EMERGING REPRODUCTIVE TECHNOLOGIES IN PIG PRODUCTION

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ABSTRACT

Without question, the implementation of artificial insemination (AI) has been the greatest achievement in swine reproductive technology during the last decade. Because AI in swine is not a new technology, the underlining technological enhancements of this procedure are most likely responsible for its recent popularity. It is widely accepted that reproductive performance as a result of AI can be as good and at times better than from the use of natural service. However, the major limitation of AI is the increased number of procedures associated with its use. These procedures are often associated with inconsistent reproductive performance in many herds. Although there are many factors that can effect the outcome of an artificial mating, the aims of this paper are to discuss the reproductive technologies that have had a positive contribution to on farm AI, their constraints to improvement, and the future need and application of new reproductive technologies in this and other areas of swine reproduction.

ARTIFICIAL INSEMINATION

Semen Analysis and Fertility Assessment

One of the advantages of utilising AI is that each boar ejaculate is assessed for some aspects of quality prior to being used in the breeding process. Generally, there are five parameters that are measured to evaluate boar semen quality: visual assessment, concentration, motility, morphology and acrosome integrity. Of these, concentration and motility are most routinely used for sorting ejaculates prior to processing. Measuring semen concentration or total numbers of spermatozoa in an ejaculate does not appear to be a valuable estimate of the overall fertility of the ejaculate (Flowers, 1997), but more so, as a tool to monitor the health an productive output of the boar. However, this parameter serves as the primary feature in processing boar ejaculates for optimising the genetic potential of a single individual.

Visual estimation of the percentage of motile and morphologically normal spermatozoa by light microscopy is the most widely used and acceptable method of semen quality assessment. The limitation to this procedure is the accuracy of the generated values. Fortunately, the fact the neither parameters are highly related to fertility and generally a surplus of spermatozoa is inseminated, allows for this inexpensive and relatively simple procedure to be currently used as means to evaluate ejaculates. Currently however, it would seem that to improve the efficiency of a boar ejaculate (produce more doses/collection), an accurate method to determine the number of viable sperm numbers in a dose would be of great benefit. Although Computerized Sperm Analyzers (CPA) are not new, they potentially are the best tools for

analysing sperm motility and in the near future, morphology. Like visual assessment, the generated results are highly dependent on the operational settings of the equipment as well is handling and care of the submitted sample. The major limitation to the widespread use of CPAs, like other technologies, are costs and repeatability. CPAs can be expensive and although they provide an objective measurement of semen quality, they remain disadvantaged compared to visual assessment because of the extra time required to process samples.

The search for *in vitro* methods and criteria to predict the fertilising ability of semen has been, and will continue to be, the subject of many investigations. It is likely that in the near future diagnostic approaches to assessing the fertility of an ejaculate will be developed in a manner similar to testing samples for pathogens with a simple diagnostic based test (PCR). However a strong correlation to a specific component or characteristic of the sperm cell or seminal fluid to fertility must first be determined (William Flowers, Personal Communication).

Semen Preservation

Developments in semen extenders for extended storage of semen have played a pivotal role in the development of AI procedures. Artificial insemination doses containing 40 x 10^9 spermatozoa (whole ejaculates) were commonly used during the early years of AI. However, these AI doses were seldom stored for more than one day without suffering a drop in reproductive performance (Rigby, 1966; First, *et al.*, 1968). Today, most semen extension media types are able to storage spermatozoa for up to three days without a drop in reproductive performance. Within most operations, this period of time is sufficient since: 1) semen can be collected and shipped to the sow farm on the same day or the day thereafter, and 2) in the case of the sow, estrus can be accurately predicted based on weaning. Semen can be collected on farm, but the centralisation of boars and modern delivery systems for semen has perhaps contributed to the growth of AI as much as any technology. Unfortunately, the major limitation in the widespread use of superior boars is the inability to store semen for more that 3 days without a drop in reproductive performance. The ability to store extended semen for longer periods of time is becoming more important because: 1) on-farm semen collection and processing is declining, and 2) AI implementation is being adapted world-wide and the demand for superior genetics is high in remote locations. In the latter, the benefits are obvious in meeting the goals of producing a high quality protein source in countries where other technologies for improving pork quantity and quality are not as widely available. Concerning the first, most commercial sow farms in the U.S. are not equipped to produce the required amount of semen during times when delivery of stud derived semen is not possible. As to not suffer a drop in reproductive performance, a viable supply of frozen semen would be of great value in most operations, however, most producers are not satisfied with the 20-30% drop in conception rates that can be expected from using frozen semen compared to fresh extended semen. Because a large number of spermatozoa are required for a swine AI and it is difficult to cryopreserve pig sperm, an increase in frozen semen on commercial farms as an alternative to fresh semen is not foreseeable in the near future.

