

Novel Reproductive Techniques in Swine Production – A Review

C. Okere* and L. Nelson

Genex Swine Group Inc., 633 Park Street, Regina, Saskatchewan S4N 5N1, Canada

ABSTRACT : The main objective of modern reproductive technologies in pig reproduction is to increase reproductive efficiency and rates of genetic improvement. They also offer potential for greatly extending the multiplication and transport of genetic materials and the conservation of unique genetic resources in reasonably available forms for possible future use. The development and refinement of these technologies is concentrating on gamete and embryo collection, sorting and preservation, *in vitro* production of embryos, culturing, manipulation of embryos (splitting, nuclear transfer, production of chimeras, establishment embryo stem cells, and gene transfer) and embryo transfer. Also, the development of these novel technologies is facilitated by modern equipment for ultrasonography, microscopy, cryopreservation, endoscopy, and flow cytometry, microinjections, micromanipulators and centrifugation. The real impact on herd productivity will come from combining new reproductive techniques with powerful DNA technologies. The new reproductive techniques will allow a rapid turnover of generations, whereas the DNA technology can provide selection, which does not need phenotypic information when the selection decisions are made. (*Asian-Aust. J. Anim. Sci.* 2002, Vol 15, No. 3 : 445-452)

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INTRODUCTION

Manipulation of farm animal reproduction is probably as old as animal domestication itself. For as long as animals have been kept in captivity, we have profoundly influenced their reproductive behavior. The main objective of modern reproductive technologies in pig production is to increase reproductive efficiency and rates of genetic advancement. Modern reproductive technologies also offer potential for greatly extending the multiplication and transport of genetic materials and conserving unique genetic resources in reasonably available forms for possible future use. The development and refinement of these technologies are concentrating on gamete and embryo collection, sorting and preservation, *in vitro* production of embryos, culturing, manipulation of embryos (splitting, nuclear transfer, production of chimeras, establishment of embryo stem cells, and gene transfer) and embryo transfer. The development of these novel technologies is facilitated by modern equipment for ultrasonography, microscopy, cryopreservation, endoscopy, and flow cytometry, microinjections, micromanipulators and centrifugation.

Assisted reproductive technologies are not as widely utilized in swine as in other large domestic animal species (table 1) (Garcia, 2001). Part of the problem is the unique features of the anatomy of the pig's reproductive tract and the high sensitivity of swine gametes to manipulation. This review will concentrate mainly on reproductive and molecular technologies that do not alter the genome other than through the standard processes of selective breeding.

* Address reprint request to C. Okere. Tel: +1-306-721-2034, Fax: +1-306-721-2528, E-mail: chucko@genexswinegroup.ca

We do not discuss transgenic or genetic modification technologies in great length as it is unlikely, in our opinion, that these techniques will be used for swine improvement in the next ten years.

SOME ASPECTS OF GENETICS OF PIG REPRODUCTION

Swine herd reproductive performance depends on complex physiological pathways which determine male and female reproductive performance such as age at sexual maturity, gamete production, libido, fertilization, embryo, fetal and piglet survival.

According to Webb (2000) genetic improvement of any trait requires two main tools:

Selection - involves identifying the best animals to be the parents of the next generation. It relies on the desired traits being inherited.

Crossbreeding - to produce a boost in performance that is often proportional to the genetic dissimilarity of the breeds. It relies on heterosis (hybrid vigor).

Selection leads to permanent and cumulative changes, whereas heterosis must be regenerated at each mating.

Reproductive traits have the distinct disadvantage of low heritability, can be measured only in a small proportion of the adult population and are age dependent. Improvements by conventional selection methods therefore, deliver slow and measured responses. Returns on investments are equally small in comparison to growth and carcass traits, because these traits are shared over all members of the litter (Webb, 1991b). Estimates of heritability (h^2) for male and female reproductive traits in the pig are shown in table 2.

Table 1. Key milestones in the evolution of reproductive technologies in the pig

Year	Event
1951	First successful surgical embryo transfer
1970	Successful intercontinental transport of embryos
1976	Embryo transfer used to establish SPF herd
1983	Embryo transfer used to repopulate swine herds in connection with the eradication of pseudorabies virus
1985	Production of transgenic piglets
1985	Piglets produced from split embryos
1986	Piglets produced by in vitro fertilization
1989	First cloned piglets born after embryo cells nuclear transfer
1990	Piglets produced after transfer of frozen-thawed embryos
1991	USDA produced first piglets with semen sorted for sex
1993	Production of piglets by non-surgical embryo transfer
1994	First piglets produced after oocyte transfer
1997	First piglets produced with embryos fertilized with sperm cells presorted for X and Y chromosomes
1998	First piglets born via cryopreserved embryos
2000	First cloned piglets born after somatic cell nuclear transfer

Source: Garcia, (2001)

NOVEL REPRODUCTIVE TECHNIQUES

Artificial insemination strategies

Artificial Insemination in pigs reached a level of practicality more than 40 years ago, but it has only developed into an important biotechnology tool in the porcine industry in the last 20 years.

