is to predict a single best time to inseminate each sow. Even so, very few operations employ farm workers round the clock during insemination periods. For this reason, at the end of each day, just before the farm staff leave the premises, PigWatch® tries to predict which sows should be inseminated in anticipation of ovulation during the night. Next morning, the system re-evaluates the need to repeat the insemination if it judges that ovulation did not occur overnight, as expected. Consequently, some sows will have two PigWatch® AI requests.

The algorithm behind the commercial version of PigWatch® is updated regularly to reflect new information obtained from clients and certain research projects. The PigWatch® system keeps a copy of the time series of sows' behavioural indicators that the system has analyzed and detected. This operating structure helps support development and ongoing improvement of the system for predicting the optimal time for insemination of sows using PigWatch®. The developer can review problems of interpretation associated with sows that received a false prediction and can also check and compare the functioning of the old and new algorithms. Until December 2015, all commercial applications of PigWatch® used the SIS4 algorithm. During this project, the PigWatch® system installed in Ro-Main Design's experimental farm was running the beta version of the new system (SIS5 Beta algorithm). As this report goes to print (May 2016) the commercial version of PigWatch® now uses the new algorithm (SIS5).

Proponents of the PigWatch® technology recommend compliance with the AI requests from the system. Available data from users of the old version of the algorithm (SIS4) show that many sows have excellent reproductive performances with the use of a single insemination dose during estrus[8]. Producers working with the old version of PigWatch® used an average of 1.8 doses of semen[8]. According to Conception Ro-Main, the SIS4 version of PigWatch® makes it possible to obtain satisfactory gestation results using less semen than traditional AI techniques, which often require more than 2 doses.

During the process of reviewing the operation of PigWatch®, the development team also looked at other animal husbandry methods with a view to system optimization and subsequent improvement in sow herd reproductive performances. For example, experience has shown that the system performs better when farm workers are present less often in the room of newly weaned sows. The golden rule to maximize the results of the system is simple and aligned with the underlying concept of the technology and the physiology of the animal: avoid disturbing the sows as much as possible so they can behave naturally. According to the product developers, the combination of the new prediction algorithm with minimization of human interaction with recently weaned sows leads to optimal performances.

[8] Information provided by Conception Ro-Main.

3 Literature Review

3.1 Reproduction in Sows

Gilts reach puberty between 150 and 220 days of age. They are usually inseminated for the first time in their second or third estrus. The resulting gestation lasts 114 to 116 days. Following farrowing, lactation lasts between 16 and 40 days during which the sows are in a period of anestrus. Estrus usually occurs 4 to 6 days after weaning. Once weaned, sows are moved into a special service area or waiting stall where they will be serviced by artificial insemination (AI) during their estrus period (mean duration of standing estrus = 2 to 3 days). Inseminations are usually done at intervals of 12 and 24 hours after the first behavioural signs indicating standing estrus. Consequently, to ensure fertilization of each sow, producers have developed various procedures that often involve two (CIPQ, n.d.), and sometimes three or four inseminations per estrus period (Poilvet, 2003).

3.2 The sow reproductive cycle

The main features characterizing estrus in sows are now well known and have been explained thoroughly in a recent critical review (Soede et al., 2011). The main concepts discussed in the present document are heavily influenced by the information presented in that review.

Estrus period in sows lasts from 18 to 24 days and is divided into two main phases, namely: the luteal phase and the follicular phase. These two phases of the estrus period refer to the ovarian functions.

Follicular phase or proestrus (4 – 6 days)
The follicular phase of the reproductive cycle describes the growth period of one or more follicles until ovulation (Figure 4). The follicular phase of the reproductive cycle follows the luteal phase or lactation. In non-pregnant sows, this phase begins four to six days before ovulation. In lactating sows, the follicular phase begins at weaning.

Luteal phase (13 – 15 days)
The luteal phase of the reproductive cycle corresponds to the period from ovulation to the functional regression of the corpus luteum. Among the non-pregnant sows, this phase begins after ovulation and ends with the onset of the next follicular phase, i.e. approximately 16 days (21 days for a cycle - five days for the follicular phase). In gestating sows, this phase lasts from fertilization until the time of farrowing, i.e. approximately 114 days.

The reproductive cycle of sows is also characterized by boar receptivity period usually described as estrus or standing estrus. Finally, all the physiological processes of the reproductive cycle converge toward ovulation which lasts approximately two hours. Figure 3 presents the main phases of the sow reproductive cycle.

Estrus period (standing estrus)
The estrus period, or standing estrus of the sow is the period of acceptance of the male and shows itself by different characteristic signs, such as a red and swollen vulva, mucous discharge, loss of appetite, characteristic growl, nervousness, seeking the boar and standing rigid and characteristic erect ears when pressure is applied to the back.

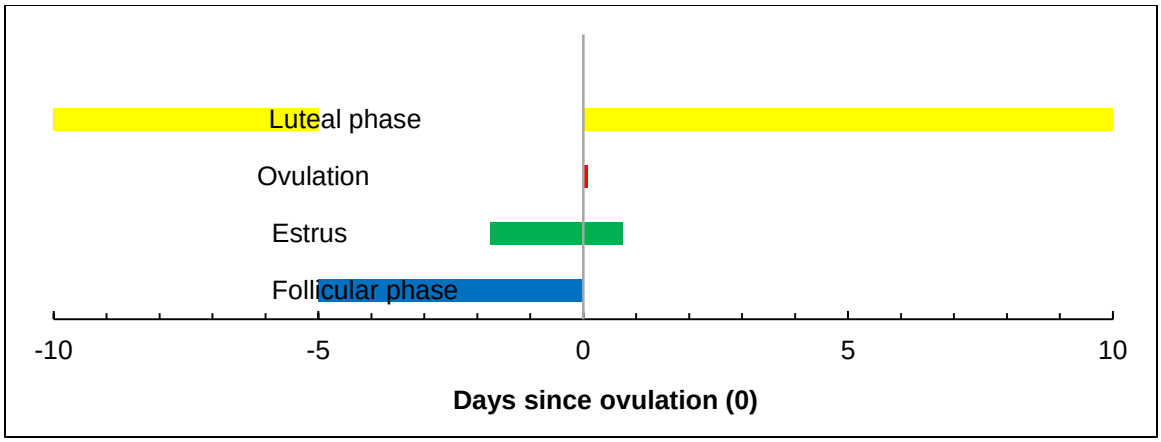

Figure 3 This figure shows the expected length (mean) of the different phases of the reproductive cycle in sows. Expected duration is 60 hours for estrus, five days for the follicular phase and two hours for ovulation. The luteal phase complements the follicular phase and lasts the remainder of the duration of the sow's 21 days estrus cycle.

3.3 Egg development and ovulation

Egg development begins at the start of the follicular phase, or approximately 5 days before the onset of estrus (Figures 3 and 4).

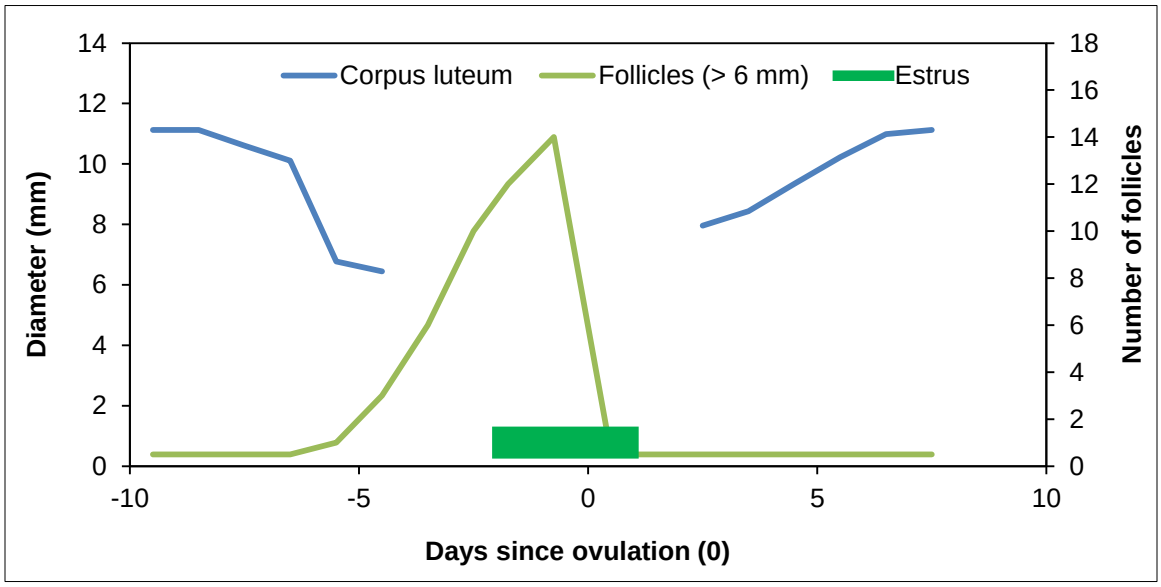

Figure 4 Diameter of the largest corpus luteum present on the ovary before and after estrus, and the number of large follicles (>6 mm) (based on the data of Noguchi et al., 2010).

At the start of this phase, a group of about 100 follicles, measuring less than 6 mm, is present in the ovaries and the largest follicles are selected to start their development (reviewed by Knox,

2005). Of the hundreds of small growing follicles, only a few (10 – 17) will continue their growth to reach ovulation as codominant follicles; the others will regress (Clark et al., 1975, Kelly et al., 1988, Guthrie and Bolt, 1990, Ryan et al. 1994 cited by Noguchi et al., 2010).

The size of most follicles forming on the ovaries is often categorized into two groups: small follicles (<6 mm) and large follicles (6 – 8 mm). Large follicles are usually described as preovulatory follicles and are easily seen using ultrasonography (diagnostic imaging). Preovulatory follicles would average 7.6 ± 0.8 mm in size (Nissen et al., 1997). The disappearance of the large follicles on the ovaries is the indicator used by researchers to identify the moment of ovulation.

Ovulation in sows that do not receive hormone therapy would last approximately two hours (1.8 ± 0.6 h (0.75 – 3.25)) (Soede et al., 1992). The duration of ovulation would be extended (4.6 ± 1.7 h (2 – 7)) in sows receiving hormonal treatments, such as injections of human chorionic gonadotropin (hCG) (Soede et al., 1992). The hCG hormone is found in products approved for commercial use in breeding sows (e.g. the drug P.G. 600[9]).

The time of ovulation usually occurs 42 hours after the start of the estrus as was shown by the studies presented in Table 1. In addition, analysis of the published data also suggests that ovulation occurs almost 72% of the way through estrus. These values are almost identical to the review of other authors, who report that ovulation takes place 70% of the way through estrus (Soede and Kemp, 1997).

