FARM TRIAL:

EFFECT OF PHEROBOAR AS A TOOL FOR HEAT DETECTION IN SOWS

MARTA MORENO (FACULTY OF VETERINARY ZARAGOZA)

ANDREA CLIMENTE (MAGAPOR R&D VETERINARY DEP.)

JUAN YEREGUI (MAGAPOR PRODUCT MANAGER)

OBJECTIVE

The objective of this trial is to compare the effectiveness of ***Pheroboar*** in terms of detecting heat in sows (gilts, weaned, delayed, aborted, etc.) and determine if it has an enhanced action on the stimulus caused by the boar when used at the same time. In addition, it has been compared with a similar product, *Competitor*.

INTRODUCTION

Heat detection in pig farms plays a fundamental role, since correct detection of heat by operators will determine the fertility rate of the sows. Generally, farms have boars as the main way to detect sows in heat, and complementary methods can be used such as the application of pheromone spray.

A correct heat detection allows determining the most suitable time for artificial insemination and it is essential to do it correctly to estimate the time of ovulation (Quiles and Hevia, 2009). Furthermore, from the moment the sow comes into heat, the insemination of the seminal doses will be determined. Despite this, the fact that many individual factors intervene in the exact moment of ovulation (genetics, number of productive cycle, management, estrus expression, etc.) means that today there is no simple method that is efficient to know the exact moment of ovulation (Roca et al., 2016); but, there are other more complex methods such as the determination of follicular size by abdominal ultrasound or the measurement of hormone levels related to the phase of the estrous cycle in serum.

1. ESTROUS CYCLE OF THE SOW

The sow is a continuous polyestrous female whose average length of the estrous cycle is about 21 days, except in periods of anestrus when the sow stops cycling. In addition, they usually have a decrease in their reproductive function during the summer and early fall known as Seasonal Infertility Syndrome, being more noticeable in gilts sows (Quiles and Hevia, 2007).

The cycle consists of two phases, a shorter follicular phase (5-7 days) and a longer luteal phase (13-15 days). The first phase comprises a proestrus stage in which the growth of ovarian follicles occurs and an estrus stage in which ovulation happens, corresponding to the moment when the female comes into estrus and is inseminated (Soede, Langendijk and Kemp, 2011) (Figure 1; Velasco Villalvazo, 2016).

At the beginning of the luteal phase (metaestrus) structures called corpora rubrum are formed in the ovary and progesterone production begins. Finally, in diestrous, a stage of longer duration, the ovary will present corpora lutea and progesterone will reach its maximum level in the blood (Figure 2; Geisert et al., 2020).

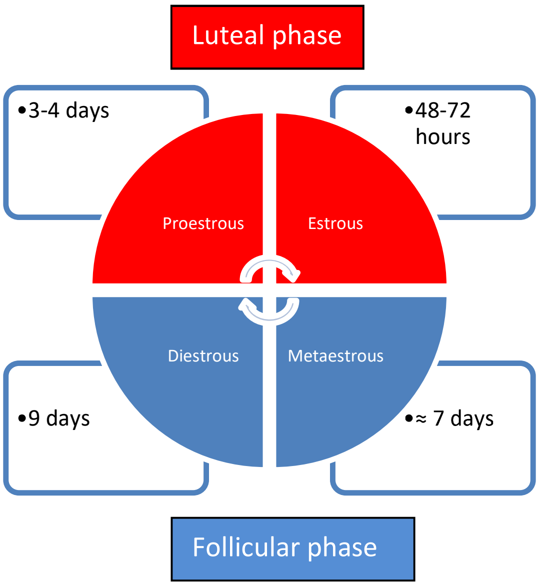


Figure 1. Estrous cycle of the sow (Velasco Villalvazo, 2016)