European producers lead the world's pork industry in AI use. Germany uses AI for over 40% of all inseminations, Norway uses AI for 85% of all inseminations, Sweden for 50%, Denmark for 32%, and the Netherlands for 70% (Foxcroft, 1996). North American usage is finally reaching levels that have been common in Europe for years. The effective use of AI in breeding programs is essential if non-European producers wish to maintain a competitive edge in the global market place.

By using AI, genetically superior boars can be used extensively, particularly at the nucleus and the multiplier levels of the pig breeding pyramid. At the nucleus level, AI has made it possible to link several units to create a large 'super nucleus', thereby increasing genetic gain at the nucleus level and a decrease in genetic lag between nucleus herds and the commercial population (Visscher et al., 2000).

Table 2. Heritability estimate for reproductive traits in the pig

Trait	Mean h^2
Male traits	
Testis weight	0.44
Semen volume	0.37
Sperm motility	0.17
Basal testosterone level	0.25
Libido	0.15
Female traits	
Age at puberty	0.33
Standing reflex	0.29
Ovulation rate	0.32
Prenatal survival rate	0.15
Age at first farrowing	0.13
Total born	0.11
Number born alive	0.09
Number weaned	0.07
Piglet survival to weaning	0.05
Litter birth weight	0.29
Litter weight at 21 days	0.17
Weaning to estrus interval	0.25
Farrowing interval	0.03

Sources: Nicholas, (1997) and Rothschild and Bidanel, (1998).

As well, AI is changing the market strategy of pig breeding companies, from selling live boars to selling semen. As with other species, some of the advantages using AI include:

- Cost efficiency in not having to buy or maintain many boars.
- Better hygiene of semen doses compared to natural service. Better fertility, since each ejaculate is tested before use.
- Convenience of being able to breed any male to any female regardless of size, temperament, or age.
- AI provides a tool to enhance leanness, growth rate and other genetic improvements.
- AI techniques are relatively simple to learn.

Conventional insemination techniques in the pig require 2 to 3 billion sperm cells/dose and a volume of insemination/dose between 80 and 100 ml. Current interest is focused on reducing sperm number per dose and intra-uterine insemination. Studies by Martinez et al. (2001) have demonstrated that the number of sperm cells per dose could be reduced significantly (50 to 200 million) sperm cells per dose using a fiberoptic endoscopic technique for deep uterine horn insemination with no effects on fertility and litter size. Low-dose insemination when combined with a single (fixed-time) insemination will have a major impact on the productivity of boar studs.

Distance between suppliers and customers remains the main obstacle to widespread use of AI. Most extenders in current usage give optimal conception rates for 72 h;

extenders need to be developed which produce reliably conception rates for at least 7 days to overcome this problem. Rozeboom (1999) suggested that the way forward is to encourage research aimed at improving fertility results associated with the use of frozen semen, which is still sub-optimal compared to the use of fresh semen.

Sperm sexing technology

One current example that illustrates the importance of reproductive technology development is a process that would allow producers to predetermine the sex of offspring. This process is based on the separation of X- and Y-chromosomes bearing spermatozoa. Two approaches have been used to try and accomplish this with varying degrees of success. First, sperm cells have been separated on the basis of DNA using flow-cytometry. Using this technique, sex ratios have been skewed in experimental and field studies swine (Johnson, 1991; Rath et al., 1997 and Johnson et al., 1999) and cattle (Seidel et al., 1997). The shift in the sex ratio is usually from the standard 1:1 to about 8 or 9:1 or vice versa.

The latest flow cytometry method is known as The Beltsville Sperm Sexing Technology developed by Dr. Larry Johnson of the USDA at Beltsville. This method involves treating sperm with a DNA-binding Fluorochrome (dye), and then flow-cytometrically sorting with laser beam based on the amount of fluorescence into separate X and Y populations that can subsequently be used for regular artificial insemination or *in vitro* fertilization to produce sexed embryos for transfer. One limitation of cell-sorting technology is that the process must be carried out one cell at a time. This makes the systems inherently slow, because billions of sperm are need for conventional AI. Improvements in the Beltsville technology in the last two years has lead to the development of a commercial high speed cell sorter which can produce 5 to 6×10⁶ sperm/hour at 85 to 90% purity for X (female producing) or Y (male producing) sperm cells in most livestock species.

