EMERGING TOOLS IN ARTIFICIAL INSEMINATION

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ABSTRACT

Artificial insemination (AI) is arguably the single most important tool in improving swine genetics. AI units select and house boars who meet their customers' needs, and collect and process semen of adequate quantity and quality for insemination. Swine producers must efficiently use semen and labour to maximise the number of females pregnant, and the number of piglets produced per litter. Both the AI units and the swine producers must drive their suppliers to undertake the research and development necessary to improve performance. For the AI units this means more semen, stored longer and producing more inseminations per ejaculate. For the producers it means a higher conception rate and larger litters year-round. Both the artificial insemination units and swine producers must prepare for the markets of the future, which could include the need for sexed semen, transgenic pigs, and a major international export market.

INTRODUCTION

In North America in 1994, artificial insemination (AI) was used in 15% of all swine breedings. That number reached 50% in 1999 (Buhr, 1999) and AI is predicted to cover 80% of all breedings by 2005 (Burke, 2000). Over these same time periods, pork production systems continue to change, striving for greater production efficiencies and to meet the demands of consumers (for product and for the quality of production), niche markets, and legislators. The North American AI industry is in the challenging position of having to meet these rapidly changing demands during its own period of phenomenal growth. In addition, the "AI industry" has two parts: the AI units producing the semen, and the swine producers using the semen. Emerging tools for AI, then, are the implements, procedures and strategies that will start build on the best of today's foundations to produce the most, highest quality sperm and the most, highest quality pigs.

PRODUCING THE MOST, HIGHEST QUALITY SPERM

To produce and package the optimal number and quality of sperm, we have to look at both the boar and the processing of the semen.

The Boar

I. Genetics

A boar is typically selected for inclusion in an AI semen production unit based on his genetic potential to produce piglets of excellent growth and conformation, and perhaps to produce female offspring with good mothering traits. It is also becoming evident that he could be, and arguably should be, selected for his semen traits, although many relevant aspects of this are poorly understood. Certainly semen quality in diary bulls can be inherited (Mathevon, Buhr and Dekkers, 1998), and boars selected for 10-11 generations on large scrotal circumference produced more sperm than boars randomly selected for the same period (Huang and Johnson, 1996). These boars also had more efficient testes, producing more sperm per gram of testes than did the controls' testes, and the bottom line is that the boars produced between 4 to 14 billion more sperm per ejaculate when collected three times per week.

Seminal plasma (the fluid minus the sperm) is a large portion of a boar's ejaculate, and some of its components might affect fertility. Seminal plasma is rich in proteins, and some of these proteins might improve the conception rate in cattle (Thérien *et al.*, 1997; Sullivan, 1999). These proteins apparently differ from one male to the next. Inseminating a female with seminal plasma from some boars, but not from others, prior to inseminating with sperm improved the conception rate and litter size (Soede *et al.*, 1998; Waberski *et al.*, 2000).

Boars can also be responsible for transmitting undesirable genetic effects to their offspring. One genetic defect leads to small litters, and at least half of the daughters would also produce small litters were they bred (Makinen *et al.*, 1999). This defective gene has been identified and can be detected with DNA screening. Hunter and Greve (1996) suggested that sires can be responsible for intersex gilts (also called pseudoherrmaphrodites or hermaphrodites). These gilts have vulvas that tip upwards, and a variety of other, less obvious and less consistently present, physical and behavioural abnormalities that interfere with normal breeding and pregnancy. No gene in the sire has yet been positively identified as responsible for this trait, and so it can only be tracked back to sires through litter reports.

The swine industry can benefit by maintaining a proper perspective on these genetic aspects. First, genetic research can eventually identify beneficial genes, and the industry can select for them, moving into such technologies as marker-based selection. AI units can benefit the Canadian swine industry by using their data (litter reports, etc.) to identify, trace and eliminate pigs carrying deleterious genes. The new Canadian Association of Swine AI Units can discuss with representatives of the Canadian swine industry the pros, cons and costs of screening all boars entering an AI unit for genetic abnormalities. Such a regulation is in place in France, and Sweden is reportedly considering a similar recommendation (in Makinen *et al.*, 1999).