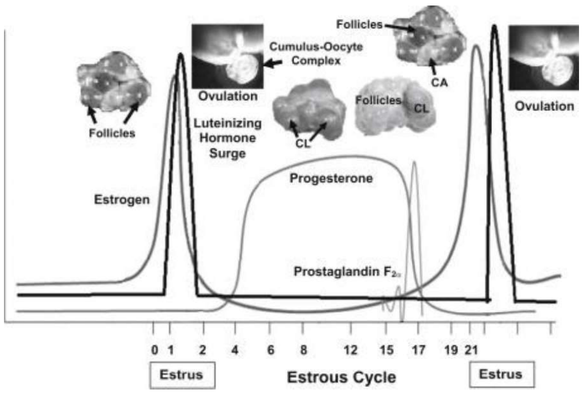


Figure 2. Hormonal changes during the estrous cycle of the sow (Geisert *et al.*, 2020)

2. THE ESTROUS AND THE MAIN HEAT SIGNS IN SOWS

Estrous is the phase of the sexual cycle in which the sow is receptive to the boar and there is a high possibility that she will become pregnant, since this is the time when ovulation takes place. The average duration of this phase is shorter in gilts sows, being between 1 and 2 days, while in sows it can last up to 3 days (Kraeling and Webel, 2015).
The sow ovulates several follicles in each cycle, so ovulation does not occur at a specific time, but over a longer time period. Therefore, sows are spontaneously ovulating females, multiple and lasting some hours (Figure 3; Porras Nieto, 2020).
It should be noted that ovulation occurs approximately when estrus reaches 85% of its total duration (Almeida et al., 2000), and according to a study by Knox et al. (2001) this would correspond to an onset of ovulation at 39 - 48 hours after the onset of estrus. It should be noted that the time at which ovulation occurs is not externally visible, unlike the external and behavioral changes that are visible (Hensel et al., 2022).

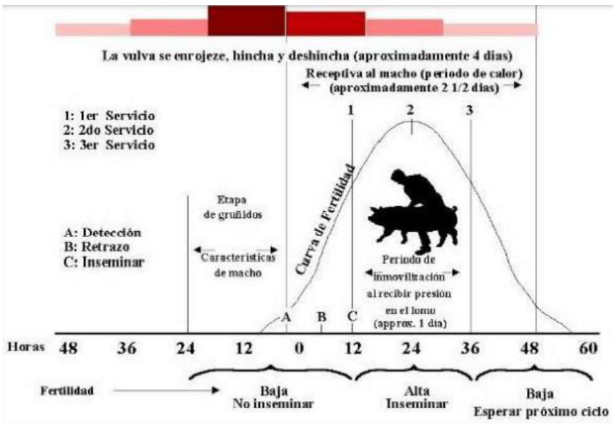


Figure 3. Heat detection in sows (Porras Nieto, 2020)

Due to the variation in estrus duration, the more times sows are heat checked, the more accurate the detection of estrus will be and, therefore, the greater the chance of successful artificial insemination.
As mentioned before, a correct artificial insemination is one of the key points in farms and, therefore, it is very important to know which are the main signs of estrus that a sow shows induced by the increase of estrogens, which will be determining factors to confirm if the female is in estrus and can be inseminated (Falceto, 2015)

- Stiffness reflex (Figure 4; Falceto, Mitjana y Bonastre, 2017)
- Pricked ears (Figure 5; Falceto, Mitjana y Bonastre, 2017)
- Lordosis
- Reddening vulva and swelling

Other signs to consider, although less noticeable, would be the possible presence of vulvar discharges (mucus), grunting, inappetence, salivation and the female mounts other sows and allows herself to be mounted (Falceto, 2015).


Figures 4 y 5. Stiffness reflex and pricked ears (Falceto, Mitjana y Bonastre, 2017).

3. HEAT DETECTION

Estrus detection is another key point and a factor with individual variability for each sow, as each female expresses the signs to a different degree. In addition, it must be considered if the sows are gilts, coming from weaning or are delayed sows with regular or irregular repetitions. In the case of sows coming from weaning, most usually show signs of estrus within 3 to 6 days after weaning (Soede, Langendijk and Kemp, 2011; Foxcroft, Patterson and Dyck, 2010).

For a correct heat detection, it is necessary to have the presence of one or two operators, one to walk the boar in front of the females (in case of applying pheromone spray, he would also be in charge of spraying it on the sow's snout) and another one to observe the signs of heat and mark the sows that are in heat for their later insemination. In the case of having only one operator for the heat detection, the corridors where the boar walks should have doors to allow the boar to pass through or to slow down the boar.