Table 1 Time of ovulation from onset of estrus and the duration of estrus (%), according to various studies

Source	Population	Onset of Estrus (h)	Duration of Estrus (%)
Martinat-Botté et al., 1997	222	43 ± 13 (6 – 88)	77 ± 16
Soede et al., 1992	21		60 ± 10
Soede et al., 1994	16	38 ± 11 (16 – 52)*	72 ± 8
Soede et al., 1995	151	35 ± 8 (10 – 58)	72 ± 15 (39–133)
Nissen et al., 1997	143		71 ± 14
Almeida et al., 2000	92	43.93 ± 7 (30 – 60)	86 ± 14
Terqui et al., 2000	29	40 ± 5.8 (25 – 50)	
Bracken et al., 2003	59	33.5 ± 12	59.5 ± 17
Expected Value (h)**		**42 ± 10 (22 – 61)**	**72 (49–95) hours**
Expected Value (d)**		**1.7 ± 0.4 (0.9 – 2.6)**	

* Mean value ± standard deviation; Values in parentheses indicate the range.
** Weighted mean value for the number of sows (95% Confidence Interval; estimate x 1.96).

3.4 The formation of the corpus luteum (luteinisation)

After the rupture of the follicles at ovulation, a remodelling and restructuring of ovarian tissue transforms the preovulatory follicles into corpus luteum. The corpus luteum start growing two days after ovulation and reach their maximum diameter (8 mm – 12 mm) one week after

[9] P.G. 600 is a drug manufactured and sold by Merk Animal Healh.

ovulation (Noguchi et al., 2010, see Figure 4). Luteinisation describes the transformation process in which the follicle develops into the corpus luteum.

The number of corpus luteum present on the ovaries is directly proportional to the number of eggs released during ovulation. Observations from various studies suggest that there are 12 – 30 corpus luteum per ovulation (19.3 ± 3.3 (14 - 25) (Soede et al., 1992), 22 ± 4 (12 – 31) (Soede et al., 1995) or 14.5 ± 0.4 (Bracken et al., 2003)).

3.5 Estrus in Sows

The estrus period of the sow is the period of acceptance of the male which is characterized by various behavioural signs that producers can recognize (Signoret, 1970; Bonneville, 2002):

- sow stands rigid in the presence or absence of a boar
- vulva is reddish and swollen
- increase and change in consistency of vaginal mucus
- ears are erect, tail shakes
- increased nervous activity, excitement
- decrease in or loss of appetite, due to increased estrogen levels
- male sexual behaviour (chases, sniffs and tries to mount other females)
- change in vocalization (vocal behaviour)

The different characteristic signs of estrus result from the production of estrogen made by the growing follicles (Soede et al., 2011).

Table 2 Duration of weaning to estrus and duration of estrus in sows, according to different authors

Source	Population	Duration: weaning-to-estrus (h)	Duration of estrus (h)
Soede et al., 1994	16	118 ± 24 (90 – 162)*	54 ± 15 (24 – 76)
Martinat-Botté, et al., 1997	222		56 (24 – 103)
Soede et al., 1995	151	93 ± 18 (65 – 153)	50 ± 13 (24 – 88)
Nissen et al., 1997	143	92 ± 13 (64 – 134)	60 ± 14 (30 – 89)
Almeida et al., 2000	92		52 (30 – 72)
Expected Value (h)**		**94 (58 – 130)**	**54 hours (27 – 82)**
Expected Value (d)**		**4 (2 – 5)**	**2.3 days (1.1 – 3.4)**

* Mean value ± standard deviation; Values in parentheses indicate the range.
** Weighted mean value for the number of sows (95% Confidence Interval; estimate x 1.96).

The producer usually detects the sows that show signs of estrus. When in the presence of the male, sows in estrus will stand rigid when the producer applies pressure on the sow's lumbar area (Soede et al., 1992; Bonneville, 2002). Various authors have studied the duration of estrus (Table 2) and it is well documented. The work of the different researcher's, shows that the duration of estrus varies greatly between sows. Analysis of the published observations suggests that the average duration of estrus is 54 hours, with expected values of between 27 and

82 hours (Table 2). These values are almost identical to the review made by other authors, who report ranges from 24 to 96 hours (Soede and Kemp, 1997).

3.6 Sow insemination relative to estrus

Scientific studies of animal physiology lead us to understand that ideally, the boar semen should be inserted into the sow's uterus in the 24 hours before ovulation (Soede et al., 1995; Nissen et al. 1997; Almeida et al., 2000; Hughes and Pope, 2001).

In practice, pork producers do not know the sow's ovulation time. Their goal is to insert sperm into the uterus at the most opportune moment to ensure fertilization of the oocytes in the sperm reservoir, which is located in the ampulla-isthmic junction, a part of the Fallopian tube. The ampulla is where fertilization of the oocytes (immature egg cells) takes place.

To fully understand the critical issue of inseminating sows at the ideal time in their reproductive cycle, it is necessary to understand the stages of the life cycle of the two gametes or reproductive cells (sperm and eggs) in the sow's reproductive system. The pathway of the oocytes (immature egg cells) and sperm is well-known and is described by various authors (Hughes and Pope 2001; Bonnes and Batellier, 2005; CFPPA, 2013). The path of the oocytes is simpler than that of sperm. The eggs are fertile immediately after ovulation. The survival of an unfertilized ovum is 12 hours at the most (Bonnes and Batellier, 2005).The sperm are not fertile when they are deposited into the vagina or uterus. They must go through a process of capacitation that takes approximately 6 hours. Sperm survival period in the sow's genital tract would be approximately 24 to 36 hours (Bonnes and Batellier, 2005).

To maximize the chances of viable sperm in the ampulla-isthmic junction at the time of ovulation, experts in reproduction and artificial insemination often recommend a process that involves two or three breeding's per estrus. For example, the recommendations of Hughes and Pope (2001) are:

1- Check twice a day if the sows in the service area or waiting stall are in estrus.
2- Inseminate all sows at least twice. Adjust both inseminations according to the sow's age and the interval from weaning to estrus. The typical sow that comes into estrus 3 to 4 days after weaning, will receive its first insemination the next day and a repeat insemination 24 hours later. Gilts and sows with longer weaning-to-estrus intervals (5 to 7days) will receive double inseminations with shorter intervals between each intervention.

The different companies specializing in swine production and artificial insemination (AI) adapt these general recommendations. Most companies will recommend two inseminations, in line with researchers' recommendations (e.g. CIPQ, n.d.), while others will recommend up to four AI inseminations per estrus period (Poilvet, 2003).

3.7 Sow insemination relative to ovulation

Researchers are not able to calculate the ideal time for insemination, but they are able to estimate an ideal time interval that can yield optimum reproductive performances.

To pinpoint the exact time of ovulation, researchers use serial transrectal or transabdominal ultrasonography (diagnostic imaging) with very short time intervals (4 – 6 hours) between each assessment (Soede et al., 1995; Nissen et al., 1997; Almeida et al., 2000). The purpose of serial ultrasounds is to identify the exact time of ovulation, which lasts less than two hours in

most sows. Ultrasound screening usually starts at the beginning of the estrus period and ends at ovulation. Consequently, sows taking part in such a project have from seven to ten transrectal ultrasounds between the onset of estrus and ovulation (42 hours on average). In this kind of project, each sow receives a single insemination dose and timing for insemination is randomly selected during the estrus. This experimental procedure makes it possible to have sow's that will have been inseminated at various times relative to ovulation (typically 36 hours before and 12 hours after).

Table 3 Optimal time interval for sow insemination relative to ovulation

Source	Population	Interval (0h = ovulation)
Nissen et al., 1997	143 sows	-28h – 4h
Soede et al., 1995	151 sows	-24h – 0h
Almeida et al., 2000	92 gilts	-24h – 0h
Expected and Retained Values*		**-28h – 4h**

* For this project, the authors concluded that the optimum interval to inseminate sows lies within the range -28 hours to +4 hours in relation to the time of ovulation (0 hours).

The information presented in Table 3 summarizes the findings of three studies that assessed the success of a single insemination performed at different times before and after ovulation. Research by Nissen et al. (1997) shows that sows inseminated in the time interval starting 28 hours before ovulation and ending 4 hours after, will have good reproductive performances (farrowing rate and number of piglets at farrowing time). These results are in agreement with the work of the two other authors that the best time to inseminate the sows lies in the 24-hour period just before ovulation (Soede et al., 1995; Almeida et al., 2000).

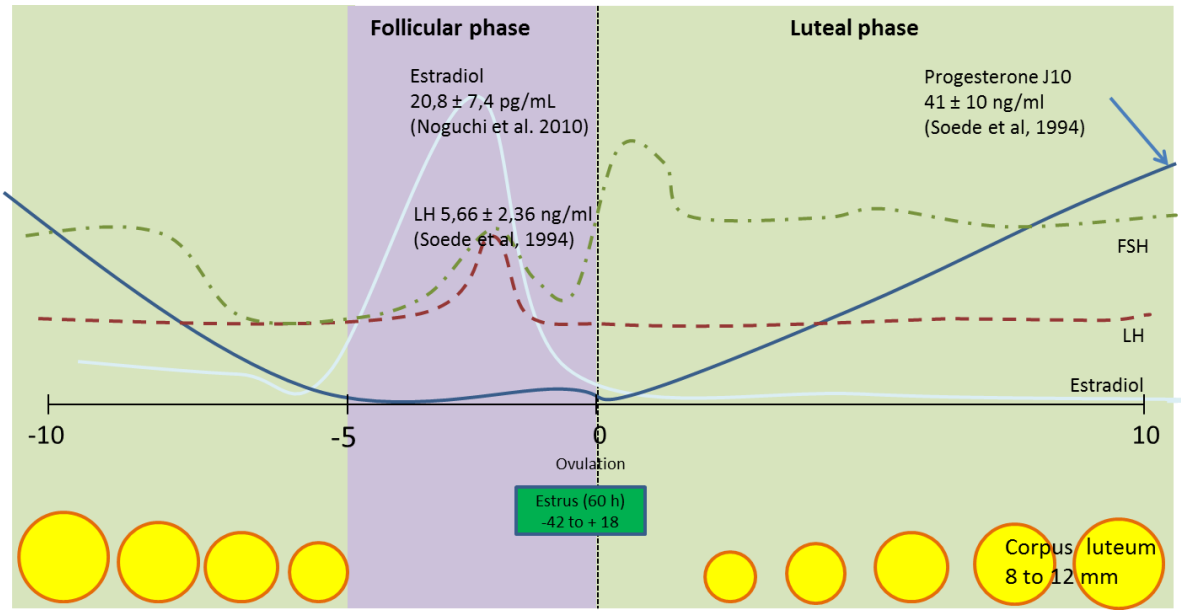

Figure 5 Diagram showing the temporal variation of the different hormones, the estrus and the corpus luteum.

3.8 Hormonal profiles during the different phases of the estrus cycle

The changes in behaviour (estrus) and tissue (ovaries, ova, corpus luteum), described earlier, are all controlled by various hormones (Figure 5). As part of this literature review, the authors will discuss the role and influence of the following hormones: gonadotropin releasing hormone (GnRH); luteinizing hormone (LH); follicle stimulating hormone (FSH); estradiol and progesterone.