II. Nutrition

Nutrition probably affects semen production, although very little directed research has been done on swine. Diet alters the membranes of bull sperm (Buhr *et al.* 1993), and diets rich in linolenic acid improved the fertilising ability of rooster sperm either temporarily (Kelso *et al.*,

1997) or permanently (Blesbois *et al.*, 1997). A recent review (Wilson, 2000) noted that different genetic lines of boars probably have different dietary needs for optimal sperm production: for example, lysine's variable effect on libido may reflect the different genetic makeup of the boars studied. Proper mineral balance is critical for strong feet and legs in a breeding boar; vitamins, fibre and 'feeling full' may enhance sperm production by alleviating stress; mycotoxins are suspected to decrease sperm quality and fertilising ability (Wilson, 2000). All of these possible influences indicate a real need for some dedicated directed research into the impact of feed on sperm production.

III. Environment

The environment of the boar is made up of many parts, but certainly includes temperature, light and handling. There is no question that high temperatures damage sperm production (volume, sperm concentration and sperm quality) in animals like the pig that have a scrotum. When nearly 30 boars in an AI unit were evaluated over 2 years of tropical temperature ranges (minimal changes in daylength), the percent of motile sperm and the percent of normal sperm dropped from the cool to the hot season. Interestingly, there was a difference among breeds, with Landrace boars having more motile and more normal sperm in the hot season than Duroc or York boars (Huang *et al.*, 2000). Because the process of producing a sperm takes approximately 60 days in a boar, the effect of a heat episode (be it a heat wave or a fever), often is felt any time over the subsequent 6-8 weeks. The idea of air-conditioning an entire swine facility is probably not cost-effective. Since pigs cannot sweat, they naturally will wallow in cool wet places to help cool themselves when it is hot. Modern housing facilities cannot provide wallows, but some provide overhead showers (either pig-operated or management-controlled) to try to allow the pigs to compensate for hot days. These can be quite effective, but work best with good ventilation and must be evaluated in any one operation for its effect on the slipperiness of floors and the possibility of foot rot or similar health issues common to damp areas.

Pigs are sensitive to season, independent of the temperature. Day length is more important than light intensity (within reasonable limits), and is driven by the nature of the modern pig's ancestor, the European wild boar. Male piglets are slower to reach puberty in the spring than in the autumn, with the testes and certain other sex glands being more mature in 141-day old autumn pigs than the spring-reared pigs at the same age (Andersson *et al.*, 1998).

Housing and handling of boars is yet another area that is believed to affect sperm production, but there is little valid information available. Culling in AI units ought to be primarily for genetic reasons, and proper handling and housing can decrease the number of animals culled for injury or behavioural reasons. Boars in crates may have an increased frequency of leg problems compared to boars in pens, although boars in pens find it easier to masturbate which reduces sperm available for collection and sale. Bedding such as straw is also recommended, as it can reduce stress and improve comfort through reducing wetness, providing rooting material, and increasing the fibre in the diet. Straw bedding may or may not reduce leg problems, but must be obtained from a reliable supplier to protect biosecurity (Glossop, 2000). Many authorities recommend regular exercise, even if that is just running up and down alleys that are not wide enough for the boar to turn around. Optimal boar housing for sperm

production is not known, but increasing the comfort of the animal will reduce stress and improve the public perception of animal husbandry, both of which are beneficial to the swine industry.

IV. Health.

The health of the boars in an AI unit is of paramount importance in maintaining biosecurity in the swine herds served by the resident boars. In general, AI semen is far less of a health hazard than natural mating, as the boars are regularly health tested, the processed semen is treated to minimise bacterial contamination, and there can be no cross-contamination of uterine infection from one sow to another as is possible in natural mating when one boar serves multiple sows. However, there is also very clear documentation of semen from infected boars transmitting classical swine fever (deSmit *et al.*, 1999), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV; Gradil *et al.*, 1996; Christopher-Hennings, 2000), and doubtless other viruses. A low percentage of the females inseminated with this semen developed the disease, but even one infected female is enough to infect and devastate a herd that was previously virus-free. Boars shedding the virus in their semen are frequently symptom-free, and thus the best way to decrease the transmission is to test boars with the highly sensitive new detection assays based on PCR technology. New and more effective vaccines are constantly being developed, and it is therefore possible to protect boars already in an AI unit. It is important to note that vasectomised boars can also be carriers of PRRSV, so producers must make sure to protect all the animals in their herds as well as insisting on the highest possible health standards from their semen providers.

Processing the Semen

I. The laboratory

A laboratory producing the highest quality semen starts with proper collection, carefully dilutes the semen with a solution ('extender') designed to maintain the sperm's fertility, and evaluates the quality of the semen before and after extension. In this way, the customer is provided with a product best able to maximise conception rates and litter size.