The second approach involves isolating a protein from the surface of the X- or Y- bearing sperm that is chromosome specific and thus sex specific. Recently, a University of Guelph spin-off company Gensel Biotechnologies Inc. announced the identification of several sex-specific proteins on the surface of sperm cells against which antibodies could be raised to enable the separation of male-producing sperm cells from female producing sperm cells. The objective is to prepare monoclonal antibodies that will be added in solution to the semen. Sperm of the unwanted sex can be made to clump together and filtered off using glass wool (Webb, 2000). Webb (2000) suggested that the main benefits of semen sexing lies in improved feed efficiency and carcass lean content. Compared to a castrate, a gilt has up to a 15% advantage in feed efficiency and is

3% leaner. An entire boar shows roughly the same advantage again over gilts. Also, he argued that single sex production also avoids the need for split-sex feeding. If successful, the semen sexing technology will be an easy and inexpensive application to on-farm AI collection. It involves no genetic manipulation, and is safe and acceptable to the consumer. In the short term sex determination probably represents the greatest single potential step forward in pig production and is therefore well worth the effort (Webb, 2000).

Embryo production, recovery, preservation and transfer technology

The basic concept of embryo technology is the ability to remove gametes from a genetically distinct but superior animal (donor) and introduce them into another animal (recipient/surrogate) who will carry them to term. The offspring will assume the genetic identity of the donor animal. Porcine embryos or immature oocytes can be collected *ex vivo* from living donors by either surgical or endoscopic flushing of the uterine horns or from slaughtered animals. Other possibilities include *in vitro* production of embryos (IVEP, e.g. from follicles collected from the slaughterhouse or from live animals), non-surgical embryo transfer techniques, embryo storage and freezing techniques and cloning. These techniques are described below.

In vitro embryo production (IVEP) : Since the early 70's great efforts have been made to produce porcine embryos *in vitro*. The initial idea was to produce a pool of pre-implantation stage embryos for other reproductive technologies. The production of a large number of *in vitro* produced porcine embryos includes numerous *in vitro* steps, which try to mimic the *in vivo* development of embryos: oocyte maturation, fertilization and culture until transfer or cryopreservation. The development of *in vitro* maturation and *in vitro* fertilization techniques remains a key step in successful IVEP programs. In contrast to results in the cow and the human, experiences to date with *in vitro* fertilization in pigs have been much less successful. A review by Gordon (1997) reveled that Japanese workers (Nagai et al., 1984) first reported evidence of fertilization by IVF in pigs. Live births were also reported in Cambridge after IVF of ovulated pig oocytes. Subsequent work by Mattioli et al. (1988) convincingly demonstrated that pig oocytes, matured and fertilized *in vitro* (IVM/IVF), could undergo normal embryonic development. They reported blastocyst formation, establishment of pregnancies and birth of live piglets.

Embryo collection techniques procedures : Embryo recovery is routinely done by flushing the uterine horns surgically or after slaughter. The nature of the sow's cervix and the uterine horns appeared to make non-surgical

recovery procedures less feasible. For each technique, manipulation of the oviducts and the uterine horns must be kept to a minimum; aseptic precautions must be taken at all stages of the recovery process. Recovery of oocytes from living donors via endoscopy or the ultrasound-guided transvaginal aspiration known as 'ovum pick up' (OPU) has been demonstrated by Besenfelder et al. (1997). Endoscopic embryo collection enables complete embryo recovery, minimizes manipulations and guarantees repeated use of donors. In addition, collected embryos may be of known genetic merit, unlike slaughterhouse production. Using techniques described above, within 6 days of ovulation, it is possible to achieve embryo recovery rates of the order of 80-90%.

Embryo-oocyte manipulation : The requirements for embryo development *in vitro* can be broadly divided into nutritional and environmental. It is now possible to produce pig embryos in the laboratory, which are suitable for transfer. Rath et al. (1995) suggested that it is preferable to transfer porcine embryos derived from *in vitro* fertilization at an early stage of development and not to culture them to the blastocyst stage before transfer.

