Recent advances in swine reproduction
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Abstract
Accurate estrus detection is an essential component of a successful breeding program in modern swine operations. It is labor intensive, time consuming, and an economically important aspect of the production system. Over the last 20 to 30 years, swine production systems have changed: there are fewer farms, with these farms holding larger numbers of breeding females. Along with this restructuring in the swine industry, application of techniques to improve reproductive efficiency and controlled breeding has occurred.1 Some of the assisted reproductive techniques now commonly utilized in modern swine operations include artificial insemination, hormonal induction of estrus in gilts, estrus synchronization of sows and gilts, and B-mode ultrasonography.2-5 Even with the addition of all of these technologies, prediction of ovulation is limited to a range of hours and is still considered a challenge for the swine industry.

In recent years, there have been several advances that have been made to improve the reproductive efficiency of the pig and increase the accuracy of inseminating at the proper time. Some of these advanced techniques include: induction of ovulation, fixed-time artificial insemination, deep uterine and post-cervical insemination, and cryopreservation of semen. These advanced techniques will be presented in this article as well as some discussion regarding production of porcine embryos and embryo transfer.

Keywords: pig, reproduction, assisted reproductive techniques, ovulation, artificial insemination

Introduction
Sows and gilts are currently artificially inseminated (AI) with multiple services (doses) of extended semen based on heat detection after estrus synchronization. Even with good estrus detection methods, it can be difficult to attain maximum fertility results since there is a wide range between timing of ovulation in relation to onset of estrus. According to Soede and Kemp6 (1997), pigs ovulate 10 to 58 hours after the onset of estrus. Best fertility is attained mating within 24 h prior to ovulation.7 The ideal time for insemination is 8 to 12 h prior to ovulation.8 The availability of these time periods provide a breeding window, however, the large variety in hours makes it even more critical for accurate estrus detection with the goal of mating as close to ovulation as possible. The broad challenge is being able to predict when the pig will ovulate.

The basis of most of the recent advances in swine reproduction has concentrated on manipulation of the estrous cycle in order to place semen as close to ovulation as possible. With this in mind, breeding programs are trying to become more efficient without adversely affecting pregnancy and farrowing rates. To increase productivity, research efforts have concentrated on induction of ovulation so that breeding protocols inseminate only once (fixed-time artificial insemination; FTAI) with a normal dose, reduced dose or cryopreserved boar semen.

Induction of estrus
The basis of all assisted reproductive strategies is for the female to be in estrus (cycling) and subsequently ovulate in a relatively predictable manner. With respect to estrus in the pig, there can be variation that occurs with the onset of estrus to ovulation and the length of estrus.9-10
Breeding management has concentrated efforts on synchronization of estrus initially to then assist in predicting when ovulation will occur.

Weaning

Traditional management and attempts to synchronize a group of females into a breeding schedule have involved weaning of piglets from lactating sows on the same day. Suckling piglets provide the stimulus for hormonal suppression and therefore, anestrus during lactation. This hormonal suppression provides the sow time for uterine involution, during which, the uterus must undergo a rapid loss of length and weight. This generally occurs during the first two to three weeks of lactation.\(^{11}\) Fernández et al. (2005) reported that the wean-to-estrus intervals are shortest for sows weaned between three and four weeks after farrowing.\(^{12}\) Sows weaned at less than 10 days of lactation demonstrate a much longer wean-to-estrus interval and in contrast, a greater percentage of sows that lactate for more than 20 days return to estrus by Day 7. The time of exhibiting estrus following weaning is linked to adequate time for uterine involution.

In general, females will show estrus 3-5 days post-weaning. The benchmark for wean-to-service interval (WSI) is less than 7 days and is directly related to the timing of behavioral estrus in the weaned female. The important thing to realize with this management practice is that sows can have a large variability when they come into estrus and the duration of estrus.\(^{9,10}\) Kirkwood (1999) has reported that sows that show signs of estrus within a short time post-weaning stay in estrus for a longer duration than those that come in later (> 5 days post-weaning).\(^ {13}\) This means that sows that come in early will ovulate later in estrus than those that come in later as they still ovulate approximately 2/3 of the duration of estrus. Without administration of an ovulation inducing agent, the manager spends a lot of time performing estrus detection and insemination based on standing estrus behavior.