There have been few changes in developing a “long” term fresh semen extender, however, some recent developments in boar semen freezing may be of some benefit to the distribution of pig semen in the near future. One of the obstacles for freezing boar semen is actually not

the extension medium, but the ability to freeze large numbers of sperm (3-5 billion) in a single container at a controlled rate. Boar semen doses have traditionally been frozen in either a pelted form or in a round 5 ml straw with modest success. Eriksson and Rodriguez-Martinez (1999) have developed a new type of flat plastic package for frozen boar semen. The Flatpack is a single flattened straw, which, similar to freezing small quantities, equalised the freezing rate throughout the whole cell population. Their system is slight modification to traditional freezing techniques and the potential gains in fertility rates appears to range from 70-80% compared to 50-60% associated with the round 5 ml straw. IMV International has recently implemented techniques to freeze boar semen in 0.5-ml straws, which improves the freezing rate of cell samples. It appears that, as a result, the viability and resulting fertility rates from the use of these frozen sperm is improved. The limitations of this technique are that 6-10 straws are handled per dose, and the equipment needed to efficiently process many collections can be expensive.

Semen Sexing

The potential benefits of pre-breeding sex determination are numerous when considering:

1. The inefficiency of castrated males within a meat producing system, and

2. The value of male or female offspring in a terminal or maternal seed stock multiplication system.

The Beltsville Sperm Sexing Technology (Johnson and Welch, 1999) has been proven as the only effective means of altering the sex ratio of offspring in livestock and humans. Commercial application of the technology will become apparent in the next few years, but this application strongly hinges on the ability of this research to develop faster technologies to sort sperm cells. Considering that just in the last 5 years the sort speed has increased over 10 fold (0.6 million/h vs. 10 million/h), this technology is still too slow to produce the enormous numbers of sperm needed for conventional AI (~2 billion). However, in cattle and horses this technology is beginning to be adapted since smaller numbers of sperm are needed for AI and bull and stallion sperm can be frozen with little complications. In the event that the sort rates continue to increase, boar semen cyropreservation technologies are improved, or practical methods to inseminate with low numbers of sperm evolve, this technology will certainly make a dramatic impact on the swine industry.

The Beltsville Sperm Sexing Technology has been perhaps the most publicised technique for sexing semen, however, other technologies could be on the market in the near future that appear to be even more promising. A new approach to immunologically sex semen has recently been reported. This technique is a non-invasive method that utilises differences in protein characteristics to sort X and Y using antibodies specific to each sperm class. It is presumable that both technologies for sorting sperm will become commercially available in the near future. The costs for utilising this technology will be initially a major limiting factor, but pork producers should be prepared to utilise this process in production areas where altering the sex ratio could be of most benefit, such as gilt multipliers or nuclease farms.

GAMATE TRANSFER TECHNIQUES

Low Dose Inseminations

Some studies have suggested that as little as 1 billion inseminated spermatozoa can maximise fertility rates in optimal conditions. However, most farms increase this number to nearly 6 billion cells per dose to mask sub-optimal breeding conditions present on most farms that cause inconsistencies in reproductive performance after inseminating with low numbers of sperm (Baker *et al.*, 1968; Steverink *et al.*, 1997). Most farms today inseminate sows with 2-5 billion sperm cells, and an obvious benefit to lowering the ultimate numbers needed for AI would be in optimising boar usage and stud efficiencies. There are three limitations to applying on farm low dose inseminations: (1) sperm transport is highly inefficient in the pig, (2) optimal insemination conditions are consistently required (technique), and (3) semen evaluations are too imprecise to accurately ensure that a required population of fertile sperm are actually inseminated.