The hormones described in this paper are synthesized in the different tissues and form part of a system of several positive and negative feedback loops (Figure 6). A positive feedback loop causes stimulation of the cells of the target organ and brings about increased hormone production. A negative feedback loop causes inhibition of the tissue cells of the target organ and brings about a reduction in hormone production.

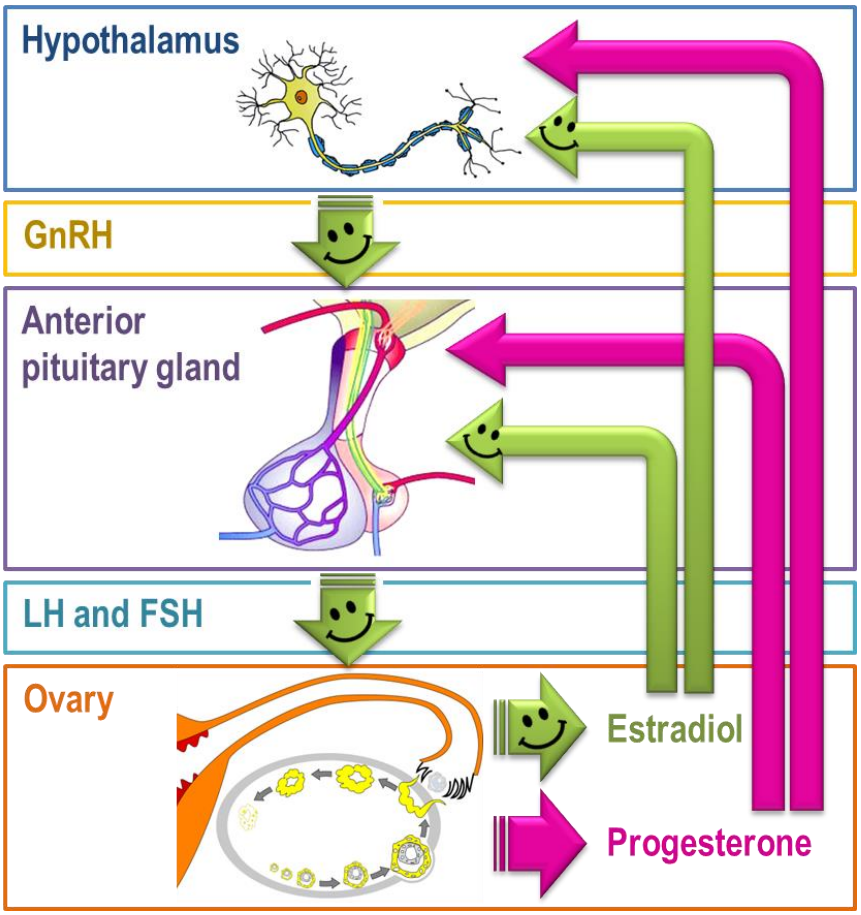

Figure 6 Positive (green) and negative (red) feedback loop of different hormones on the cells of target organs.

3.9 Gonadotropin releasing hormone (GnRH)

Gonadotropin-releasing hormone (GnRH) is produced in the cells of the hypothalamus. GnRH stimulates the synthesis and secretion of the luteinizing hormone (LH) and the follicle-

stimulating hormone (FSH) by the anterior pituitary gland[10]. The estradiol produced by the ovarian cells increases the production of GnRH by the hypothalamus. Conversely, the production of progesterone by the cells of ovarian corpus luteum reduces the synthesis of GnRH by the hypothalamus cells.

3.10 Luteinizing hormone (LH)

Luteinizing hormone (LH) is produced by the anterior pituitary gland as a result of stimulation by GnRH and is one of two gonadotropic hormones concerned with the regulation of the gonads (sex glands)[11]. LH receives positive feedback from 17-beta estradiol and negative feedback from progesterone. LH mediates recruitment of the preovulatory follicles and triggers ovulation (Soede et al., 2011). The Figures 5 and 7, and Table 4 show the temporal variation in serum concentration of LH.

Table 4 Results of certain studies on the positioning of the peak of LH versus the onset of estrus and ovulation

Source	Population	Peak serum LH (ng/ml)	After onset of estrus (h)	Before ovulation (h)
Martinat-Botté et al., 1997	222 sows		4 ± 12 (-23 – 32)	(24 – 64)
Soede et al., 1994	16 multiparous sows	5.66 ± 2.36* (2.49 – 11.18)*	8 ± 11 (-10 – 22)	30 ± 3 (26 – 34)
Terqui et al., 2000	29 gilts	17 ± 3.3	9 ± 0.5	28
Expected and Retained Values **			**8 – 9 ± 3**	**30 ± 3**

* Mean Value ± standard deviation; Values in parentheses indicate the range.
** For this project, the authors concluded that: 1) the interval between the onset of estrus and the pre-ovulatory LH peak is 9 hours; 2) the interval between the pre-ovulatory LH peak and ovulation is approximately 30 hours.

Basal serum LH during the luteal phase was 0.98 ± 0.35 (0.5 – 1.73) ng/ml (Soede et al., 1994). Early in the follicular phase, a slight increase in basal LH was recorded, followed by a peak that occurred 8 to 9 hours after the onset of estrus, and approximately 30 hours (24 – 48) before ovulation (Soede et al., 1994; Martinat-Botté et al., 1997; Terqui et al., 2000; Noguchi et al., 2010). The LH surge is a hormonal feedback loop that triggers ovulation (Noguchi et al., 2010).

3.11 Follicle stimulating hormone (FSH)

The follicle stimulating hormone (FSH) is a pituitary hormone operating in conjunction with luteinizing hormone (LH). FSH is involved in the maturation of ovarian follicles in females and sperm development in the tubules of the testes of males[12]. FSH is produced by the pituitary as a result of stimulation by GnRH and is subjected to negative feedback control by inhibin.

[10] Adapted from: http://www.britannica.com/science/gonadotropin-releasing-hormone
[11] Adapted from: http://www.britannica.com/science/luteinizing-hormone
[12] Adapted from: http://www.britannica.com/science/follicle-stimulating-hormone

Concentration of FSH decreases from 6 to 3 days before ovulation and is at its maximum concentration 2 days after ovulation (Noguchi et al., 2010). Figures 5 and 7 show the temporal variation of the serum concentration of FSH.

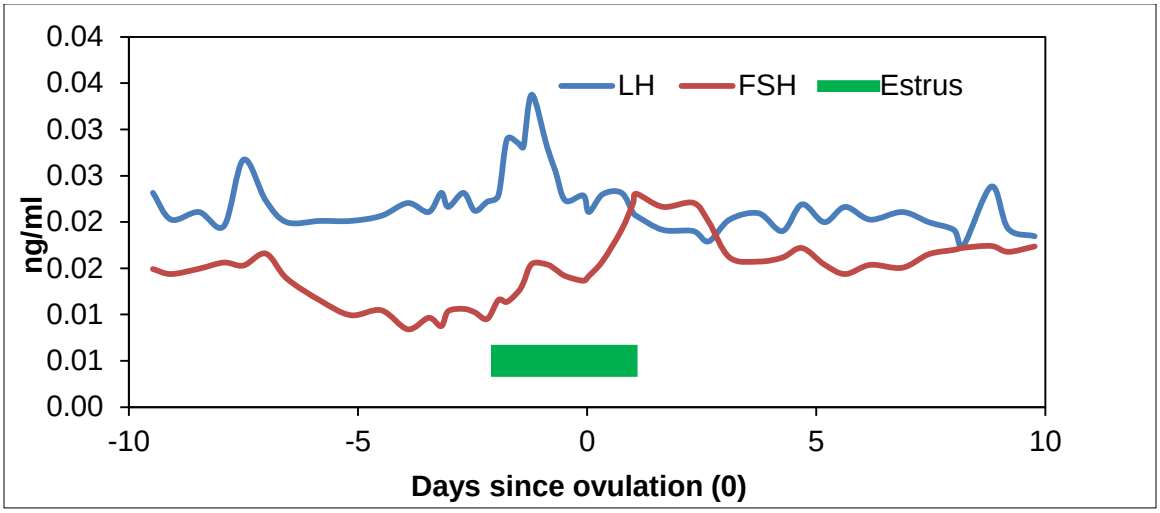

Figure 7 Serum levels of FSH and LH during the sow's hormonal cycle (based on data from Noguchi et al., 2010).

3.12 Estradiol, an estrogen family hormone

Estrogens are primarily produced by the ovaries. There are three forms of natural estrogens: 17-beta estradiol, estrone and estriol. The most common is 17-beta estradiol (Gayrard, 2007). Estradiol is responsible for triggering the symptoms of estrus. The Figures 5 and 8, and Table 5 show the temporal variation of the serum concentration of estradiol.

Estradiol concentration is very low from 10 to 7 days before ovulation and then increases as the follicles grow, to peak approximately 3 hours before onset of estrus, and approximately 40 hours (35 – 48) before ovulation (Soede et al., 1994; Noguchi et al., 2010).

Table 5 Results of certain studies on the positioning of the estradiol peak versus the onset of estrus and ovulation.

Source	Population	Peak serum estradiol (pg/ml)	Before onset of estrus (h)	Before ovulation (h)
Soede et al., 1994	16 multiparous sows	27 ± 17* (4 – 70)*	3 ± 11 (14 – 26)	41 ± 4 (34 – 48)
Noguchi et al., 2010	11 cycles (8 sows)	20.8 ± 7.4		48
Expected and Retained Values**			**3 ± 11**	**40 ± 4**

* Mean Value ± standard deviation; Values in parentheses indicate the range.
** For this project, the authors concluded that: 1) the interval between the onset of estrus and peak estradiol is 3 hours, and 2) the interval between peak estradiol and ovulation is approximately 40 hours.

3.13 Progesterone

Progesterone is a steroid hormone that is secreted mainly by the corpus luteum cells of the ovaries and is involved both in the gestation and embryogenesis, and the estrous cycle of various mammalian species[13]. Figures 5 and 8, and Table 6 show the temporal variation of the serum progesterone concentration.

An elevated serum progesterone concentration is regarded as one of the best indicators of ovulation in mammals because this hormone is synthesized by the corpus luteum, a tissue that develops in the ovarian follicles after ovulation. The sow data measured and presented by several authors show that serum progesterone increases immediately following ovulation (Soede et al., 1994; Terqui et al., 2000; Noguchi et al., 2010).

Before the advent of ultrasonography, an increase in serum progesterone was the primary means of gauging the time of ovulation. For example, Terqui et al. (2000) identified time of ovulation as an increase of one standard deviation compared to basal progesterone (0.3 ng/ml).The correlation coefficient of the regression with the time of ovulation identified by ultrasound was r^2 = 0.98. In fact, the interval between ovulation detected by ultrasound and a 0.1 ng/ml increase in progesterone is 3 ± 6 h (1–18 h). In addition, for most sows (17/26) the increase in progesterone was reported during the hour just prior to ovulation as estimated by ultrasound. In another study, Martinat-Botté et al. (1997) also identified ovulation as the first peak level of progesterone (mean basal level + 1 standard deviation).

