The state of the art in **porcine artificial insemination**

Artificial insemination has allowed the swine sector to reach its current levels of world meat production while also facilitating work on farms, advancing genetic improvement in breeding animals and reducing the spread of sexually transmitted diseases.

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In recent years, the swine sector has tended to decrease the number of spermatozoa contained in each semen dose used in artificial insemination. This is now possible thanks to post-cervical insemination, in which semen is deposited directly into the body of the uterus. However, given the difficulty in pinpointing the moment of ovulation during estrus, several inseminations are usually carried out to ensure maximum fertility and prolificacy in sows.

The current challenge is to reduce the number of semen doses used during each estrus. However, this can only be achieved by improving estrus detection methods and deepening our understanding of the duration and characteristics of estrus on each farm. Ovarian ultrasound can help us better understand the follicular and luteal dynamics in sows. However, the successful scheduling of a single artificial insemination can only be ensured via ovulation induction using gonadotropin-releasing hormone (GnRH) agonists. Perhaps new research advances will soon allow us to perform a single insemination using encapsulated semen.

Introduction

The history of artificial insemination dates back to 1930 on farms in Russia, but it was not until the 1970s and 80s when artificial insemination with chilled semen would gradually replace natural mating on most pig farms.

Without artificial insemination, the swine sector would not have reached its current levels of pork production, which have increased to try to satisfy the protein needs of a continuously growing world population. Artificial insemination has not only facilitated work on pig farms but has also allowed for greater advances in the genetic improvement of breeding individuals. Unlike natural mating, this technique allows between 20 and 60 sows to be inseminated with the ejaculate of a single boar, enabling faster dissemination of genetic improvements.

Increased biosecurity and hygiene in insemination centers have minimized the spread of sexually transmitted diseases to inseminated sows.

Prerequisites for insemination

Hygiene

Good hygiene during insemination is essential to prevent uterine diseases. Before insemination, to avoid introducing microorganisms into the genital tract when inserting the catheter, the vulva must first be cleaned with disposable wipes moistened with a non-spermicidal disinfectant.

Estrus detection

Estrus detection is the stage prior to insemination and must be as correct as possible. The purpose of this is to determine the beginning and end of the estrus. It is one of the most important tasks on the farm and should be carried out by staff with specific training and with the aid of teaser boars. These boars should be manageable and have a high sex drive. All the sows on the farm should be checked for estrus once or twice every day. Sows who do not present the standing reflex should never be inseminated.

Semen quality

Until recent years, semen quality analysis for the preparation of semen doses was limited to recording its volume and subjectively evaluating motility and concentration using cell counting chambers. Insemination centers now have sophisticated software linked to microscopes that allow for the precise, repeatable analysis of every ejaculate, to quickly and objectively evaluate sperm motility, concentration and morphology. These techniques allow semen doses with higher-quality spermatozoa to be obtained.

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The use of catheters on farms

Catheter sterilization requires time, special equipment and specific training. Thus, single-use, disposable catheters have been substituted into use on farms. Another milestone in terms of hygiene is the use of individually packaged catheters. However, the disadvantage of using all this disposable material is that it increases environmental pollution. Thus, research is underway to create catheters made of biodegradable materials, in order to reduce plastic use on farms.

Without a doubt, semen quality is a critical factor during insemination; only when the semen quality is excellent can sperm concentrations be decreased in the semen doses used on farms. This is one of the challenges insemination centers are currently working on.

Insemination techniques

Perfect insemination technique is the key to reproductive success. Artificial insemination uses catheters and semen doses to deposit spermatozoa directly into the genital tract of sows, with the aim of impregnating them. There are three artificial insemination techniques: intracervical, post-cervical intrauterine and deep intrauterine.

Intrauterine techniques require strict hygiene during insemination because semen is deposited directly into the uterus, while in intracervical insemination the cervix acts as a significant barrier to the entry of germs.