Altrenogest

Altrenogest is an orally active progestogen which inhibits gonadotropin release, imitating the biological activity of progesterone (Matrix\(^{\circ}\); Merck Animal Health distributed by Intervet American Inc., Millsboro, DE). It does not prevent luteolysis, but it blocks the onset of estrus even after luteolysis and allows for continued follicular growth and development after its removal.\(^{14}\) In order to synchronize estrus in gilts, Altrenogest is administered orally as a top-dressing in feed at a dose of 15-20 mg/day/gilt for 14-18 consecutive days. Estrus can be expected 5 to 7 days after the last day of Altrenogest treatment.\(^{14,15}\) The majority of these females have been observed in estrus previously and considered to be cycling.

Altrenogest can be used to synchronize estrus in weaned sows – with most of the efforts being concentrated on primiparous sows as they have the most difficulty in resuming cyclicity post-weaning. There have been several protocols, involving varying doses and length of treatment, examined for treatment of these females. Investigators have compared length of treatment ranging from 4 up to 15 days with the initial day of treatment beginning the day before weaning.\(^{16-18}\) From review of these studies, it appears as if a longer treatment (15 days total) yielded the most acceptable farrowing rates compared to treatment lengths of 4 and 8 days.\(^ {18}\) Continued research occurs in this area (is ongoing) to determine follicular size and reproductive hormone profiles at various points during and post-treatment with altrenogest in primiparous sows.
P.G. 600®

The combination of gonadotropins, follicle stimulating hormone and luteinizing hormone, are used by the female to stimulate follicular development to an appropriate size and maturity to stimulate rupture of these follicles (ovulation). In swine breeding programs, this combination of these naturally occurring gonadotropins is marketed as a product (P.G. 600®; Merck Animal Health, distributed by Intervet American Inc., Millsboro, DE). This product is composed of 400 IU pregnant mare serum gonadotropin (PMSG) and 200 IU human chorionic gonadotropin (hCG) and is labelled to induce estrus in prepubertal gilts and return anestrus sows to heat. The PMSG acts like FSH and stimulates follicular development and the hCG acts like LH and induces ovulation. But since these compounds are given simultaneously, there is not an accurate time that ovulation is induced.

Administration of P.G. 600® to sows on the day of weaning stimulates ovarian follicle development and results in a greater expression of estrus with a shorter wean-to-estrus interval.19 Additionally, this leads to an increase in estrogen, an LH surge, and improves return to estrus in multiparous sows and primiparous sows.19-20 The average time of ovulation is not normally affected by treatment with P.G. 600® and sows will typically ovulate 45 hours after the onset of estrus.19

Induction of ovulation

The use of exogenous hormones to induce ovulation is a common practice in other species such as the equine where follicular development is visualized using B mode ultrasonography.21 Within the past 10-15 years, ultrasonographic imaging of the ovarian structures of gilts and sows has become more common and can be followed throughout the estrous cycle.22-24 With this increased information regarding follicular dynamics and assessment of formation of corpora lutea, the timing of when ovulation occurs can be more accurately predicted. In order to predict ovulation, breeding managers use products such as GnRH or LH-like compounds in combination with estrus synchronization protocols.

PMSG + hCG

Although these hormones are used in combination in the P.G. 600® product, they are used in a very specific protocol for the induction of ovulation. The ovulatory process involved in an estrus synchronization program using P.G. 600® is not as exact as when these hormones are given separately and at specific times.11 For induction of ovulation, the PMSG is given to stimulate the follicles to develop (follicular development) and then the hCG is given to cause ovulation. PMSG (usually in the form of P.G. 600®) is given to the sow at weaning or day 15 of the estrous cycle and the hCG is given 80-96 hours later.25 Various breeding programs have been implemented after ovulation is synchronized and may involve single or multiple inseminations.