When estrus is detected in a sow, it can be due to either one or several stimuli (visual, tactile, olfactory or auditory) that the sow receives at the same time. The use of the boar to detect estrus would encompass all types of stimuli, whereas, in the case of pheromones, the stimulus would be olfactory.

The maximal estrus expression response has been described previously and is achieved by the combination of tactile, visual, olfactory-chemical and auditory stimuli (Signoret et al., 1975) (Figure 6; Sankarganesh et al., 2021).

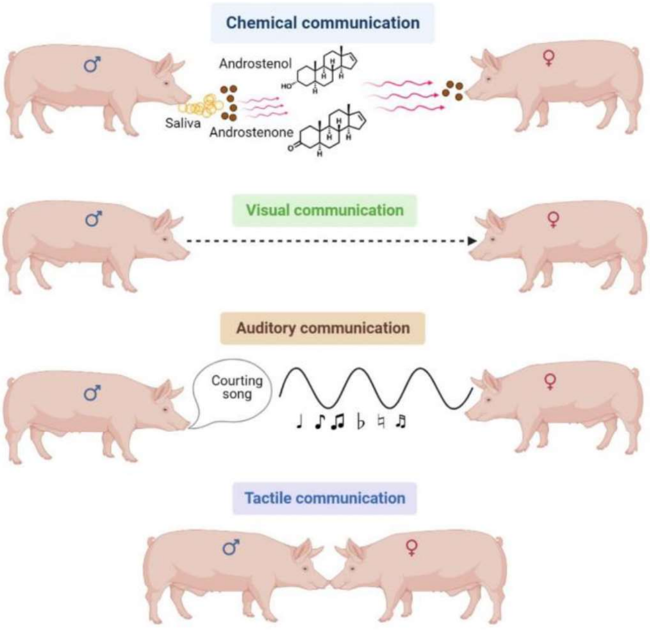


Figure 6. Types of communication – stimuli in pigs (Sankarganesh *et al.*, 2021)

The boar is the method commonly used in heat detection, which consists of walking the boar in front of the sows' cages as an operator checks the sows for signs of heat.

One study indicates that exposure of the boar to the sows is necessary to detect if the sows are in estrus and may influence the uptake and transport of sperm once they are inseminated (Langendijk, Soede and Kemp, 2005). However, this exposure should only be done for a few minutes a day, since several studies, such as that of Knox et al. (2004) show that if the boar is always in the same room as the females, i.e., there is a continuous stimulus, the time in which estrus occurs is shorter and the detection of estrus is more complicated.

It is also proposed that the most important stimulus is the olfactory-chemical one, which is produced by the high concentrations of androstenol and androstenone formed in the salivary glands of the boar, which are an important source of pheromone production (Perry et al., 1980), and that, previously it had been demonstrated that when these substances were applied to the snout of gilts and multiparous sows, the signs of estrus were more noticeable (Hillyer, 1976).

Thus, the salivary glands (submandibular, sublingual and parotid) are the main source of pheromone production that serve as an olfactory-chemical stimulus. Patterson et al observed that the submandibular gland is the most involved in this type of stimulus, since it is responsible for producing the highest concentration of androstenol and androstenone.

The importance of this structure was subsequently demonstrated in several studies using males that had the submandibular gland removed and showed an altered response to estrus, and that gilts exposed to these males reached puberty at an older age than those exposed to intact males, but at a younger age than those without contact with boars (Perry et al., 1980; Pearce, Hughes and Booth, 1988).

4. PHEROMONES

The porcine species is one of those with the highest number of olfactory receptors (Nguyen et al., 2012), so pheromones play an important role as olfactory-chemical stimuli for females of this species.

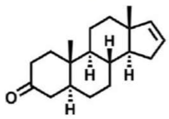
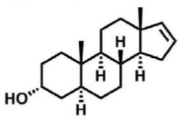
Pheromones produced by the submandibular gland constitute an olfactory-chemical stimulus per se, which can be used for estrus detection or as a supportive tool for estrus detection when flinching with boars. These can be either natural or synthetic (mimicking those of the boar, such as ***Pheroboar*** which simulates the chemicals secreted by the pig through its salivary glands: androstenol and androstenone). Both types produce both oestrus behavioral and endocrine responses in the sow.