Recent reports suggest that as few as 10 million sperm cells can be inseminated surgically without significant drops in fertility (Rath *et al.*, 1999), and similar results have been obtained using non-surgical deep uterine insemination with specialized equipment (Struthers Inc. 2000; Vazquez *et al.*, 1999). These approaches are being developed to achieve reasonable fertility when using biotechnologies such as sperm sorting where larger numbers of sperm are not available. Obviously, the time required to perform an insemination and the inconsistencies in reproductive performance resulting from these procedures, limit the on farm use of these technologies. Their application in the future will greatly depend on the advancements and costs of the equipment needed to quickly perform a deep uterine insemination. There have been numerous press releases related to the development of a fiber optic scope for performing a deep uterine insemination with low numbers of sperm (Gourley Scope) that bears mention. Although the Gourley scope may hold a small competitive advantage today, researches in Germany have shown that the success of a low dose insemination is not dependent on a specific placement in uterine horn and that the only obstacle for improving sperm viability remains the cervix and the major uterine body. Therefore, placement of semen at the beginning of the uterine horn should yield similar results as placement of semen much deeper in the uterine horn cavity. Although there is no published evidence of performance improvements due to insemination beyond the cervix, it is conceivable that a 20 to 40% improvement in sperm survival or overall AI efficiency from adapting this technology is possible. Because it is not that difficult to transverse the cervix with a small catheter, I anticipate that within the next year a disposable apparatus that serves the same function as the Gourley scope for insemination will be introduced to the market. Producers should be aware that regardless of this technique becoming affordable, more skill is required to perform this procedure and if conducted inappropriately, a loss in reproductive performance will occur due to the sensitive nature of uterine tissues to abrasion. Producers should definitely consider their technician's ability before rapidly adapting this technology.

Reductions in Inseminations

As an alternative to semen placement techniques, methods to improve the viability of the sperm deposited into the uterus using the traditional insemination technique seem more promising. Although spermatozoa capable of fertilising an egg have been recovered from the reproductive tract of the sow nearly 2 weeks following insemination, the fertile lifespans of sperm are generally considered to only be 12-36 h following deposition (Polge, 1978). Our current knowledge of the limiting factors to prolonged sperm survival in the pig has only recently become expanded. Accumulating evidence from our laboratory suggests a post-breeding uterine inflammatory response, which appears to be vital for clearance and preim-plantation preparation, may also be one on the most significant limiting factors for prolonged sperm survival in the female tract as well (Rozeboom *et al.*, 1999). Sperm are only viable in the female reproductive tract for about 24 h and, thus, insemination intervals shorter than 24 h may not be necessary on most farms if each AI is performed with a sufficient population of fresh, viable sperm. However, viability or longevity of sperm in the female tract appears to be dependent on the components of the insemination dose. Seminal fluid, which is often diluted extensively during semen processing, seems to protect inseminated spermatozoa and may reduce the potentially negative impacts of inadvertent inseminations performed at the end of estrus. Our *in vitro* data suggests that a minimum of 10-12 % of the entire AI dose should consist of seminal plasma to protect and improve the viability of spermatozoa once it is in the female reproductive tract. Therefore, we suggest that boar studs and/or producers collect the entire ejaculate, because the entire fluid portion in an ejaculate is seminal plasma, and dilute semen to reach target seminal plasma volumes in AI semen doses. Future applications of synthetic seminal components to AI doses are inevitable, since reduced external shelf life (storage) is a major constraint to large volumes of natural seminal plasma present in AI doses.