Ia. Semen collection

Semen is collected from a mature boar two or three times a week, using the gloved hand technique and having the boar mount a specially built 'dummy' sow. Proper collection procedures are outlined in several publications (e.g. Buhr, 1999). Briefly, the boar's prepuce is emptied of any retained fluid and the underline is cleaned with a disinfectant and then water, to reduce the chances of bacterial or other contamination. The disposable gloves are made of a non-spermicidal material and changed after cleaning the boar, between boars, and whenever they become soiled. It seems unlikely that an artificial vagina will be developed for boars in the near future, due to general satisfaction with current methods. Semen is collected into a container whose temperature is maintained at 35°C, to prevent the semen from experiencing a rapid temperature change. Boar sperm will easily suffer 'cold-shock' if abruptly chilled as

little as 3°C. Cold shock will kill or damage the vigour of sperm, and reduce the sperms' longevity (the length of time a sperm remains able to fertilise). Chilling injury can result from drafts in the collecting room, cold hands, an improperly warmed collection vessel, etc.

The sperm rich portion of the ejaculate is collected through sterile gauze to filter out gel particles. Gel particles in the semen will attract and bind the sperm and, if present in sufficient numbers, will reduce the fertilising ability of the semen by tying up the sperm. The person collecting the semen and/or handling the boar definitely influences the amount and quality of semen collected (Mathevon, Dekkers and Buhr, 1998). A good handler makes the animal comfortable, confident, and stimulated, and, by carefully noting the beginning and end of the sperm-rich portion of the ejaculate, collects all, and only, the sperm-rich fraction. The sperm-poor seminal plasma that precedes and follows the sperm rich fraction is currently collected separately and discarded, but may some day provide a value-added product for the AI industry. The seminal plasma of some boars will, when inseminated into some gilts and sows, enhance conception rates and litter size (Waberski *et al.*, 2000). When we understand what the important factor(s) is/are to stimulate the female response, certain boars may have their seminal plasma harvested for use as a fertility enhancement treatment.

Ib. Extender

The best extender is always a controversial topic, and commercial suppliers are always developing new products (e.g. Kuster and Althouse, 1999). There are usually approximately 3 billion (3×10^9) sperm in 70-100 ml of any extender plus antibiotics for one insemination dose, and most females are inseminated at least twice. Boar semen is currently extended in extenders described either as 'medium-term' or 'long-term', meaning that they will keep sperm viable for up to 3 days (72 hours) or up to 5 days (120 hours) from the day of collection, respectively. Most extenders recommend storage at approximately 16-18°C, and stored sperm should be gently remixed in the bottle every 24 hours. Carefully controlling the rate at which freshly-collected semen is cooled during processing can allow semen to be stored at a temperature not lower than 12°C (Althouse *et al.*, 1998). A producer needs to estimate when his females will be in heat, when the semen from the desired boars will be available, and ask the AI unit if they can supply semen that will meet those storage conditions. Everyone involved must recognize that the longer term extenders are more expensive, and boars are always different, so that some boars will produce semen that lasts quite differently when compared to semen from another boar in the identical extender.

A major problem for any comparison of any semen processing procedures is to get sufficient inseminations to adequately determine if the new treatment does indeed have an effect. This requires hundreds or thousands of inseminations, and so is frequently carried out at a commercial operation. A commercial unit needs adequate conception rates to remain economically viable, and so insemination is frequently done with 3 or more billion sperm in each of two or three inseminations. This is considerably more sperm than necessary to ensure maximal fertilisation, and so frequently an effect of a treatment is not seen because so many sperm used that their sheer numbers overwhelms any small but important difference among treatments (Tardif *et al.*, 1999).