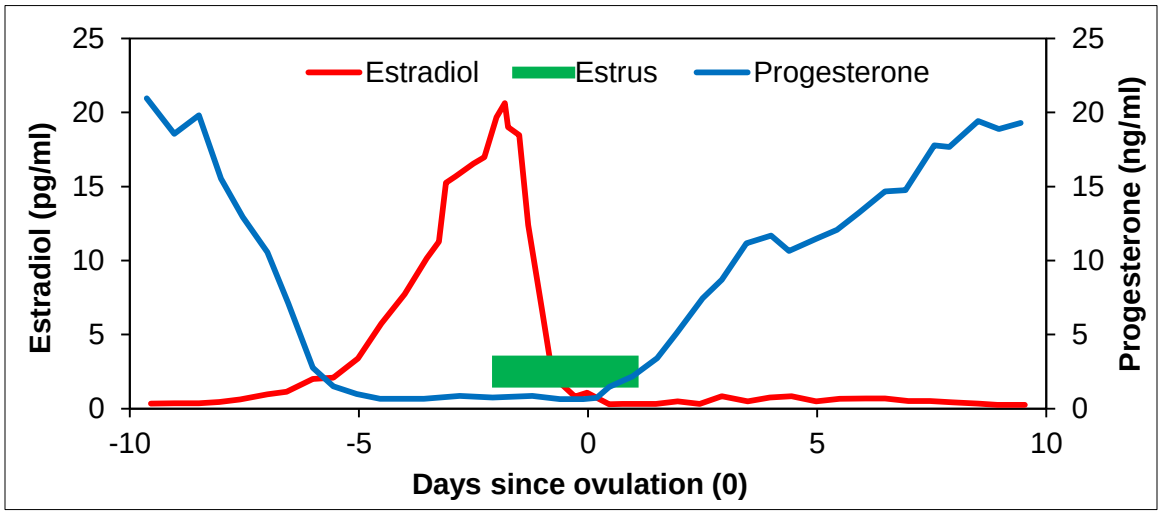

Figure 8 Estradiol and progesterone hormonal profiles throughout the sow hormonal cycle (based on data from Noguchi et al., 2010).

[13] Adapted from : https://fr.wikipedia.org/wiki/Progest%C3%A9rone

Table 6 Characteristics of the increase in progesterone versus onset of estrus, and time of ovulation.

Increase in serum progesterone (ng/ml). 14 multiparous sows (Soede et al., 1994)	**After onset of estrus (h)**	**After ovulation (h)**
Increase of > 0.1 ng/ml versus baseline concentration	40 ± 12* (18 – 58)*	3 ± 7 (-20 – 10)
Increase of > 1.0 ng/ml versus baseline concentration	50 ± 11 (27 – 67)	13 ± 4 (6 – 19)
Expected Values to observe an increase of 1 ng/ml in 95% of sows**	**50 ± 11 (28 – 72)**	13 ± 4 (5 – 21)

* Mean Value ± standard deviation; Values in parentheses indicate the range.
** As part of this project, the authors retained these values as a reference.

3.14 The hormones during the follicular phase (5 days before ovulation)

During the follicular phase, progesterone is at its lowest level (often close to the detection level of 1 ng/ml) in most sows. Development of the largest follicles takes place during this phase (Figure 4). Follicular development increases serum 17-beta estradiol (Figure 8). Peak serum estrogen brings about the onset of estrus (Table 5). Peak estrogen (≈ 40 hours before ovulation) also brings about peak LH (≈ 30 hours before ovulation). This LH surge initiates the luteinization process that starts by stopping the synthesis of estradiol, continues with ovulation and ends with transformation of the follicle into the corpus luteum.

During the follicular phase, pulsatile GnRH released by the hypothalamus, sets in motion the release of LH or FSH by the pituitary gland. FSH increases the number of follicles and enables them to grow to recruitment size (Figure 6).

Recruitment is done by the pulsatile GnRH/LH. The LH is essential for the follicles to develop into pre -ovulatory follicles. LH stimulates the development of the follicles large enough to have LH receptors. The 17-beta estradiol, secreted by the large follicles, has a negative influence on the hypothalamus, which causes a reduction in GnRH and consequently a decrease in LH and FSH (Figure 6). The smaller follicles, not having enough LH receptors and with too much FSH, regress.

3.15 The hormones during the luteal phase and the final stage of lactation

By definition, the luteal phase begins at ovulation. At the start of the luteal phase (hour 0), progesterone concentration is minimal but increases rapidly and exponentially (Figure 8). Progesterone will reach maximum level by day 10 of the estrus cycle. In pregnant sows, serum progesterone remains high until the end of gestation (114 days). In non-pregnant sows, there will be a decrease in serum progesterone that commences ten days before the next ovulation (Figure 8).

In part two of the luteal phase (day-10 to day-5), the number of small follicles increases in preparation for the follicular phase. Similarly, in the final stage of lactation, most sows have waves of follicular development when the follicles reach 4 – 5 mm and then regress. After lactation, production of pulsatile LH starts again and enables the follicles to increase in size in anticipation of ovulation.

3.16 The effect of stress on the sex hormones

As part of this project, the authors had to handle animals to carry out blood tests and ultrasonography. To take blood samples, the plan was to install a permanent catheter in the sow's ear. However, where sample taking via catheter proved impossible, researchers caught the sow by the snout and took the sample right in the jugular; a procedure that is more distressing for the animal.

Prolonged or chronic stress can suppress secretion of GnRH (Tilbrook et al., 2000) which may influence production of the hormones associated with reproduction (see Figure 6). The effect of a short-term stressor on reproduction is not that obvious. In fact, repeated application of electric shocks to the gilts increased cortisol levels, but did not affect reproductive factors (Turner et al., 1998, cited by Tilbrook et al., 2000).

3.17 Measurement of serum hormone levels as indicators of biological functions

By definition, a hormone is a biologically active chemical substance secreted by an endocrine gland, acting at a distance and by way of the bloodstream on specific receptors of a target cell.

Given this way of functioning, determination of the serum concentration of the different hormones appears the right method for gauging hormonal activity. However, since blood sampling is time-specific and the secretion of many hormones is pulsatile, the reality is not so simple. In addition, several hormones have a very short half-life (minutes). Therefore, measurement of serum hormone levels is a good indicator of actual activity only for those hormones that are secreted on a continuous basis and have relatively long half-lives (several hours).

The information summarized in Table 6 illustrates the diversity of the secretion mechanisms and the metabolism (half-life) of the different hormones involved in reproductive function modulation. In light of this information, the scientific community recognises the impossibility of using serum measurement to gauge GnRH activity. Alternatively, measurement of estradiol, progesterone and serum FSH is likely a good indicator of the function of these hormones. Finally, evaluation of serum LH as an indicator of the tissue function of this hormone is more complex because the looked-for production peak appears in the serum for a brief period of time (≈ 8 hours (Figure 7). This short duration peak can partially be explained by the short half-life of this hormone (Table 7).

Table 7 Secretion type and half-life of the main hormones involved in modification of reproductive functions

Hormone	Secretion Type	Half-life	Reference (half-life)
GnRH	Pulsatile	2 – 4 minutes	Ehlers and Halvorson, 2013
LH	Pulsatile	30 minutes	Esbenshade et al., 1986
FSH	Pulsatile	5 days	Esbenshade et al., 1986
Estradiol	Pulsatile	15 hours	Wikipedia[14]
Progesterone	Continuous	17 hours	Wikipedia[15]

[14] Adapted from : https://en.wikipedia.org/wiki/Estradiol
[15] Adapted from : https://en.wikipedia.org/wiki/Progesterone

3.18 Characteristic values of the typical sow retained by the authors

Displayed in Table 8 is the timing of the various stages of the reproductive cycle of a typical sow.

Table 8 Characteristic values of the typical sow, as retained by the authors. There is some variability between sows for each target parameter.

Cycle stages	Estrus (h 0)	Ovulation (h 0)
1- Peak estradiol	-3	-43
2- Onset of estrus	0	-40
3- Peak LH	9	-31
4- Start of ideal time for insemination*	12	-28
5- Start of sperm fertility (stage 4)**	18	-22
6- Ovulation**	40	0
7- End of ideal time for insemination	44	4
8- Apoptosis (degeneration) of unfertilized ova (stage 4)	48	8
9- Start of sperm fertility (stage 7)	50	10
10- Apoptosis (degeneration) of unfertilized ova	52	12
11- Increase in progesterone level (1 ng/ml)	53	13
12- End of estrus***	57	17

* Start and end of ideal time for insemination of sows was established as situated between - 28 hours and 4 hours.
** Sperm must pass through a capacitation (maturation) stage before they become fertile.
*** Ovulation in the typical sow occurs 70% of the way through estrus.

4 Adjustments to the methodology

4.1 Technical adaptations

The aim of this project was to determine whether the PigWatch® (SIS5 Beta) system issues insemination requests at the right time in relation to sow ovulation. In the original project, the work team intended to assess the time of ovulation using two techniques: 1) transrectal or transabdominal ultrasonography, and 2) analysis of the temporal variation of reproductive hormones during estrus.

Ultrasonography was eliminated from the protocol because it necessitated too much human presence in the weaned sow room. This would have altered sow behaviour and, in so doing, changed the functioning of the PigWatch® system.

4.2 Best timing for sows insemination

As the literature demonstrates, sow ovulation is an event that takes place over a short period (approximately 2 hours). To achieve a high fertility rate, sow insemination must be done within an optimal time period relative to the time of ovulation. As part of this project, the authors decided that the optimal interval to inseminate sows lies in the range of -28 hours to + four hours relative to time of ovulation (0 hours). This optimum range, was inferred from information about the survival of the gametes (eggs and sperm) in the sow's genital tract and from certain experiments by other researchers (see Table 8).

4.3 Hormone selection

As part of this project, it was necessary to determine the time of ovulation by analyzing the temporal variation of the reproductive hormones during estrus. The literature review recommended three candidate hormones:

1- **Luteinizing hormone (LH):** Serum concentration of LH reaches a pre-ovulatory peak approximately 30 hours before ovulation. This peak is usually of short duration (less than eight hours). In theory, detection of this peak would predict the time of ovulation. However, this approach also includes two uncertainty factors: 1) the observations of other researchers show that the time between peak LH and ovulation varies greatly among sows (24 to 64 hours, see Table 4), and 2) peak LH can easily go unnoticed with infrequent serum evaluations (more than 8 hours between samples) because this peak appears in serum over a short time period (less than 8 hours).
2- **Estradiol:** Serum concentration of this hormone peaks approximately 40 hours before ovulation and reaches its minimum level at ovulation (Figure 8). As is the case for LH, observations by the other researchers show that the time between peak estrogen and ovulation varies greatly between sows (34 to 48 hours, see Table 5).
3- **Progesterone:** Elevated serum concentrations of progesterone are the result of luteinization of the follicles that contain ova. It is a known fact that luteinization starts at the peak of pre-ovulatory LH, continues with ovulation and finishes with the rupture of the follicles and subsequent release of ova from the corpus luteum. During the 5 – 7 days before ovulation, serum progesterone is at its lowest level (less than 1 ng/ml) for most sows (see Figure 8, and Tables 6 and 8). Progesterone increases rapidly after ovulation (increase of 1 ng/ml 13 ± 4 hours after ovulation).