GnRH analogues/agonists

Gonadotropin releasing hormone (GnRH) causes the release of FSH and LH from the anterior pituitary and has been used in a variety of species to induce ovulation.11 The use of GnRH in swine breeding programs has only recently been re-evaluated for its effectiveness at inducing ovulation and application in fixed-time AI programs.26 The administration of GnRH for induction of ovulation has involved intramuscular injection as well as deposition of a gel intravaginally.27-30 The injectable formulation (Receptal, Intervet, Angers, France) contains 10
µg buserelin, a GnRH analogue, that has been administered at between 77 and 120 h post-weaning or synchronization protocol to induce ovulation. Sows treated with 10 µg buserelin at 86 ± 23 h after weaning and inseminated 30 to 33 h later had farrowing rates (87% vs. 84.5% in treated vs. control, respectively). Additionally, similar litter sizes (13.6 ± 3.8 vs. 13.7 ± 3.2 in treated and control, respectively) were reported.28,30

The transvaginal formulation of GnRH may be the preferred route of administration as it would potentially minimize injection stress, carcass quality effects, and reduce the loss of GnRH from the site of deposition.29 The GnRH intravaginal gel product (Ovugel®; JBS United Animal Health; Sheridan, IN) contains triptorelin acetate which is a GnRH agonist. It contains 100 µg of triptorelin and is administered intravaginally 96 h post-weaning.28-30 According to the label, a fixed-time AI is performed approximately 24 hours post-treatment (day 5 post-weaning). Further studies are in progress to determine if including a gonadotropin at weaning or increasing the dose of triptorelin to 200 µg could improve the synchrony of follicular development and subsequent induction of ovulation so that FTAI breeding programs can be implemented.29

**Artificial insemination techniques**

Most swine breeding programs adopt the strategy of administering two to three individual inseminations, every 12-24 hours after the detection of estrus. Performing multiple inseminations can be critical due to the relatively short viability of oocytes and spermatozoa in the female reproductive tract and also because time of ovulation in the sow is highly variable, and unpredictable.31 Fertilization results are highly dependent on the time of insemination relative to ovulation; however, the moment of ovulation may vary between 35 to 45 hours after the onset of estrous behavior.6 Variation in the onset of estrus to ovulation interval limits the chance that insemination is occurring close to the optimal time, within 12-24 hours of ovulation.11 Breeding managers have increased the possibility of achieving this goal by performing at least 2 artificial inseminations with 3 billion sperm cells during standing estrus. This practice can be time consuming and many producers are interested in reducing the number of sperm cells in an insemination dose as well as performing a single insemination without having negative effects on reproductive parameters (farrowing rate and litter size).29

**Single fixed-time insemination**

With the advancement in protocols to induce ovulation in sows and gilts, the modern swine producer is interested in performing a fixed-time artificial insemination (FTAI) and still achieve high farrowing rates and litter sizes. Many of these breeding strategies have depended on the success of the protocol used to induce ovulation and the method of insemination – traditional cervical, post-cervical, or deep uterine insemination.

**Cervical insemination**

Traditional AI in swine has involved the placement of the insemination catheter into the cervix and “locking” it into the folds – similar to that of the glans penis of the boar.31 Semen is deposited via gravity flow combined with contractions of the female reproductive tract, under the influence of estrogen and oxytocin, into the cervix. The semen then migrates up the uterine horns into the oviducts where fertilization occurs. Because of backflow in the post-insemination period, catheters have been designed to allow for post –cervical and deep uterine semen deposition.32-33
Post-cervical insemination

This method of insemination has the goal of depositing semen in the uterine body. The main obstacle that must be overcome with using post-cervical insemination (post-CAI) compared to the traditional cervical approach is the cervical folds. This methodology introduces an inner catheter into the uterus by placing it in the lumen of the standard catheter that locks into the cervix. With the success of the design of these catheters, further investigation occurred with decreasing the dose of sperm cells (1-1.5 x 10^9 sperm in a total volume of 30-50 ml) without a significant effect on farrowing rates or litter sizes. Studies with this method allowed for further development involving boar semen, fixed-time AI, and deposition of semen further into the uterine horn.