Ic. Sperm dose

This brings up the interesting question of sperm dose - how many sperm are required to produce the most pregnancies with the most piglets. This is not an easy question to answer, as semen from different boars responds differently, and many other factors (timing of insemination, age of the female, etc) affects the success of the fertilisation. However, weaned sows inseminated with 1, 3 or 6 $x10^9$ sperm at a variety of times before ovulation had similar pregnancy rates, but there was a trend to bigger litters with more sperm (Steverink *et al*., 1997). Pubertal gilts induced to ovulate with PG600 had similar pregnancy rates and litter sizes with 3 and 0.3 x 10^9 sperm (Tardif *et al.*, 1999). All these results were obtained with 'normal' cervical inseminations, but progress in deep intrauterine inseminations may make inseminations with substantially fewer sperm economically viable. Prepubertal gilts induced with PG600 were surgically inseminated with 0.002 to 0.5 billion sperm placed directly into the oviduct. The lowest doses tended to reduce litter size and percentage of normal embryos, but did not affect conception rates (Krueger *et al.*, 1999). Producers are certainly not likely to widely embrace surgical insemination, but several commercial suppliers have designed and recently released a deep intrauterine insemination rod. This could permit insemination doses to contain fewer sperm while maintaining excellent conception rates and litter sizes, thus allowing more litters from the most popular boars. Such technology will also facilitate the use of sexed semen and frozen semen, and possibly embryo transfer.

II. Predicting fertility

Every semen processing lab assesses the freshly collected semen, measuring the concentration of sperm (usually by a spectrophotometer, Coulter Counter or haemocytometer), the volume of semen, and the percent of motile sperm by visual estimation of how many sperm are moving under a microscope. Many labs will at least occasionally evaluate sperm morphology, which involves microscopic examination and classification of the shape of at least 100 sperm from an ejaculate. If all freshly-collected ejaculates, regardless of apparent quality, are used for insemination, the fertility of the semen is related to the percent of motile sperm. This is particularly evident if the inseminations are done with a relatively low number of sperm. The conception rate in prepubertal induced gilts was correlated with visually-estimated motility when the gilts were inseminated with 0.3 x 10^9 sperm, but not when they were inseminated with 3 x 10^9 sperm (Tardif *et al.*, 1999). Much work has, however, confirmed that if the worst-quality ejaculates are eliminated from such a breeding trial, then the percent of motile sperm is only poorly correlated to fertility. In other words, in a group of medium-to-good quality ejaculates, a change in the visually evident motility does not necessarily lead to a change in fertility – and the same holds true for morphology. This means that the tools used to evaluate sperm quality do not relate to the single most important aspect of quality – the fertilising ability. Therefore there is considerable interest in developing a test that does predict fertility.

Several sophisticated sperm motility analysers are now available. These computer-assisted sperm analysers (CASA) machines can analyse many different aspects of sperm motility (how fast, how straight, how vigorous) and can even be set to analyse morphology. CASA machines are very expensive (normally over $50,000.00 Canadian), require a well-trained

technician, and require relatively expensive slides (but with these slides the machine can assess sperm concentration as well). When analysed in this way, some aspects of motility are related to both conception rate and litter size (Holt *et al.*, 1997). However, this analytical system is probably too expensive for most AI units. Other new tests are constantly being developed, found capable of differentiating among boars in the lab, but then do not succeed in predicting fertility (Tardif *et al.*, 1999; Holt *et al.*, 1997). Therefore AI labs continue to measure sperm quality by the best methods they have, but do so recognizing that there is little relationship of these measures to practical fertility. They therefore compensate by including more sperm than ought to be necessary in order to ensure that their customers have optimal conception rates and litter sizes, thereby sacrificing the number of doses produced. There will be a considerable market for the first inexpensive easy-to-operate semen quality analyser.

PRODUCING THE MOST, HIGHEST QUALITY PIGS

No artificial insemination programme (and no natural breeding programme, for that matter) is going to achieve maximum fertility if the females are not properly selected, handled and prepared, and if the semen is not properly handled and delivered into the female. New insights into these aspects are improving conception rates, litter sizes and piglet vigour.

The Gilt and Sow

Clearly there are differences between gilts and sows, most particularly with gilts being in heat a shorter time (Steverink *et al.*, 1999) and producing smaller litter (Peltoniemi *et al.*, 1999) than sows. However for optimal success in AI, gilts are not so different from sows and so all information here can be assumed to apply equally to both types of pigs – any differences will be clearly specified.

I. Selection and handling

Females entering the breeding herd can and should be selected for their estimated breeding value (EBV) for litter size. When sows whose EBVs for litter size were inseminated, their fertilisation rate days after breeding, and their percent of normal embryos, was related to their EBV and the number of piglets born (Steverink *et al.*, 1997).