Finally, as part of this project, the authors retained the measurement of estradiol and progesterone. To prevent interference with the prediction algorithms, blood sampling to assess the serum concentration of both hormones was done after PigWatch® launched the insemination request.

5 Materials and method

5.1 Animal selection and management

This study was conducted from September 2014 to March 2015. The project was carried out in a commercial breeding-gestation-farrowing facility, with a holding capacity of 1,600 sows (Genetiporc, Landrace x Large White), on the farm of A.G & R. Labrecque inc. in St-Bernard-de-Beauce. This facility operates in batches every four weeks and weans piglets over three days (Wednesday, Thursday and Friday). Sow breeding takes place in two adjacent rooms with 208 and 120 places respectively.

For each batch involved in the project, 39 of the 90 sows weaned on Wednesday morning were randomly selected and routed into the gestation room assigned to the project. The original protocol called for the monitoring of all the sows in three consecutive weaning's to meet a target of approximately 120 sows. The number of sows monitored per batch was revised downward (20 –30) and the number of monitored batches increased from three to five. This methodological adjustment was required to minimize worker presence in the room assigned to the project.

The duration of all human intervention (feeding, blood sampling and inseminations) had to be limited to two hours maximum. Sows were fed twice daily on a fixed schedule (5:30 am and 2 pm). Blood sample collection began immediately after feed distribution and sow insemination was done during this same period.

The total number of piglets born and sow fertility at 35 days served as indicators of reproductive performance. Sow fertility was assessed by transabdominal ultrasonography (diagnostic imaging) between 35 and 40 days post-insemination.

5.2 Use of the Pigwatch® System

Farm installations and the work methods of the research team were adapted to comply with the recommendations of the designer of PigWatch®.

First, a room was set up just for this project and 40 PigWatch® units were installed. Throughout the entire project, farm workers minimized their daily activities in the room in order to reduce interference with the sows.

During this project, the PigWatch® system installed in Conception Ro-Main's experimental farm operated using the beta version of a new insemination prediction algorithm (SIS5 Beta).

5.3 Estrus detection and sow insemination

To stimulate the sows and detect those that were in estrus immediately after weaning, heat detection was performed manually in the mornings of Days 0 (weaning), 1 and 2. The PigWatch® system has not collected sufficient data in the first two days post-weaning to

correctly detect this type of estrus. Sows in estrus during those days were inseminated using the traditional method (one daily insemination (AI) over a two or three day period).

From Day 3 onward, the PigWatch® system carried out detection of the best time for insemination. As recommended by the system designers, sows were inseminated once only. All inseminations were Post Cervical AI with 2 billion sperm per dose.

5.4 Measurements

5.4.1 The hormones in the study, number of samples and sampling frequency

Blood samples were taken from all eligible sows selected for the project (20 to 30 sows per batch) with the aim of establishing time of ovulation for each sow by analysis of the serum hormones, progesterone and estradiol.

The study authors hypothesized a priori, that the PigWatch® system issues a request for insemination just before ovulation and that these requests are based on analysis of sow behaviour. Therefore, by sampling blood at the first system insemination request, it would be possible to confirm the decrease in serum estradiol and detect the time of increased serum progesterone, the indicator retained to identify time of ovulation. In order to obtain sufficient data to estimate the time of ovulation from the serum levels of both estradiol and progesterone, researchers planned to take a minimum of five blood samples per sow over a 48-hour period, at a rate of two blood samples per day.

After collection, blood samples were kept on ice and centrifuged within an hour. The serum was frozen and shipped for analysis to the Endocrine Research Laboratory of Western College of Veterinary Medicine (WCVM) at the University of Saskatchewan. Serum progesterone and estradiol were measured by radioimmunoassay. Quantitative analysis of serum progesterone was carried out using the Progesterone Coated Tube RIA Kit, sold by MP Biomedicals[16]. Quantification of serum estrogen was conducted using a method developed by the laboratory staff and described in two different publications (Joseph et al., 1992; Kingsbury and Rawlings, 1993). The detection threshold of the hormones in the serum was 0.1 ng/ml and 0.1 pg/ml for progesterone and estradiol respectively.

5.5 Blood samples

Blood samples were taken by inserting a catheter into the ear vein. The technique proved effective for most sows. In some sows, the series of blood samples was completed by taking blood in the jugular vein.

5.6 Assessment of time of ovulation

Probable time of ovulation was determined by statistical analysis of serial measurements of serum progesterone. Detection of time of ovulation by analyzing the serial measurements of serum progesterone is based on the following considerations:

1. After ovulation, there is an exponential increase in serum progesterone.

[16] See: http://www.mpbio.com/product.php?pid=07270102&country=38

2. Elevated serum progesterone results from the luteinisation of the follicles containing ova.
3. During the follicular phase, which starts about 5 days before ovulation and ends on the day of ovulation, the serum progesterone level for most sows is very low and often close to the detection limit (0.1 ng/ml) (Soede et al., 1995).
4. For reasons not yet established, the base-line level of serum progesterone in a few sows remains higher during the follicular phase (e.g. Soede et al., 1994).

It is generally conceded that the inflexion point of the exponential increase in serum progesterone is a good indicator of the time of ovulation. This concept has even been validated with ultrasonography (e.g. Martinat-Botté et al., 1997; Terqui et al., 2000).To measure the position of the inflexion point, one or more assessments of serum progesterone before and after the time of ovulation, need to be obtained. Pre-ovulatory assessments are needed to calculate the base-line level of progesterone.

The first challenge was to identify the first data point of the serial measurements, which was part of the luteal phase, i.e. post-ovulatory. Another way to present the same problem is to identify the last data point that is part of the follicular phase. Statistical analysis was used to identify for each sow, the number of progesterone measurement data points that were part of the follicular period. The first data point that differed statistically from the preceding points was identified as the first point of the luteal phase.

The second challenge was to identify the most probable time of ovulation in a time period before the first data point of the luteal phase. Time of ovulation was defined as the mid-point of a time period with a 95% chance of containing the true period of ovulation. Assessment of probable time of ovulation was performed in four steps:

1. Determination of probable ovulation period with a first method (A).
2. Determination of probable ovulation period with a second method (B1/B2).
3. Determination of the probable ovulation period by merging information from both the previous methods (A and B), using Bayesian inference (method C).
4. The mid-point of the interval, assessed with the most precise methodology (A, B or C), served as an indicator of ovulation.

5.6.1 Assessment of probable ovulation period (Method A)

The first method for estimating the probable ovulation period is based on the results of the study by Soede et al. (1994) who observed that in 95% of cases, the time of ovulation lay in a period from 5 to 21 hours before the moment where the serum progesterone increased by 1 ng relative to the base-line level. In the present case, researchers determine which period is defined by two consecutive measurements containing the desired value outcome (i.e., base-line level + 1 ng). Next, they interpolate the progesterone level over the period, using a straight line connecting the progesterone levels in both measurements, and calculate the moment of reaching the required value from the right (point P at Figure 9). By moving backward 13 hours from this moment, the mid-point of the probable ovulation period according to the first method is found. Since the duration of the 95% confidence interval for this method is 16 hours, the beginning and end of the time period are obtained by subtracting and adding, 8 hours from, and 8 hours to, the mid-point of the time period, respectively (Figure 9).

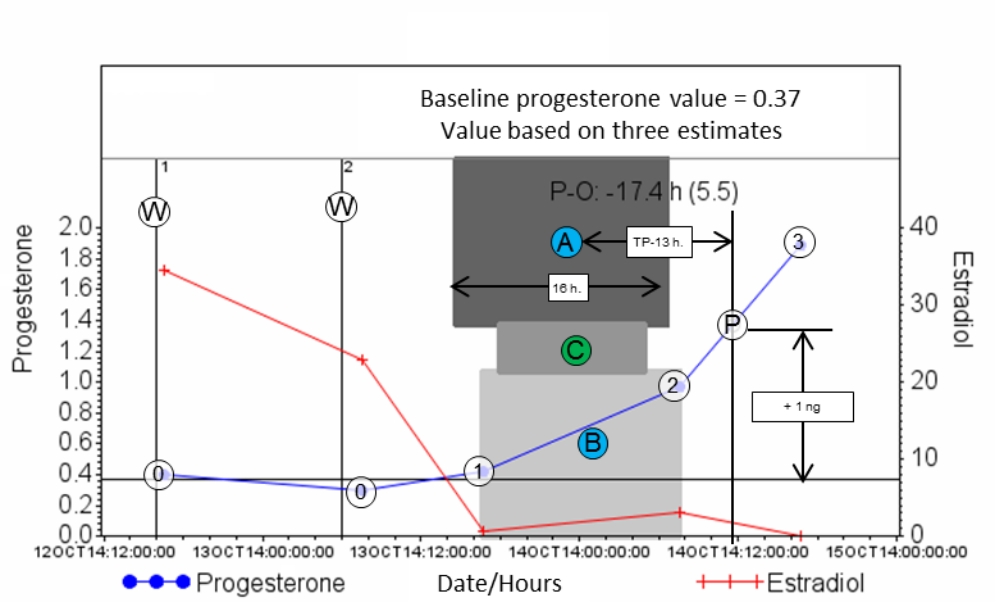

Figure 9 Steps to obtain probable period of ovulation using Method A. For methodological details, see text.

5.6.2 Assessment of probable ovulation period (method B1)

The second method is to place the probable time of ovulation between the first measurement of the luteal phase (data point # 2) and the last measurement of the follicular phase (data point # 1) (see Figure 10).

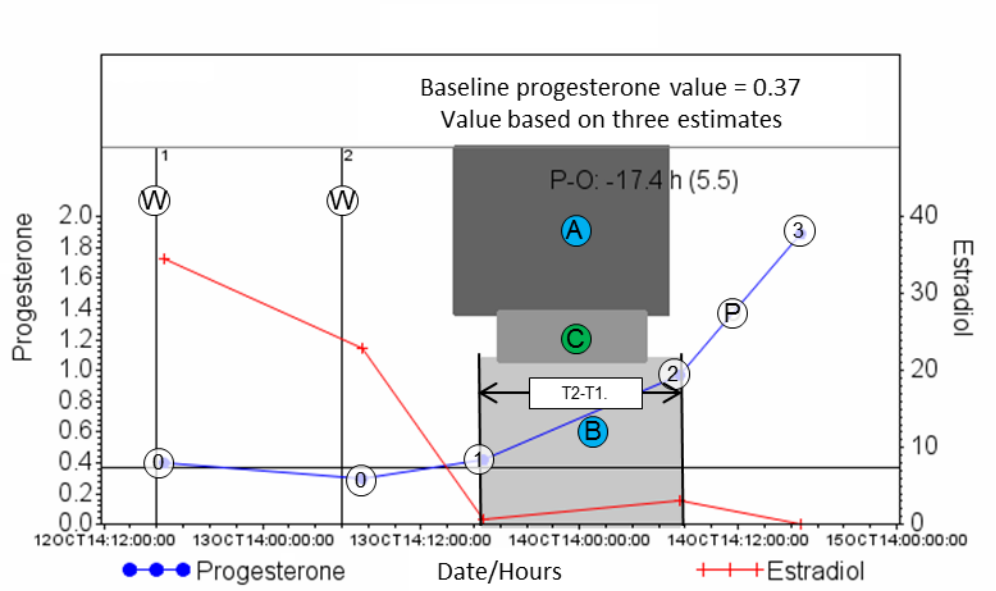

Figure 10 Steps to obtain probable period of ovulation using Method B1. For methodological details, see text.