1 Intracervical insemination

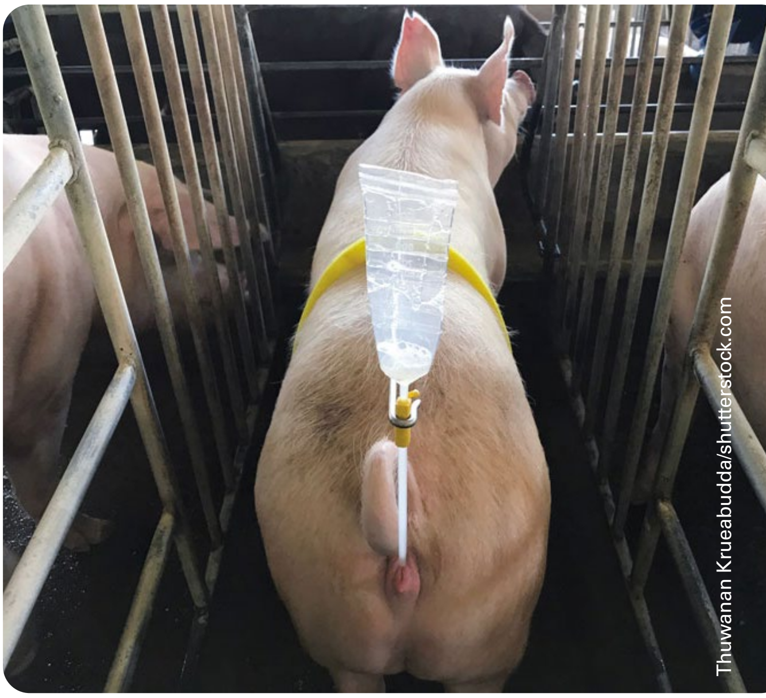
In intracervical artificial insemination, spermatozoa are deposited in the uterine cervix of sows, just as the boar would during natural mating. By the end of the 20th century, the use of this technique had already completely substituted natural mating in the white pig sector and in Spain is now also progressively becoming the breeding method of choice for rearing Iberian pigs.

Intracervical insemination is a well-known traditional technique that yields good productive results. Spiral catheters are introduced by gentle counterclockwise rotation into the cervix, while sponge or multiring catheters are introduced directly without rotation.

After a high-volume semen dose (80–100 mL) containing an elevated number of spermatozoa (3 billion) is introduced over a long insemination time of 3–8 minutes, the sow will slowly draw the semen towards the uterus from the cervix. The presence of a teaser boar during insemination helps this process. Once finished, we can leave the catheter in place for a few minutes before removing it and closing with an anti-backflow stopper.

2 Post-cervical intrauterine insemination

This is currently the most commonly used technique on farms in Spain. Since the start of this century, post-cervical insemination has progressively replaced traditional insemination. This new technique has been the determining factor in improving genetic dissemination in breeding animals. In addition, its use can reduce labor costs by 4.66% and semen costs by 16.18% (Bolarin, 2016).



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If there is excessive backflow from the vulva immediately after insemination, the process should be repeated. However, if the backflow is limited and delayed, it is considered physiological.

Post-cervical insemination deposits the semen directly into the body of the uterus via a cannula inserted through an intracervical catheter. Because they do not need to pass through the barrier of the uterine neck, this technique allows for the use of half the volume and number of spermatozoa per semen dose. Therefore, with a given ejaculate, twice as many multiparous sows can be inseminated using the intracervical technique, without negatively affecting the productive performance of the farm.

The genital tract in sows

The genital tract of a gilt is not as developed as that of an adult sow. Therefore, the post-cervical technique was initially thought to be unsuitable for nulliparous or even primiparous sows. However, catheters designed for these ages are now available and the technique works with the same production results.