McGlone et al. evidence an aromatic hydrocarbon, quinolone, as another possible salivary gland pheromone responsible for sow estrous behavior (McGlone, Devaraj and García, 2019) which is still under analysis, as other authors (May, 2016) only found quinolone in the saliva of boar from one of the two farms in their study.

Based on the above, all synthetic products should contain androstanol and androstenone, while quinolone could be added or not as more tests are still needed to confirm its presence in boar saliva.

In addition, tests relating the concentration of these pheromones to the influence they have on the immobility reflex of the sow in estrous should be performed to create the perfect combination to achieve the highest possible percentage of detected sows (Table 2; Melrose, Redd and Patterson, 1971, as cited in Sankarganesh et al. 2021).

Table 2. Relationship between pheromone concentration and % of shows showing immobility reflex (Melrose , Redd y Patterson, 1971, as quoted in Sankarganesh et al., 2021)

Compound	Concentration	Standing response percentage in sows
5α-androstenone 	1.14 μg/mL 4.56 μg/mL	56% 48%
3α-androstenol 	0.86 μg/mL 4.30 μg/mL	31% 53%

It has been demonstrated that androstenol is transmitted via local and humoral pathways from the nasal cavity to the pituitary and central nervous system via humoral pathways (Figure 7; Krzymowsky et al., 1999) (Stefáncyk – Krzymowska, Waswka and Jana 2002)

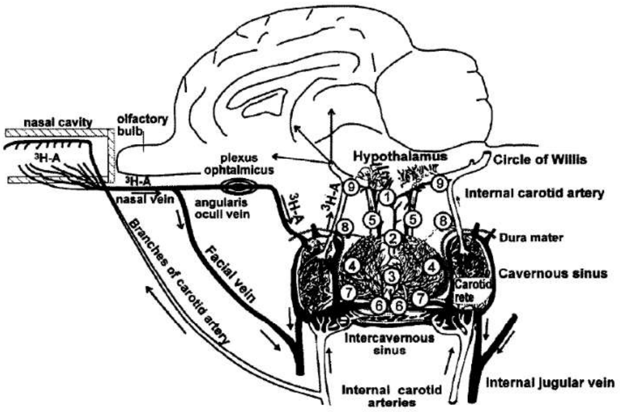


Figure 8. The putative humoral pathway for transmitting a priming pheromone from the nasal cavity to the hypophysis and the neural centres: nasal mucosa → venous blood of nasal vein → angularis oculi vein → plexus ophtalmicus → cavernous sinus → arterial blood of carotid rete → inferior-, middle-, and superior-, hypophyseal arteries (to the hypophysis and hypothalamus) → internal carotid artery → circle of Willis (to the hypothalamus and other neural centres). For better understanding of the processes that accompany the action of pheromone diagram includes: 1) infudibulum, 2) infundibular stem, 3) infundibular process, 4) adenohypophysis, 5) long portal vessels, 6) inferior hypophyseal veins, 7) inferior hypophyseal arteries, 8) middle hypophyseal arteries, 9) superior hypophyseal arteries.

Figure 7. Transmission of the pheromones from the boar to the female, humoral route (*Krzymowski et al.*, 1999)

Synthetic pheromones can be used to complement the heat detection with the boar which would be of great help if the boar itself produces a low level of pheromones, or to apply in places on the farm where the boar cannot access, as in the case of some quarantines or maternity areas, to identify animals that are coming into heat before being weaned.

Another situation in which pheromones can be used is in the case of operators with little experience in heat detection, which would serve as an extra support while passing the male, since it should not be forgotten that this is a complementary method to the boar.

MATERIAL AND METHODS

The trial was conducted on two sow farms, Farm 1 and Farm 2.