Embryo Transfer

Embryo transfer in other species (cattle, horses) has been for some time a very affordable and successful means of genetic dissemination. In pigs, however, the need to implement this tech-nology into practice has only recently surfaced. In light of pork production's health related challenges, the means to preserve genetics while eliminating devastating diseases has now become a higher priority. There have been recent reports of successful embryo transplants using non-surgical approaches. A reliable means to transfer pig embryos non-surgically will greatly increase this practice in critical situations of genetic multiplication where health issues can influence large numbers of breeding stock throughout the swine industry. Surgical transfer of pig embryos remains the most reliable choice for embryo transfer in pigs. A team of Canadian researchers is at the front of this emerging reproductive technology to enable producers to improve the health status of existing genetic lines and multiply the genetic potential of superior sow lines (Rohman, 2000). It is important to note that in the past these procedures relied mainly on the practice of transplanting freshly recovered frozen embryos, since pig embryos have been notoriously difficult to cryopreserve. However, research in this area in a variety of locations (Dobrinsky, 2000) has made tremendous strides in improving the embryo transfer success rates of frozen pig embryos. During the next decade, one should expect this technology to be practical for genetic companies to improve health status, and to preserve and distribute existing female lines here and throughout the world.

BIOTECHNOLOGIES

In Vitro Fertilisation, Embryo Cryopreservation, and Cloning

One may argue that these technologies have very little place in commercial swine production since pigs are a litter bearing, highly fertile domesticated species where genetic progress can be made rather quickly because of a short generation interval. However, two of the limitations to the widespread use of swine genetics throughout the world are: (1) the current health status of most of the world swine population, and (2) the transportation costs associated with the transfer of genetics (Pollard and Plante, 1998). From a discovery standpoint, advances in the development of *in vitro* produced embryos should ultimately lead to better research on fertility and embryo survival issues that directly impact swine production and efficiency. Developments in this area have been slow and its impact on the swine industry is not likely in the near future. Methods in female gamete transfer and long term storage have rapidly progressed during the last decade. Pregnancy rates associated with these procedures are at least now measurable (~40-50%) and may be applicable to swine systems in the near future. Their potential benefits may include: rapid development of genetically superior lines of animals, the eradication of certain pathogens in developing disease free operations, international sales of genetics through frozen embryo transport, and the development of transgenetic animals for the use in human xenotransplantation medicine. Commercial application of all these technologies is limited, however, to advancements in procedural efficiency. Most of the procedures involved in these technologies often require expensive laboratory equipment, surgical expertise, indirect costs and time for trial and error.

It is also important to recognise the potential medical applications of the swine species that are evolving through cloning and organ transplant research. It has now been reported that two separate teams have successfully cloned pigs, a tricky accomplishment that opens the door to breeding herds of genetically engineered pigs to farm for organ transplants to people. But other scientists said they had found tough barriers to such an attempt -- saying they had shown that human cells could be infected with potentially dangerous viruses from pigs. So far sheep, cattle, goats, mice and monkeys have been cloned. One idea is to breed genetically identical farm animals that can produce human products such as proteins for use in medicines. They have also been seen as a potential source of organs and tissue for transplant into people.

PREGNANCY DIAGNOSIS, INSEMINATION TIMING, AND UTERINE PRIMING

Pregnancy Testing

Historically, daily boar exposure is the earliest and the best means to diagnose open sows and gilts. The development of ultrasound techniques for pregnancy diagnosis was a great stride in improving overall herd reproductive performance, since daily boar exposure does not catch all open sows and gilts in heat. The most common types of ultrasonic equipment for these purposes are A-mode and Doppler ultrasound. A-mode ultrasound machines are programmed to emit a beep in response to fluid in the reproductive tract, confirming pregnancy. Doppler machines allow the user to actually hear movement associated with the fetal heartbeat and the

pulsing blood flowing through the uterine artery, indicative of pregnancy. They are accurate beginning around day 28 to 35 of gestation (day 0=day of first breeding), however, each has limitations. Both instruments are 'yes' or 'no', there is little room for judgement. As a result, each may yield erroneous diagnoses.