To reduce the insemination time per sow, boars should be removed from the vicinity during insemination so that the sow's cervix will be more relaxed, making it easier to insert the post-cervical catheter into the uterine body. The application time for the seminal dose is just 1 minute per sow. With correct technique, there should be no backflow. Otherwise, the catheter should be removed and correctly re-inserted before continuing the insemination. The catheter is removed immediately after insemination.

3 Deep intrauterine insemination

Deep intrauterine insemination deposits the semen towards the end of the uterine horn, which requires less than half the spermatozoa used in the post-cervical technique. However, this technique is not usually used on commercial farms because it is more labor-intensive and requires more expensive catheters.

Deep intrauterine insemination is very useful when frozen semen is used in genetic selection programs.

Number of inseminations per sow in estrus

Fertility and prolificacy are maximized during the hours leading up to ovulation. Spermatozoa can fertilize eggs for up to 24 hours and oocytes remain fertile for 6–8 hours. If inseminations are performed outside of these windows, the gametes will already be sub-optimal when they meet in the oviduct. In these situations, it is very likely that the sow will not get pregnant or that the embryo survival rate will be lower, thus reducing the litter size.

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Estrus lasts between 1 and 3 days and sows are usually inseminated several times at 24–hour intervals during this period, starting from when the standing reflex is detected in sows in the presence of a teaser boar and continuing until the end of estrus. The goal is for one of these inseminations to be as close as possible to the moment of ovulation.

Inseminations should always cease when sows no longer present the standing reflex. On some farms, a third or fourth insemination is sometimes forced. But even a second insemination might be superfluous if the first one was delayed because estrus was not detected. We must keep in mind that late inseminations can lead to vulvar discharge, a return-to-estrus and promote infertility. Inseminating a sow more than twice during estrus is not recommended.

Undoubtedly, one of the current challenges in swine production is to reduce the number of inseminations per sow in estrus and thus be able to inseminate more females with each ejaculate collected from genetically selected boars. In addition, reducing the number of inseminations decreases staff overexertion and costs.

An average of 2.4 inseminations per sow in estrus are carried out in Spain, although it is likely that they will be fertilized by only one of the semen doses.

Estimating the moment of ovulation

To reduce the number of inseminations per estrus, we need know when the moment of ovulation occurs.

Although many methods to identify the moment of ovulation have been studied, to date, no effective, cheap and easy to use solution has been identified. The techniques studied thus far monitor variations in the different estrus cycle parameters: luteinizing hormone (LH) levels, vaginal cytology, vaginal mucus resistivity, vulval temperature, ovarian ultrasound, etc.

The key to reproductive success is perfectly scheduling inseminations according to age and the day post-weaning she came into estrus.

A technique currently used on many farms is to estimate ovulation based on the average estrus duration in multiparous sows. Doing this requires noting the average length of the sows' estrus cycles on the farm over a given period. Considering that ovulation occurs once 70% of the estrus period has elapsed (Kirkwood and Kauffold, 2015), ovulation can be estimated with respect to the average estrus duration recorded for the farm. Thus, insemination is recommended 8–12 hours before the estimated ovulation time. This is because the time estrus starts and its duration are usually similar among sows in the same group in response to the common factors they all experience on the farm: genetics, feeding, lighting, temperature, humidity, ventilation, well-being, teaser boar management, etc. However, we must not forget that this estimate is based on potentially subjective information, depending on the training of the farm's workers.

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In general, the number of inseminations can be reduced on farms that perform estrus-detection twice a day, allowing 24 hours to elapse between estrus detection and the first insemination for any sows that go into estrus before four days post-weaning.

Ovarian ultrasound

Undoubtedly, diagnosis by ovarian ultrasound is very precise. However, its disadvantage is that it requires highly qualified personnel and a lot of time on the farm must be spent evaluating the sows. Current abdominal ultrasound equipment makes it possible to check whether the usual insemination routines used on each pig farm are adapted to the moment of ovulation. Ovarian follicular growth is monitored every 6–12 hours, starting from the beginning of estrus until the follicles reach their pre-ovulation size. Ovulation is detected when the large follicles disappear from the ovary and *corpora hemorrhagicum* appear.