Females, like males, experience seasonal changes in their fertility, due both to daylength and temperature. Gilts are up to 10 days older at puberty if they are growing through the spring and summer, so while the average age at first breeding is around 234 days, gilts reach puberty at 230 days of age between January-June, and at 237 days July-December (Peltoniemi *et al.*, 1999). Farrowing rates are lowest in August to September, although there is no difference in litter sizes in those sows that do farrow. The percentage of females coming back into heat after breeding is highest in August to November. This includes both those females presumed not to catch and coming back into heat around 21 days, and those that presumably got pregnant but lost the pregnancy, coming back into heat around 25-30 days (Peltoniemi *et al.*, 1999). The late return to estrus may be caused by high temperatures interfering with the

normal embryo-dam communication necessary to maintain an early pregnancy (Peltoniemi *et al.*, 2000).

Housing and feeding bred females, not surprisingly, affects their ability to hold a pregnancy. Newly bred sows housed in groups are more likely to come back into heat 25-37 days after breeding than are sows in stalls, probably because of the stress associated with establishing a social hierarchy (Peltoniemi *et al.*, 1999). This difference between group and stall penning is even more evident in the period of seasonal poor fertility. Interestingly, sows in stalls have more late abortions (55 – 107 days; Peltoniemi *et al.*, 1999) than group-penned sows, which may be related either to a lack of exercise or stress from behavioural frustrations. Straw bedding reduced the percentage of sows coming back into heat after breeding, and reduced the impact of season on fertility. Feeding roughage (hay or straw) similarly decreased the percentage of sows coming back into heat, suggesting that the effect of straw bedding may be either due to it serving as dietary roughage, or reducing stress by providing material in which the sows could root. (Peltoniemi *et al.*, 1999). Current recommendations are that sows should be full-fed through lactation until they are bred, and then intake should be restricted (Peltoniemi *et al.*, 2000). However, the restriction should not take the form of complete feed denial, as denying food to females for 48 hours after breeding slowed embryo development (Mburu *et al.*, 1998).

II. Insemination

Successful pregnancies depend upon vigorous sperm encountering recently-released eggs in the oviduct of the female. Behavioural heat precedes ovulation, being nature's way of increasing the chance that sperm will be present and waiting when the eggs are ovulated. For successful AI, or any controlled breeding system, the critical factors controlling successful conception and maximum litter size are the proper timing of insemination and the proper placement of semen in the tract.

IIa. Timing of insemination

Heat detection is critically important in the proper timing of AI. Heat detection is most successful if females are subjected to a back-pressure test in the presence of a boar once every 12 hours or more frequently; snout to snout contact with the boar is best, particularly for gilts. For sows, the weaning to estrus interval should be approximately 4 to 5 days (3.8 ± 0.6 days for 115 sows on one farm; 5.4 ± 3.5 days for over 12,000 sows on 55 farms, Steverink *et al.*, 1997, 1999). As this interval extends beyond 4 or 5 days, the actual heat period shortens, and conception rate and litter size decreases (Steverink *et al.*, 1999). The average length of estrus varies due to parity, season, weaning-to-estrus interval and farm management (Soede *et al.*, 2000), but can be thought to average 40 hours in gilts and 48 hours in sows, ranging from 12-88 hours (Steverink *et al.*, 1997).

The best conception rates and litter sizes are achieved when the female is inseminated anywhere from 24 hours before, to 4 hours after, ovulation (Waberski *et al.*, 1994), with ovulation occurring anywhere from 10-85 hours after the onset of estrus (Soede and Kemp, 1997) depending upon parity and farm. Ovulation can be assumed to occur approximately 2/3

of the way through the period of standing heat of the female, but that is not helpful because the length of estrus cannot be calculated until it is too late to successfully inseminate the female. However, sows within a farm have quite consistent estrus periods when the length of heat is calculated taking into account the days from weaning to estrus, so carefully kept records allow ovulation to be predicted with good results (Soede *et al.*, 2000).

Inseminating once in the 24 hours before ovulation with 3 x 10^9 sperm in a good extender within 48 hours of semen collection will fertilise 90% of all eggs released (Soede *et al.*, 2000). However, anything that reduces the number or quality of sperm in the oviduct, narrows that 24 hour window preceding ovulation. Therefore it is best to inseminate at least twice, 12 hours apart, in the period preceding the anticipated ovulation. Double inseminations on average improve farrowing rates (from 80.8 to 85.1% for sows and from 81.2 to 88.2 for gilts) and litter sizes (Steverink *et al.*, 1999). Inseminating as often as possible is not recommended, as inseminating late in estrus or just after the end of estrus can actually decrease the farrowing rate of gilts and second-parity sows, and decrease the number of total and live-born piglets in all pregnancies (Rozeboom *et al.*, 1997). In addition, the uterus in late estrus is less able to resist bacteria, and so late inseminations also increase the risk of uterine infections.