Deep intrauterine insemination

Deep intrauterine insemination (DUI) is the deposition of semen further up the uterine horns compared to post-CAI. There have been various devices designed to accomplish this goal, with the current one being similar to that of the post-CAI catheter, but has a longer length (1.8 m vs. the 15-20 cm in the DUI and post-CAI, respectively). This technology has allowed more opportunities to use a 20-fold reduction in the number of sperm as well as volume and still achieve acceptable farrowing rates and litter sizes. It has been shown that sperm deposited deep into one uterine horn will migrate to the contralateral horn and fertilize oocytes that were ovulated from that ovary. This technology definitely lends itself to be a useful strategy for using other technologies such as frozen semen or sex-sorted semen.

Laparoscopic insemination

The most advanced method of insemination currently being developed for use in swine is the use of laparoscopic technique to inject a very low dose of sperm cells directly into the oviduct. This technology has limitations as it is not currently considered a field procedure as the sow needs to be anesthetized and specialty equipment is necessary, but could certainly be utilized by breeding companies to advance genetic progress. This technique allows visualization of the oviducts of the female pig and placement of 0.3 to 0.5 x 10^6 sperm into the oviduct. If higher doses of spermatozoa are used, polyspermy has been noted and this can be detrimental to the development of the porcine embryo. This technology shows the most promise when using sex-sorted semen.

Advances in semen processing

Boar semen is currently being collected and extended to a dose of 3 x 10^9 in 80-100 ml for artificial insemination via cervical methods. As discussed previously, there has been interest in reducing the number of spermatozoa in each insemination dose. This would increase the efficiency of the boar:sow ratio resulting in the reduction of boar numbers within the stud facility. With the reduced dose and volume, cryopreserved semen could be utilized. Frozen boar semen has the advantage over fresh semen in that it has an indefinite storage life. Other advantages of frozen boar semen includes the usage of semen from animals from all over the world, the banking of superior genetics, and offering another level of biosecurity when introducing new genetic material to a herd.

The main issue that needs to be addressed with the use of frozen semen in the swine industry is the cold-induced damage that occurs to boar sperm. The plasma membrane of boar spermatozoa is very sensitive to the extremely cold temperatures required for cryopreservation as
well as ice crystal formation during the thawing process. \cite{34,39} Investigators have concentrated their efforts to improve the actual freezing process, the cryoprotectant in the extender, and the components of the thawing extender. \cite{39-40} Until these aspects are improved, frozen-thawed boar sperm will be a research tool or utilized by genetic companies as the its lowered reproductive efficiency will not allow it to be used commercially although there is some very promising research using frozen-thawed boar semen and FTAI. \cite{41-42}

**Advances in other biotechnology**

The use of assisted reproductive technologies such as *in vitro* production of embryos, cloning, and embryo transfer has been investigated in the pig for several decades. The success of these biotechnological techniques has been fraught with many difficulties. \cite{32} Pig oocytes and embryos do not develop and mature well in vitro, polyspermic fertilization still occurs at a high rate, and cryopreservation of porcine embryos is subpar. The main reason for much of the failure is the high lipid content within the cytoplasm of these cells. There are a couple of review articles \cite{43,44} discussing the advances in the technologies involved in swine *in vitro* production technologies and cryopreservation of porcine embryos. Continued research in these areas will likely yield improved results in the future.

**Conclusion**

With artificial insemination being near 100% in the swine industry, advancements to improve the reproductive efficiency of the female as well as the male will continue. There needs to be a high level of success for an assisted reproductive technology to be adapted by breeders/producers. The industry has become accustomed to high farrowing rates (>85%) with large litter sizes (> 12 piglets). Although producers are interested in implementing new technologies, they are not going to accept a decrease in their reproductive parameters. Some of the technologies discussed in this article have promise in the industry: induction of ovulation and fixed-time artificial insemination may become as common as that of bovine reproductive programs. There is still a lot of room for improvement in the use of frozen semen and embryo programs before they will be used commercially.

**References**

35. Watson PF, Behan JR. Intraterine insemination of sows with reduced sperm numbers: results of commercially based field trial. Theriogenology 2002;57:1683-93.