Single insemination at a fixed time

The main advantage of a single insemination is that it facilitates the use of semen from one boar per sow in estrus, or even the insemination of a batch of sows, thus widely spreading the genetic characteristics of the selected boar. However, this requires optimal management of the insemination technique and excellent seminal dose quality.

Other advantages of a single insemination are: the reduced time and labor required, as well as decreased catheter costs and environmental contamination via plastic waste per artificial insemination. In addition, knowing how to plan for a single insemination is very useful in case a situation arises in which the farm has difficulty accessing semen doses. For example, in the event of a production failure at the insemination centers supplying them.

Advantages of a single insemination at a fixed time

- The genetic characteristics of the selected boar will be widely spread.
- Reduced time and labor required.
- Decreased catheter costs.
- Less environmental pollution via plastic waste.
- Improves planning for possible unexpected events.

Two different technologies are currently available to perform a single insemination per sow in estrus. The first, which is widely commercially available, is the hormonal induction of ovulation with GnRH analogues, accompanied by a single insemination at a fixed time. The second, which is still under development, is the use of a single dose of encapsulated semen that is released slowly and sequentially throughout estrus, thereby ensuring that spermatozoa are present in the oviduct at the moment of ovulation.

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Hormonal synchronization of ovulation

The induction and hormonal synchronization of ovulation allows us to predict the time interval between administration of the hormone and the moment of ovulation so that a single insemination can be scheduled 10–12 hours later. This time allows the sperm to capacitate and wait for the oocytes, so that they can be fertilized immediately after ovulation. Human chorionic gonadotropin (hCG), porcine LH and GnRH analogues can be used: buserelin, triptorelin, etc. Buserelin is currently commercially available in Spain.

Using a single insemination at a fixed time has many potential benefits.

- The work on the farm can be better organized.
- Insemination of all the sows in a batch with semen from the same boar, thereby decreasing variability in the birth weights between litters.
- More farrowings can be grouped together because all the sows ovulate at similar times.
- Inseminating more sows with semen from the same boar allows genetic progress to be made faster.

Use of semen doses with encapsulated spermatozoa

The use of a single artificial insemination with encapsulated spermatozoa ensures that viable male gametes are present in the oviduct at the time of ovulation. The sperm are harbored in a matrix-type network that maintains their cellular structure and functionality while they are preserved at 15 °C. After insemination, once the dose reaches a temperature of 38 °C, the spermatozoa are slowly and incrementally released into the genital tract. This allows them to be available for longer periods. In addition, the capsule protects the sperm from vaginal backflow and uterine phagocytosis. It also prevents premature sperm capacitation (Sánchez *et al.* 2014).

Future perspectives

Among the most modern techniques used for semen comparison, flow cytometry, the separation of sperm populations and techniques for sperm penetration of pig oocytes are the most noteworthy. It is likely that these tests and cytogenetic studies to identify chromosomal abnormalities will become part of the standard work conducted at pig artificial insemination centers (PAICs) in the future. This will aid process optimization and enable prediction of the fertilization potential of boars.

Indeed, the results of future research could facilitate the commercial incorporation of artificial insemination using sex-sorted semen. Selecting the sex of litters could allow for better selection programs, by shifting to one sex or the other according to the needs of each farm. In this case, cost reductions using a single insemination at a fixed time after inducing ovulation with GnRH agonists would be essential.

The usefulness of frozen semen as a genetic reserve is undeniable but in practice, the resulting fertility and prolificacy in pigs are inferior to those obtained through artificial insemination with chilled semen, while the cost of frozen doses also far exceeds that of chilled doses. However, keeping frozen doses in PAICs will ensure service for farms if there is a disease outbreak at the center and all the boars have to be culled. Moreover, it allows semen doses to be safely transported worldwide (Rodríguez-Gil and Estrada, 2013).

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