IIb. Insemination technique

What are the latest improvements in insemination techniques?

The use of seminal plasma is being explored from many different perspectives. Seminal plasma has been inseminated into females 12-24 hours prior to inseminating semen, and has also been used experimentally in place of an extender to dilute semen. Seminal plasma appears to stimulate a rapid response, presumably from the uterus, that speeds up ovulation in those females who regularly ovulate an extraordinarily long time after estrus (Waberski *et al.*, 2000). Ovulation after seminal plasma infusion in these sows then occurs much closer to the normal interval after the start of estrus. The effect is not consistent (Soede *et al.*, 1998) and may indicate a difference among boars in the exact nature of the stimulant they naturally produce in the semen. Certain boars stimulate, and others inhibit, embryonic growth from an early stage of development (Ramsoondar and Christopherson, 1998). Seminal plasma from some boars may have more of the component(s) that stimulate the uterus to protect the new embryos from the dam's immune system (Rozeboom *et al.*, 2000). Others have suggested that seminal plasma may contain compounds that stimulate uterine contractions, thus helping sperm to reach the oviduct (reviewed by Soede *et al.*, 1997). The lining of the oviduct prior to ovulation is deeply folded and produces viscous mucus (Mburu *et al.*, 1996) that readily traps and holds sperm in stasis for up to 24 hours awaiting the ovulated oocytes. These various studies all support the idea that seminal plasma has valuable components which might benefit establishment of pregnancy, but it is clear that both the components and their actions must be much better understood before commercial application is possible.

Injection of $PGF_{2\alpha}$ into the vulva tissue, or adding 5 mg into the extended semen just prior to insemination, may promote sperm transport to the oviduct and thereby enhance fertility (Pena *et al.*, 1998, 2000). Vulvar injection of $PGF_{2\alpha}$ increased average conception rate and litter size

(Pena *et al.*, 1998) and addition of $PGF_{2\alpha}$ to the inseminate improved the summer conception rate and annual litter size (Pena *et al.*, 2000). The authors suggested that $PGF_{2\alpha}$ might advance ovulation, but that it would be of little benefit in well-managed herds except perhaps in periods of seasonal infertility.

Backflow of semen during or shortly after insemination has long been a concern, but recent evidence suggests that some of the concern is misplaced (Steverink *et al.*, 1998). Semen backflow occurs within 5 minutes in 66% of all inseminations, and the volume lost in this time period contains the exact amount of sperm you would expect: if 70 ml of extended semen containing 3 x 109 sperm has been inseminated, and 5 ml flows back out in the first five minutes, there has been [5/70 x (3 x 109)] = 0.2 x 109 sperm lost. So if a large volume flows back out in the first 5 minutes, there is indeed cause for concern, but a small volume is not a concern. The female tract is very effective at moving sperm out of seminal plasma and up into the oviduct, and so backflow over the next 30 minutes, which happens in 98% of all inseminations, contains less and less sperm per ml fluid lost. In fact, Steverink *et al.* (1998) suggested that this backflow is one of the pig's natural mechanisms for removing the large volume of inseminate from the tract.

Many new devices are constantly being introduced. Some of them will be valuable to some operations, but not to others. There is no device that can completely substitute for good management, but many tools can improve many levels of management.

THE FUTURE

What will the future bring? Here's a list of a few insemination-related things that might be available to the swine industry within the next 10 years:

1. Sexed semen (Johnson and Welch, 1999). Producers will be able to purchase semen that will guarantee the sex of at least 85% of a litter.
2. Transgenic pigs (Niemann, 2000). These will be pigs that carry genes of commercial interest, which will most likely be pigs whose organs can be used for transplantation into humans with little or no risk of rejection.
3. The use of sperm to actually transfer genes of interest into eggs (Lavitrano *et al.*, 1997). This will facilitate the creation of transgenic pigs.
4. Low dose insemination. This has many ramifications, including more semen doses available from popular boars and less costly sexed or transgenic semen.
5. Embryo transfer (Day, 2000).
6. Frozen semen (Buhr, 1999; Ericksson and Rodriguez-Martinez, 2000). This will create an international export market for semen.

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