The objective in Farm 1 was to compare the efficacy of ***PheroBoar*** against another product already marketed, Competitor, since the infrastructure of the facilities allowed the groups of sows in which each product was tested to be in separate areas. The band of sows to be inseminated was divided into 4 different groups. The heat detection in sows in two of these groups was done after application of the synthetic pheromone products; the heat detection on the other two groups was done by applying the pheromone and the "teaser" boar together. In addition, after the time in which the pheromone ceased to exert its effect in the groups in which only this technique had been applied, the sows were again exposed to the boar to compare the efficacy of this olfactory stimulus against the contact with the male (Figure 8).

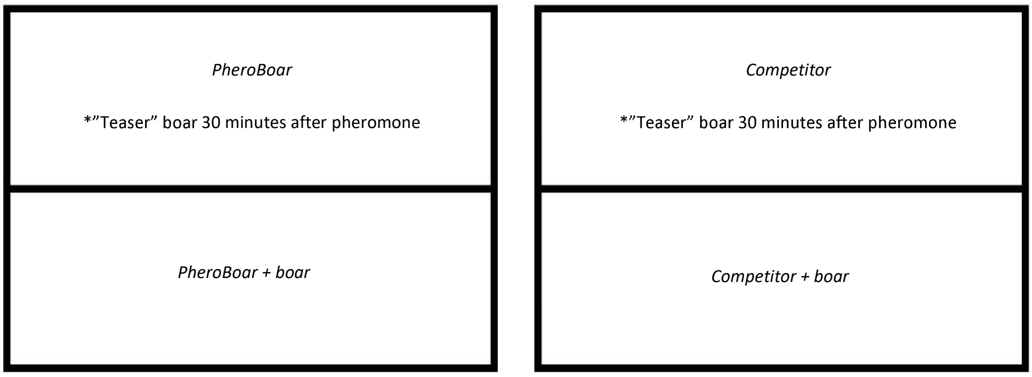


Figure 8. Trial scheme in Farm 1

In the other test, on farm 2, only the efficacy of PheroBoar was tested. The technique was the same as in Farm 1, except that this time the group was divided into only two different test groups (Figure 9).

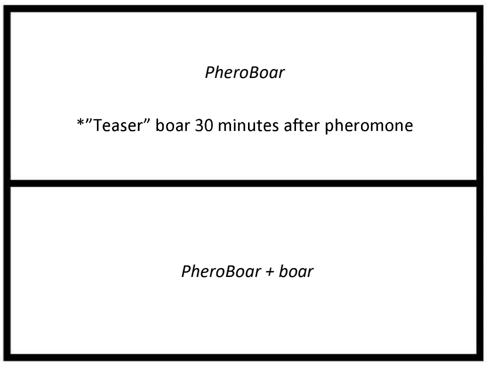


Figure 9. Test development in Farm 2

In total, 316 sows made up the study, 116 belonged to Farm 1 and 200 to Farm 2.

They are distributed in the following groups:

- PheroBoar
- PheroBoar + boar
- Competitor
- Competitor + boar

The signs of estrus to be evaluated corresponded to those routinely checked on the farm:

- Stiffness reflex
- Lordosis
- Pricked ears

Results

FREQUENCY OF HEAT DETECTION ACCORDING TO DIFFERENT SIGNS

Lordosis:

	% sows in heat detected by LORDOSIS								
Pheromone/Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**	**% LOR/batch**
COMPETITOR	0%	0%	0%	0%	16.6%	41.7%	41.7%	100	65%
COMPETITOR + BOAR	0%	0%	0%	0%	23.3%	37.2%	39.5%	100	95,5%
PHEROBOAR	0%	0%	0%	1.4%	20.9%	40.1%	37.6%	100	76,2%
PHEROBOAR + BOAR	0.3%	0.6%	1%	1.3%	30.8%	35.6%	30.4%	100	93,7%

Stiffness:

	% sows in heat detected by STIFFNESS								
Pheromone/Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**	**% STIFF/batch**
COMPETITOR	0%	0%	0%	0%	16%	40%	44%	100	67,56%
COMPETITOR + BOAR	0%	0%	0%	0%	23.3%	37.2%	39.5%	100	95,5%
PHEROBOAR	0%	0%	0%	1.4%	20.9%	40.1%	37.6%	100	76,2%
PHEROBOAR + BOAR	0.3%	0.6%	1%	1.3%	30.2%	34.8%	31.8%	100	95,5%