5.6.3 Assessment of probable ovulation period (method B2)

The third method requires a minimum of three data points in the luteal phase. The projection of the regression line which connects these points on the reference line of the baseline measurement can estimate the position of the upper limit of the period of probable ovulation (see Figure 11).

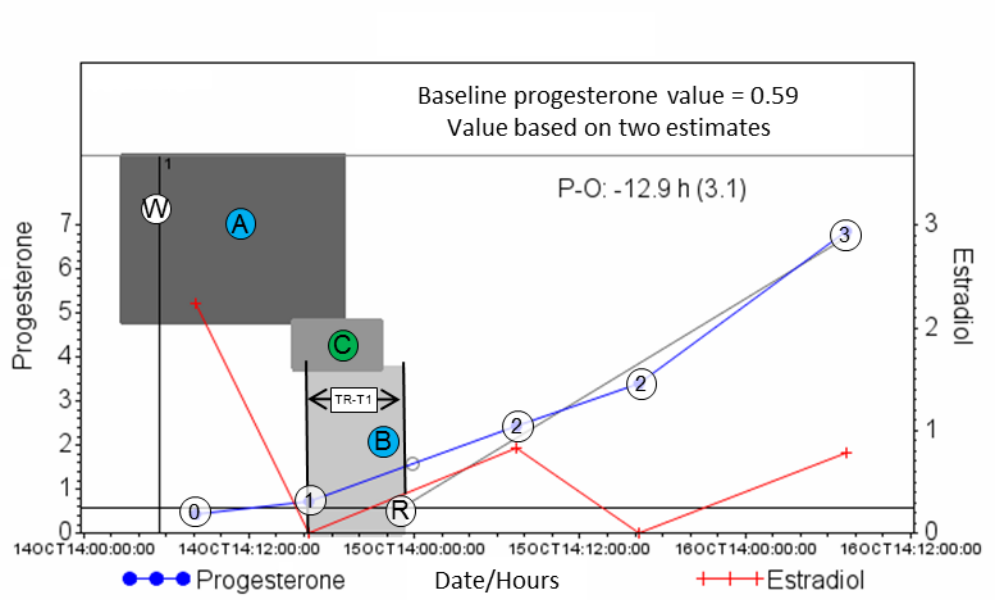

Figure 11 Steps to obtain probable period of ovulation using the method B2. For methodological details, see text.

5.6.4 Assessment of probable period and time of ovulation (Method C)

According to this method, information (mean and standard deviations) from the first two methods is combined according to Bayesian concepts. This methodology assumes that the two intervals defined above, and the third interval resulting from the combination the first two, are associated with an estimate with a normal distribution and that they are independent.

For both methods (A and B) the mid-point of the time period serves as estimate of the average value and the estimate variance is calculated as:

$$\text{Variance estimate 1} = \sqrt{\frac{Half\ of\ the\ interval}{1.96}}$$

The intervals obtained by the two methods above, are combined to produce a final time period using the following relationships:

Whereas w1 and w2, are defined as follows:
w1 = indicator variable 1/(indicator variable 1 + indicator variable 2)
w2 = (1-w1)

Then:
Composite mean indicator = w2 mean indicator variable1 + w1 mean indicator variable 2
Composite indicator variable = $w2^2$ indicator variable1 + $w1^2$ variance indicator 2

The limits of the probable ovulation period for indicator 3 are obtained as follows:

- Lower Limit = Mean composite indicator -1.96 $\sqrt{\text{Variance composite indicator}}$
- Upper Limit = Mean composite indicator +1.96 $\sqrt{\text{Variance composite indicator}}$

The mid-point of the probable ovulation period is used as estimate of the time of ovulation (point C of Figures 9, 10 and 11).

5.7 Reproductive performances, Pigwatch® request and ovulation

The length of time between the Pigwatch® request and the presumed time of ovulation (period P - O) was divided into 4-hour and 8-hour categories.

The number of piglet born and sow fertility at 35 days served as indicators of reproductive performance. The relationship between the time period from the PigWatch® request and the time of ovulation, regarding the number of total born and the gestation rate was first investigated visually and then by estimation of the linear, quadratic and cubic temporal effects.

Finally, P-O durations were divided into two categories, optimal and suboptimal, according to two definitions of optimum in the literature. The first optimal period was defined as the time period starting 28 hours immediately before ovulation and ending 4 hours after ovulation (Nissen et al., 1997).The second optimum period was defined as the period starting 24 hours before ovulation and ending at the time of ovulation (0) (Soede et al., 1995; Almeida et al., 2000). Performance comparison between optimal and suboptimal PigWatch® requests according to these optimum definitions was done using an analysis of variance (ANOVA) for the total born, and logistic regression for the fertility rate at 35 days.

6 Results

6.1 Project implementation

Five batches of 39 sows for a total of 195 sows were randomly selected and routed into the gestation room and assigned to the project. The number of sows selected for more intensive monitoring (blood sampling) was between 20 and 30 sows per batch. This number was required in order to match the available human resources with the time length constraint on farm worker presence in the room assigned to the project (maximum two hours in the morning and two in the evening). In the end, 122 sows were selected for analysis (20 sows in Batch 1, 22 in Batch 2, 27 in Batch 3, 23 in Batch 4, and 30 sows in Batch 5).

Table 9 Causes of elimination of 73 sows not selected but part of the five batches of 39 sows present in the rooms during the experiment (73/195 = 37%).

Causes of elimination of non-selected sows	
Sows not monitored by PigWatch®	20
Sows not selected due to lack of time for blood sampling	36
Non-inseminated sows	10
Sows with incomplete data	7
Total	**73**

Table 9 shows the main justifications for non-retention of sows. Overall, the project went very well because only seven sows were eliminated due to incomplete data (Table 9).

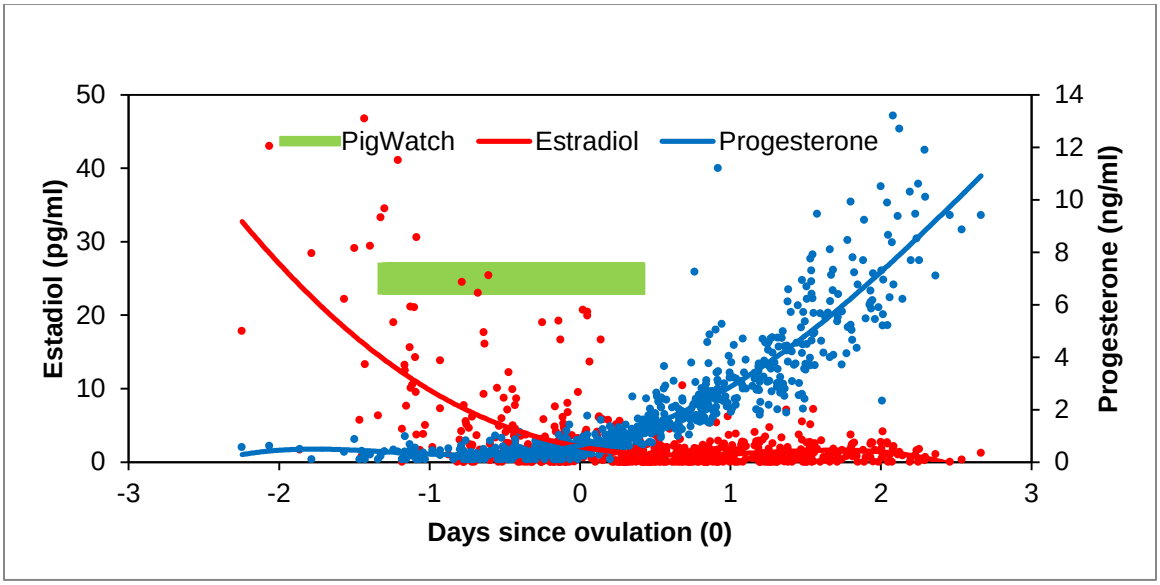

Figure 12 Temporal variation in progesterone (blue) and estradiol (red) measurements, before and after ovulation. Green line indicates the time period of PigWatch® requests for the majority of sows (116/122, 95%).

Table 10 Distribution of base-line progesterone data points in sows with different data points during the follicular phase (0 = no data point in follicular phase; 1 = sows with one data point in follicular phase; 2+ includes sows with 2 – 4 data points in follicular phase).

Base-line progesterone (ng/ml)	0	1	2+	Total
0.1	0	6	9	**15**
0.2	0	7	10	**17**
0.3	5*	9	17	**31**
0.4	0	5	8	**13**
0.5	0	5	8	**13**
0.6	0	3	5	**8*** **
0.7	0	3	3	**6*** **
0.8	0	7	2	**9*** **
0.9	0	2	1	**3*** **
1	0	2**	2***	**4**
> 1	0	2**	1***	**3**
Total	**5***	**51**	**66**	**122**

* These 5 sows had no progesterone data point during the follicular phase. The first progesterone data point of these five sows was high (> 0.8 ng/ml) and all had low serum estradiol levels (<7 pg/ml). For these 5 sows, the expected baseline value was set to the expected value for an average sow (0.34 ng/ml).
** These 4 sows had high serum estradiol levels (> 15 pg/ml).
*** These 29 sows had low serum estradiol levels (<7 pg/ml).

Figure 12 is a scatter graph showing the serum progesterone and estradiol measurements over time. The temporal variation of serum progesterone was in line with expectations and the observations reported by other researchers (Soede et al., 1994; Terqui et al., 2000; Noguchi et al., 2010). During the follicular phase, serum progesterone was low (<0.5 ng/ml) for the majority (84/122, 69%) of sows (Table 10). Several sows had higher progesterone values (0.8 to 1.0 ng/ml). Progesterone values during the follicular phase were low and they showed little variability. The distinction between the last follicular phase value and the first luteal phase value was visually and statistically easy to distinguish (Figure 13).

The temporal variation of serum estradiol showed greater variability and uncertainty than that of progesterone (Figure 12). Measurement of estradiol proved useful to detect those sows with only one data point during the follicular phase and elevated serum progesterone levels (4 sows, Table 10).

6.2 Operation of the PigWatch® system

The PigWatch system worked well during this project. Most of the 195 sows monitored by the system received an insemination request from the system.