Within the last decade, the use of another means of pregnancy diagnosis, real-time ultrasound (RTU), has become more widely applicable. Similar to other methods, RTU utilises the same principles of emitting and receiving sound waves. However, RTU displays the resulting information as a two-dimensional image allowing the user to see the reproductive tract and its contents, thereby reducing the chance for diagnostic error. This may impact producers as earlier diagnosis has the potential to decrease non-productive days and reduce the time-spent heat checking, positively altering animal flow. RTU's major disadvantage is cost, but its accuracy (nearly 100% at 23 d) and benefits in reducing non-productive sow days are significant in most herds of reasonable size (>600 sows). It is also important to note that other technologies are also emerging as potential low cost, simple and accurate means to diagnose pregnancy in swine. A competitive inhibition-type enzyme-immunoassay (EIA) has been developed for direct measurement of hormone levels in swine urine. In a field trial with a group of 387 sows (7 in estrus, 16 non-pregnant and 364 pregnant sows at several stages post service), it was shown that the assay is potentially an accurate pregnancy test in assessing the viability of the fetoplacental unit from day 23 up to day 30-post service. This type of assay is potentially well suited for routine testing, particularly as a swine early pregnancy diagnosis test since urine sampling is easier and does not disturb the animal, while in the present assay there is no restriction in the time of sampling and the sample storage conditions (Stefanakis *et al.*, 2000).

Techniques to Improve Insemination Timing

In addition to pregnancy testing, real time ultrasound has also helped define insemination strategies by characterising ovulation patterns in the sow retrospectively. Initially, some researchers have felt that it may be useful in tracking follicular growth, and hence, use this technology in a prediction model for ovulation and subsequent timing of a single fixed time AI. Unfortunately, it does not appear to be effective in predicting ovulation ahead of time, that is, when ovulation will occur, and this is a major constraint in using this technology. Nevertheless, countless hours of research have been conducted on retrospective analysis of the moment of ovulation and on defining relationships between the time of ovulation relative to estrus behaviours. These relationships have ultimately led to methods that allow producers to more accurately time inseminations. Even though there is large variation in the time that ovulation occurs after estrus is first detected, ovulation consistently takes place at a relatively fixed two-thirds of estrus length in most herds and females. Thus, even if a female's estrus length is known, then we have a pretty good idea when ovulation will occur. However, there is some variation in ovulation time relative to estrus length, but this response can be verified using real time ultrasonography. Briefly, once estrus is detected transcutaneous flank ultrasonography (Weitze *et al.*, 1989) can be performed using an ultrasound machine, preferably with a with a 5 MHz micro-convex probe (Universal Medical Systems, Bedford Hills, NY) to detect the presence (pre-ovulation) or absence (post-ovulation) of tertiary follicles greater than 6 mm in diameter. When pre-ovulatory follicles are present, ultrasound should be repeated

morning and afternoon until these follicles disappear. The absence of large follicles indicates that ovulation occurred. Females should then be checked for estrus at the same interval until they are no longer in standing heat. Subtract 6 h from each time (ovulation and estrus length) and an estimation of ovulation relative to estrus length can be accurately established in this herd. The number of females required to accurately establish this relationship should be around 10 % of the total population of the breeding herd. This description has been developed from subcutaneous ultrasonography, whereas, some researchers have suggested that rectal sonography can allow faster and more detailed means of assessing fine reproductive structures of varying echogenicity (Knox *et al.*, 1999). We have in our laboratory, however, used only subcutaneous ultrasonography, since rectal sonography requires specialised transducer rods and our current system up to now only requires a gross structural evaluation of the ovaries. It is become increasingly evident in our research program that ultrasonography is perhaps a more valuable as a tool in retrospective analysis of established insemination timing schedules than as a tool for predicting when to inseminate.