Pricked ears:

	% sows in heat detected by PRICKED EARS								
Pheromone/Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**	**% P.E./batch**
COMPETITOR	0%	0%	0%	0%	16.6%	41.7%	41.7%	100	65%
COMPETITOR + BOAR	0%	0%	0%	0%	23.3%	37.2%	39.5%	100	95,5%
PHEROBOAR	0%	0%	0%	1.8%	21%	40.2%	37%	100	75,9%
PHEROBOAR + BOAR	0.3%	0.6%	0.9%	1.3%	30.2%	34.9%	31.8%	100	95,5%

The most identified sign is the STIFFNESS reflex, and it is important to note that there is a greater response for all signs when used in conjunction with the boar.

COMPARISON BETWEEN PHEROMONES (PHEROBOAR AND COMPETITOR) WITH THE BOAR AS A CONTROL:

All sows:

The following table shows the percentage of sows detected with the different pheromones (PheroBoar and Competitor) and the boar (30 minutes after the use of the pheromone)

	% of heats detected by the pheromone compared to the boar at different days			
Pheromone/Days	**4**	**5**	**6**	**7**
COMPETITOR	-	44,4%	50%	54.5%
PHEROBOAR	80%	84.7%	47.8%	47.6%

Competitor:

- No heat was detected in the Competitor group before day 5.
- Of the 100% of the sows detected with the boar, only 44% were also detected with the competitor pheromone on day 5
- On day 5 and 6 the heat detection with the Competitor pheromone did not reach the 55%.

PheroBoar:

- On day 4, 80% of the sows detected with the boar, were also detected by PheroBoar
- On day 5, almost the 85% of the sows detected by the boar were also detected by PheroBoar

Gilts:

The following table shows the percentage of gilts detected by PheroBoar and then with the boar (30 minutes later).

	% of heats detected by PheroBoar compared to the boar		
Pheromone/Days	**5**	**6**	**7**
PHEROBOAR	100%	100%	100%

As can be seen, the gilts were only evaluated with PheroBoar and 100% of them detected with the boar were also detected by PheroBoar. However, more trials will be conducted to increase the number of gilts and confirm this result.

Sows:

The following table compares the percentage of heats detected in sows compared to the boar (30 minutes later).

	% of heats detected by the pheromones and the boar			
Pheromone/Days	**4**	**5**	**6**	**7**
COMPETITOR	-	44,4%	50%	54.5%
PHEROBOAR	80%	83.8%	45.9%	45%

As can be seen, the group exposed to PheroBoar showed the heat before the Competitor group (either because the sows showed the heat naturally or because they were more stimulated by PheroBoar and the heat was induced by it). On the other hand, the Competitor does not show a detection rate higher than 50% before day 7 compared to the boar.

CONCLUSIONS

1. Estrus detection is an essential and sometimes complex task that will determine the timing of insemination and, therefore, the fertility rate on the farm.

2. The use of synthetic pheromones at the farm level can replace the male in situations where it is impossible for him to access the rooms where the females are, since only with the presence of this product these signs can also be detected.

3. The most frequently observed sign, taking into account all methods of heat detection, is the stiffness reflex.

4. As can be observed, multiparous sows show a higher percentage of signs of estrus, confirming what is usually detected on the farm, that gilts generate more problems in estrus and provoke more doubts.

5. PheroBoar is better than Competitor at detecting estrus without the presence of a boar, reaching 84% diagnosis of sows in estrus out of 100% of sows in estrus (detected by the boar) at day 5.

6. 100% of the gilts detected with the boar had been detected with the Pheroboar product. More trials will be carried out to establish a pattern of use in gilts, since this is usually the group of sows that creates the most difficulties in detecting estrus on farms.

7. The use of synthetic pheromones is always recommended when accompanied by the boar, because a greater effect is achieved when, in addition to the pheromone, there is also a reaction with the animal, so that their use is recommended as a complementary tool, and not as a substitute.

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