Blood samples probably interfered with sow behaviour estimates made by PigWatch®. As a matter of fact, during blood sampling phase, all the sows in the room were standing. This one-time stress may also have influenced the sows' natural behaviour outside the blood sampling periods. The designer of PigWatch® observed and reported some odd behavioural curves during the experiment, but no sows were removed from testing because of these anomalies. These behavioural changes likely caused the PigWatch® system algorithm to encounter some difficulties in analysing certain noisy (meaningless) data corrupted by external factors.

6.3 Assessment of the first data point of the luteal phase

Figure 13 shows four examples of the serum progesterone profile data points for certain sows.

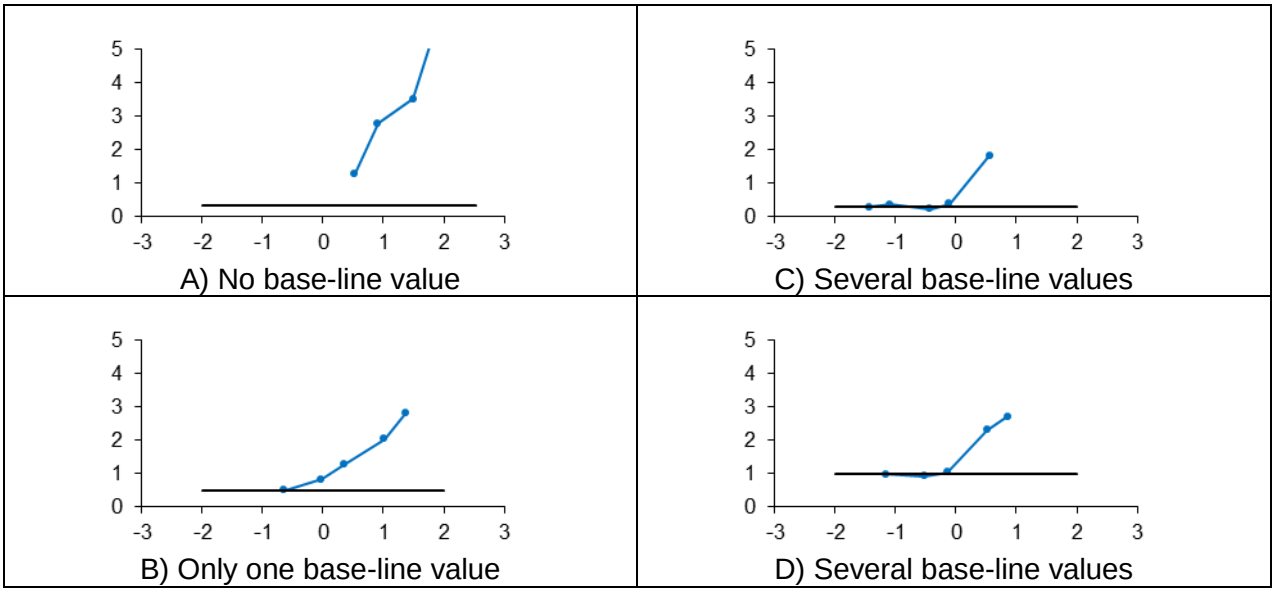

Figure 13 Four progesterone profile patterns

The first pane (A) shows the progesterone profile of a sow whose first blood sampling was obtained during the luteal phase. The second pane (B) shows the progesterone profile of a sow that had only one blood sampling during the follicular phase. The two other panes (C and D) show the progesterone profile of two sows that had multiple blood samplings during the follicular phase. Pane C shows a case with low base-line progesterone (a common situation, see Table 10). Pane D shows a case with elevated base-line progesterone (≈ 1 ng/ml). Table 10 presents the distribution of base-line progesterone mean values measured during the follicular phase.

Just over half the sows monitored in this project had more than one base-line value (66/122, 54%). Fifty-one sows had only one base-line progesterone value (51/122, 42%) and five sows (5/122, 4%) were probably sampled after ovulation. The expected base-line value for an average sow was 0.34 ng/ml. This value served as a reference for the 5 sows without follicular phase progesterone estimates.

6.4 Assessment of time of ovulation

The different methods (A, B1 and B2) were used to assess the most likely period of sow ovulation. Table 11 shows the relationship between the applied methods and the number of data points available in the follicular and luteal phases.

Table 11 Distribution of the methods used for assessment of probable ovulation period according to the number of values available during the follicular phase

Number of values (follicular phase)	Sows (n)	Method A	Method AB1	Method AB2*
0	5	5	0	0
1	51	51	0	0
2	41	0	4	37
3	17	0	13	4
4	6	0	6	0
5	2	0	2	0
Total	**122**	**56**	**25**	**41**

* The B2 methodology was only applicable for the sows with more than three estimates in the luteal phase.

6.5 Analysis of the length of time between the PigWatch® request and ovulation

The time of ovulation was calculated for 122 sows in the study. This time was estimated as the mid-point of the most likely period of ovulation, according to the method best suited. Method A was best for 56 sows (46%) and Method C for 66 sows (54%).

Figure 14 shows that in 95% of the sows, the time period between the presumed ovulation and the PigWatch® request lies in the period 32 hours before ovulation and 8 hours after ovulation.

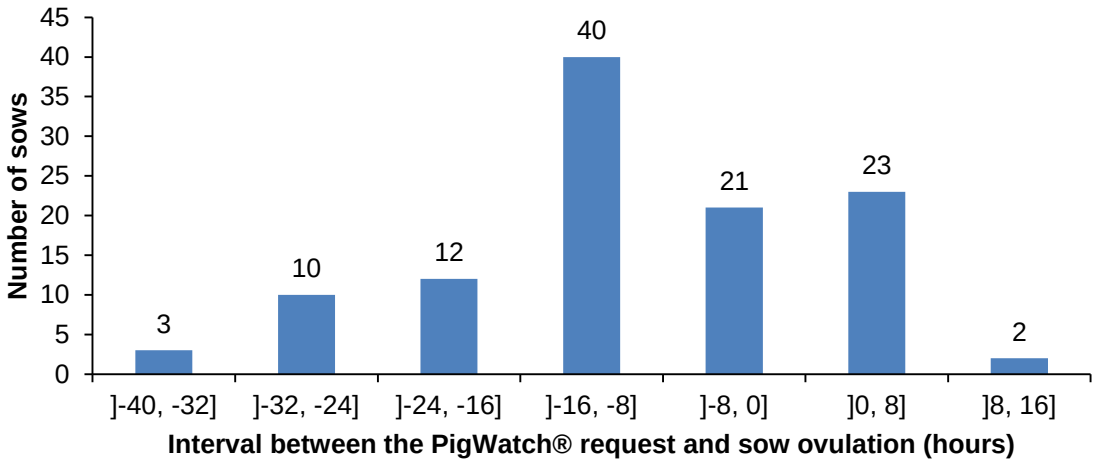

Figure 14 Interval between the PigWatch® request and sow ovulation

6.6 Performances and the length of the interval between the PigWatch® request and ovulation

The 122 sows kept for analysis were inseminated on average 1.16 times following requests from PigWatch®, and they had a 95.1% fertility rate and 15.29 piglets born per litter. The sows were inseminated an average of 10.1 hours before ovulation; with a standard deviation of 10.8 hours (between 41 hours before and 11 hours after ovulation). Median is 10.65 hours before ovulation and 95% of insemination requests are between 31.5 hours before ovulation and 8 hours after.

Table 12 shows the results for mean total born and gestation rates by group of eight hours. Figures 15 and 16 present the same data in bubble charts, where the diameter of the bubbles represents the number of sows in each 8-hour group.

Table 12 Number of piglets born per litter and sow fertility of the 122 sows at 35 days

Groups	Number of Sows	Total Born	Fertility
]-40, -32]	2	10.50	100.0%
]-32, -24]	13	15.10	84.6%
]-24, -16]	12	15.18	100.0%
]-16, -8]	45	16.09	95.0%
]-8, 0]	22	16.24	100.0%
]0, 8]	24	13.74	95.8%
]8, 16]	3	15.50	66.7%

The visual and statistical analyses did not enable identification of any linear ($p = 0.7378$), quadratic ($p = 0.3379$), or cubic ($p = 0.1757$) relationship between gestation rate and the interval between the PigWatch® request and ovulation.

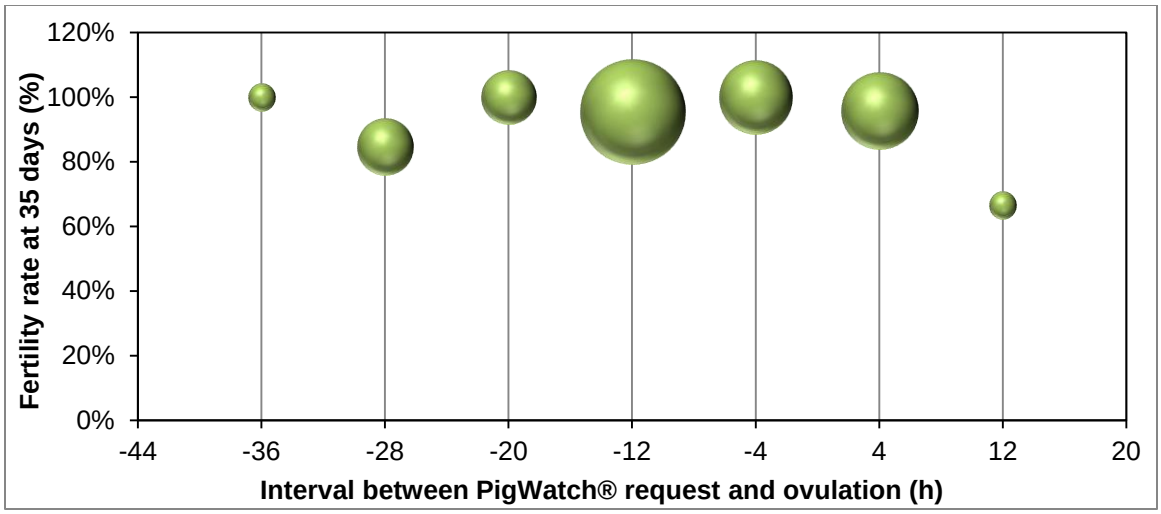

Figure 15 Fertility rate by group (mean values)

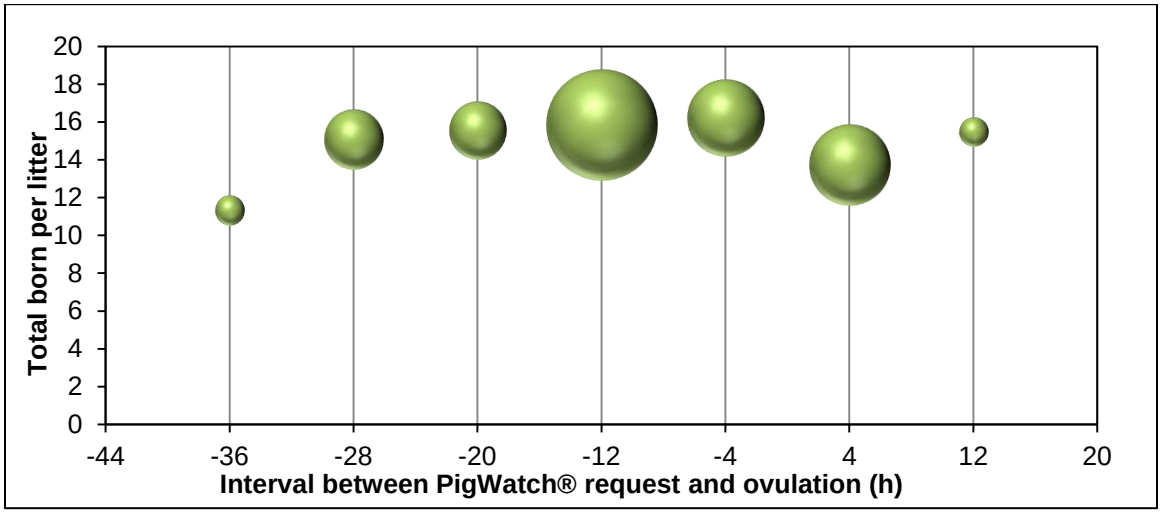

Figure 16 Total born by group (mean values).