Uterine Priming

Priming the uterus for mating before or during insemination with either synthetic or natural products to enhance subsequent fertility has been attempted through various means during the past 25 years. Leucocytes, estrogens, oxytocins, and prostaglandins have been either added to semen or injected into the female as a strategy to enhance reproductive performance of artificial matings. The effectiveness of additions of these types of compounds has yielded only small improvements or no positive effect in most published trials. In most instances where improvements have been noted, the positive effect has been attributed to masking or covering up sub-optimal fertilisation conditions such as old or poor quality semen, poor AI techniques, and poor estrus detection (Flowers and Esbenshade, 1993). There has, however, recently been a renewed interest in this area because of recent reports of a positive influence of "priming" the uterus with dead semen or synthetic seminal plasma during the estrus period just prior to mating. In both cases, these reports have reported about a 0.5 pig advantage to this technique, however, there is no scientific literature to support these claims as of yet and one could perhaps expect that the advantages to implementing these types of programs across many farms would yield highly variable results. Nevertheless producers should expect further publicity of these types of insemination technologies and, as with most new procedures, approach them with optimistic caution, since simple changes in either estrus detection, or AI procedures that can yield similar results without sacrificing extra labor or product costs would certainly be considered more cost effective. However, a positive cost/beneficial procedure like this could be easy implemented on most sow farms and therefore should never be overlooked.

Hormonal Therapy

Historically, gonadotropins and progestens have been used with limited success to improve reproductive performance in swine and this author does not recommend using them on a routine basis. Nevertheless, application of these hormones in specific swine management areas has helped reduce the reproductive lag associated with these recent management trends that can potentially improve sow reproductive performance. Two hormonal strategies using

PG600® (400 I.U. PMSG + 200 I. U. hCG) and Regumate® (progesterone) have been introduced to counteract the negative effects of shortening lactation lengths. PG600® can be injected at weaning to stimulate follicular growth, speed return to estrus intervals and reduce the incidence of anestrus. However, costs are a major limitation and therefore this approach may only be beneficial in herds where extended wean to estrus lengths (>10d) or high frequencies of anestrus are occurring. Some producers only treat problem groups of sows such as those with low lactation feed consumption or of low parity to improve the efficiency of this technology.

Extended weaning to estrus intervals and anestrus following weaning in parity 1 sows are probably the most noticeable effects of poor lactational feed intake, short lactation lengths, and heat stress on reproduction. The combination of heat stress, parturition, lactation, and poor feed intake contribute to poor reproduction in all sows; however, P1 females also have a metabolic demand for growth. One strategy to minimise these impacts on overall herd reproduction is to adjust female replacement schedules to avoid large numbers of P1 farrowings during July and August. It may also be possible to treat this subpopulation of females with hormonal therapy during lactation and at the time of weaning to stimulate the reproductive system. A single injection of PG600® at the time of weaning has been effective in reducing weaning to estrus interval in sows. However, a recent field report suggests that a vulvular injection of 1/2 cc. of Estrumate (not currently labeled for swine use) within 24 hours after farrowing in conjunction with PG600® at weaning may be even more effective at reducing weaning to estrus interval and the incidence of anestrus than the use of either of these components alone.

Continual feeding of Regumate® suppresses follicular growth and estrus until withdrawn. Its usage appears to be useful in estrus synchronization of cycling females and as a strategy to improve reproduction performance after a short lactation length (feed throughout lactation and withdraw at weaning). In addition to costs, the delivery system is a major limitation. Regumate® is currently produced in an oil-base form that is difficult to handle and constrain.

CONCLUSIONS

Reproductive technologies have and will continue to benefit swine production systems. I would argue that in the advent of large scaled operations with a lack of skilled labour and management, the recent application of both new and old technologies actually masks the potential losses associated labour deficiencies. It is with certainty that other reproductive technologies will continue to be developed in addition to the ones mentioned here. The impact of present, developing and other conceptual technologies is certainly dependent of the cost/benefit relationship of their application directly on the sow or boar unit. When developing new reproductive technologies for the new millennium, I urge science to ask the right questions. What is the problem in the production unit (industry, operation or female) that needs improvement and will the benefits of the technology outweigh the limitations in terms of time, money and market influences? Traditionally, the application of reproductive technologies into swine production is slow and one must understand that there are very few technologies that can or will make dramatic changes in a short period of time.

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