The visual and statistical analyses identified a significant quadratic relationship ($p < 0.05$) between the number of total born and the time period between the PigWatch® request and ovulation. This relationship indicates that the maximum number of total born is obtained when the PigWatch® request occurs 12.7 hours before ovulation (Figure 17).

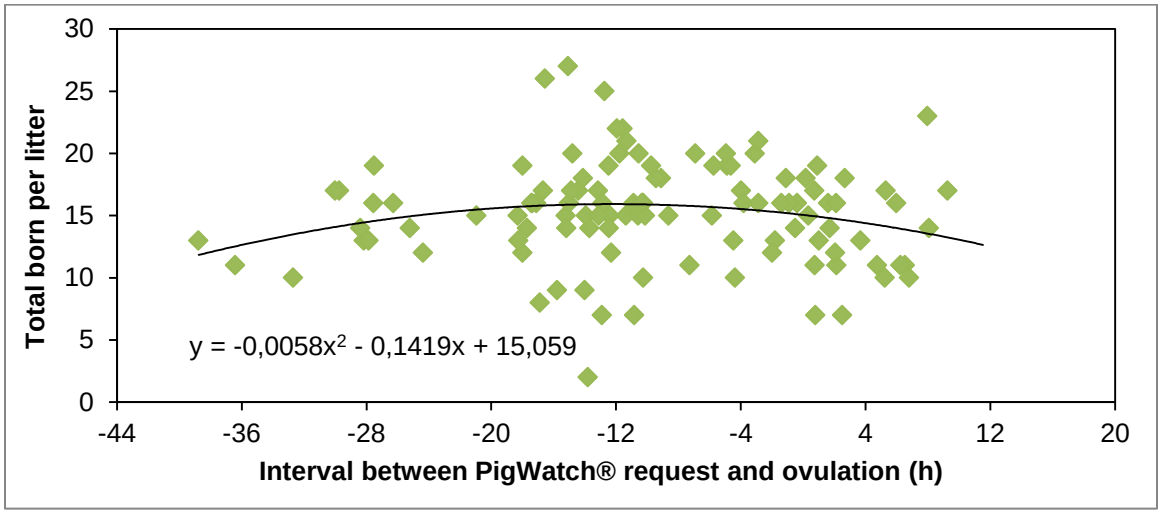

Figure 17 Quadratic regressions for the number of total born according to length of the interval between PigWatch® request and ovulation.

Table 13 shows the sow reproductive performances according to their position in the two optimal time periods proposed by other researchers. The number of total born is higher when the length of time between the PigWatch® request and the time of ovulation lies in the optimum time period (Table 13). For its part, the gestation rate is not significantly affected by the categorization, ideal vs. non-optimal.

Table 13 Farrowing performances of sows inseminated in the hours before or after ovulation compared to other studies.

	Insemination period: 24 h before ovulation *			**Insemination period: 28 h before to 4 h after ovulation ****		
	Inside target period	**Outside target period**	**Difference**	**Inside target period**	**Outside target period**	**Difference**
Number (percentage) of sows	79 (65%)	43 (35%)	---	103 (84%)	19 (16%)	---
Fertility rate	97.47	90.7	6.77 (p = 0.122)	96.12	89.47	6.64 (p = 0.265)
Number of total born	15.92	14	1.92 (p = 0.019)	15.55	13.78	1.77 (p = 0.094)

* Ideal period for sow insemination according Soede et al. (1995) and Almeida et al. (2000).
** Ideal period for sow insemination according to Nissen et al. (1997).

7 Economic analysis

7.1 Discussion on the potential economic impact of the system

The group of sows monitored in this project had excellent growth performances, with a fertility rate of 95.1% and 15.29 total born per litter[17]. In addition, these performances were achieved using only 1.16 doses of semen per breeding, which is not many.

It is actually more common to see the use of 2 doses of semen (for the most part) to 3 doses (for some sows) per breeding. So, for a herd, this would translate into an average of more than 2 doses per breeding (2.2–2.4).

This decrease in the number of doses could result in potential savings for a farrowing operation due primarily to:

1. A reduction in insemination (AI) tasks in the breeding-gestation and farrowing pens
2. A decrease in the number of doses of semen that is required.

7.1.1 Decreased work time in breeding-gestation and farrowing barns

Lowering the number of inseminations required per breeding will have an impact directly proportional to the time allotted to inseminations. Also, since PigWatch® should facilitate the job of estrus detection, the amount of work time devoted to estrus detection should also decline.

In addition, a system that manages sow estrus detection should be an asset to operations with a high employee turnover. The PigWatch® system should facilitate the training of employees who work in breeding-gestation and farrowing barns. Greater uniformity of task performance between employees (weekday shift vs weekend, holidays, replacements) might also be achieved.

7.1.2 Falloff in the needs for semen

The PigWatch® system should make it possible to lower artificial insemination costs, especially if the number of doses per breeding drops to the level observed during this project. However, the impact may vary depending on whether a company purchases the semen or produces it itself.

However, for a company that buys semen, the savings stemming from the falloff in the number of inseminations, may decline over the long term. Should this technology become widespread, it will bring about a significant drop in the number of doses of semen that are required. The insemination industry would experience a fall in sales, which could lead to a restructuring of the industry. An increase in semen prices could ensue, which would negate a part of the savings.

On the other hand, the impact on a company that produces its own semen would be more noticeable. A company with its own AI centre would lower its operating costs. It could keep a smaller number of boars in its centre, which would reduce work time, e.g. for semen collection, and lower the overall cost of housing the animals.

[17] In comparison, farrowing barns in the Coop network displaying the best 2014 provincial productivity results, had an average fertility rate of 91.8% and 14.29 total born per litter on average (*Le Coopérateur agricole*, July -August 2015).

In the medium term, a technology that reduces the number of boars in this way will also result in accelerated genetic progress. Indeed, the semen of boars with the highest selection index could be used to inseminate a greater number of sows. For example, Dr. Sonderman is of the opinion that the transition from conventional insemination of 3 billion sperm in post-cervical insemination to 1.2 billion sperm, allows an organization with its own insemination center to reduce by 40% the number boars in inventory and improve the progress index of the equivalent of one generation (Sonderman, 2015).

Finally, even though this project did not allow for an exhaustive economic analysis, at the same time it still provided food for thought. Accordingly, an effective system of estrus detection in sows could result in:

- Reduced work time for insemination and estrus detection;
- Easier training of employees and improved uniformity of work;
- Fewer semen doses needed for insemination.

For those organizations with their own insemination centre, the number of boars in inventory should also decrease. If the average index of the boars that are maintained increases, genetic progress would also accelerate.

8 Discussion

The results of this research show that the PigWatch® system makes insemination requests in a period around ovulation that is consistent with known information and is conducive to obtaining good animal performances results. In fact, 95% of PigWatch® requests were made within the period of 31.5 hours before to 8 hours after the presumed ovulation. The fertility rate at 35 days was 95.1% and the number of piglet born per litter was 15.29. These reproductive performances were obtained with 1.16 inseminations per sow. These finding indicates that it is possible to determine a good time to inseminate sows and get good reproductive performances from a statistical evaluation of sow behaviour in the stall, as is done by the PigWatch® system.

By classifying the sows according to optimal time periods for insemination, such as those established by the studies of Soede et al. (1995), Almeida et al. (2000) and Nissen et al. (1997), it is possible to see that the fertility results of sows inseminated outside the optimal time periods are not statistically different from the fertility results of sows inside the so-called best time periods. These observations support to the fact that the PigWatch® system is able to determine a good time to inseminate each sow.

Using the same type of classification, it can be seen that the resulting number of total born to sows inseminated outside the optimal time period proposed by Soede et al. (1995) is statistically different from the resulting number of total born to sows within this so-called optimal time period. Sows inseminated in this time period (65% of total) had an average of 1.92 more piglets ($p = 0.0187$). By doing the same exercise for the optimal time period suggested by Nissen et al. (1997), the difference was not statistically significant ($p = 0.0939$), but the data suggest the same tendency.

This information suggests that to obtain the maximum number of total born, insemination within the 24 hours pre-ovulation should be a top priority. Sows inseminated outside this optimal time period have a slightly lower number of piglets without significantly affecting fertility rates.

The method of statistical analysis of the temporal variations of progesterone and estradiol, developed in this project, appears to be sufficiently accurate to correctly estimate the time of ovulation in sows from a collection target of five blood samplings at a rate of two a day, starting when the PigWatch® system signals a request to inseminate. The fact that some requests occur very close to ovulation, or slightly after, complicates the analysis because the lack of data to establish the baseline progesterone level necessitates the use of certain approximations.

The animal performances results tend to support the findings of Nissen et al. (1997) and Soede et al. (1994). The quadratic relationship observed between the total number of piglets born and the interval between the PigWatch ® request and ovulation, shows that the maximum number of piglets was obtained when insemination was performed 12.7 hours before ovulation. This ideal time is in agreement with the results of Nissen et al. (1997) which showed that the maximum number of total born was obtained when insemination was performed 10 ± 6 hours before ovulation. Although sow fertility is not significantly affected by a request outside the ideal times, the digital data available suggest lower fertility rates, in agreement with the work of the other researchers (Soede et al., 1994; Nissen et al, 1997).

9 Conclusion

The PigWatch® system is a technological innovation developed jointly by the Quebec company, Conception Ro-Main, and the Italian company, LPS Electronics. The goal of this technology is to predict the best time to inseminate weaned sows based on real-time analysis of sow behaviour in their stall. Product development has been achieved mainly through measurement of sow behaviour before and during estrus, the development of electronic modules and of mathematical algorithms to analyze behavioural data and predict the time of insemination.

The results of this project show that 95% of insemination requests made by the PigWatch® system were issued in a time period between 32 hours before and 8 hours after ovulation. Each sow was inseminated 1.16 times on average, and the average fertility rate was 95.1% for an average of 15.26 total piglets born.

In conclusion, the PigWatch system, which works according to an analysis of the sow's behaviour, can predict the right time to inseminate. Its use also reduces the number of semen doses required per sow, while maintaining good animal performances.

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