In vitro fertilization and In vitro maturation (IVM) of oocytes : Collection of oocytes by either follicular dissection or aspiration is possible. Although the collection of oocytes by dissection is labor intensive, it ensures that oocytes are recovered from non-atretic follicles. After collection, the selection of good quality cumulus-oocyte complexes (C-O-Cs) is important. Studies also suggest that only maturation culture systems that involved the presence of abundant follicle wall components were able to create conditions for competent oocyte maturation (Mattioli et al., 1988). A review by Nagai (1994) noted that IVM of pig oocytes was possible in a simple medium (mTALP) supplemented with pig follicular fluid and cystine. Hunter (1990) observed that the challenge of IVF of pig oocytes demands proper understanding of the factors contributing to the oviductal microenvironment during *in vivo* fertilization in pigs. However, the most important problem in pig IVF is polyspermy (multiple sperm penetration). Funahashi and Day (1993) showed that pre-fertilization incubation of boar sperm in suitable concentrations of pig follicular fluid could reduce the incidence of polyspermy in an IVF system. Foxcroft et al. (1995) reported significant boar effect on sperm penetration, monospermy and male pronuclear formation.

Embryo Cryopreservation : In contrast to cattle, pig embryos are difficult to adapt to the cryopreservation conditions. It is becoming clear that the sensitivity of pig embryo to cooling and freezing is probably the result of their high lipid content (Gordon, 1997). Deep freezing protocols have been developed which tried to optimize the cooling rates, temperatures, cryoprotectants (including

nutrients and supplements) and the embryonic stages. Freezing of pre-hatching-stage pig blastocysts and their subsequent storage in liquid nitrogen was reported by Kashiwazaki et al. (1991). Live piglets were born after transfer. In another study, pregnancies and live-born piglets were generated from early stage embryos (two-cell) which had been deep-frozen (-196°C) after centrifugation and the removal of cytoplasmic lipids (Nagashima et al., 1995). Because the removal of cytoplasmic lipids by micromanipulations is labor intensive, research efforts now target procedures that improve the ability of embryos to tolerate freezing by reducing their lipid content and/or increasing the fluidity of the cell membranes. The results from Dr. John Dobrinsky's lab at Beltsville (USDA) were encouraging in this area. He uses a new technique called vitrification or freezing pig embryos in a solution that remains in a liquid state even at -196°C. His laboratory can produce 7-8 live offspring per litter with a conception rate of around 50%.

Embryo transfer : The embryo transfer (ET) includes the collection or production of embryos (*ex vivo* or *in vitro*) from donor pigs, the temporary culture and/or manipulation and reintroduction into a recipient animal (Besenfelder et al., 1998). Embryo transfer in pigs usually involves surgical intervention. There are very few reports of laparoscopic or transcervical transfers. Surgical transfer of porcine embryos into the oviduct and uterine horns was developed to introduce new genetic materials into specific-pathogen-free pig herds (Cameron et al., 1989). Reported pregnancy rates after surgical transfer range from 60 to 85%. In addition, significant stress of the animals results from anesthesia, surgery, and manipulation of the genital tract. Several attempts have been made to establish non-surgical or minimal invasive methods.

As with ruminants, exposure of the pig embryo to asynchronous uterus can be detrimental to further development. Assessing synchrony requirements, Polge (1982) showed that there was no reduction in pregnancy rates when donors were 1 day earlier than recipients but transfer to recipients more advanced than donors did result in a decrease. Collection and transfer of one-cell stage and two-cell stage embryos to oviducts of synchronized recipients can achieve acceptable embryo survival rates. The difficulty at this stage of embryo development is in determining whether they were fertilized or not.

Kim and Oguri (1990) examined various factors affecting pregnancy rates and litter size after surgical transfers. They reported pregnancy rates of 75% and 90% and litter sizes of 5.3 and 6.1 after transfers to recipients at 3 and 4 days after estrus, respectively. For transfer of embryos of 5 and 6 days of age, they reported pregnancy rates of 66.7% and 100% and litter sizes of 6.5 and 5.6, respectively. Maintenance of pregnancy is unlikely through

embryo transfer unless recipient pigs receive at least four embryos.

Non-surgical embryo transfer : The cervix is a barrier for transferring porcine embryos non-surgically. Polge and Day (1968) reported some of the first efforts in non-surgical embryo transfer in pigs and obtained viable conceptuses from one gilt at d 17 after transfer. Li et al. (1996) described a novel embryo transfer technology which enabled embryos (four-cell to blastocyst stages) to be delivered into the lumen of the uterine horn of recipient gilts. Five of 16 recipients became pregnant (31%) and farrowed an average of 6.2 ± 3.1 pigs per litter. This technology involves a four part transfer instrument: (i) a modified commercial AI spirette which is used to produce a cervical lock; (ii) stainless steel tubing with a curved tip that can be manipulated over folds of the cervix; (iii) a testing bar to ensure that the tip of part (ii) reaches the bifurcation of the uterus; and (iv) a disposable tubing complex for embryo delivery. This report claimed that a technician can easily learn to operate the instruments.

In the Netherlands, Hazeleger and Kemp (1999) and Van der Lende (1998) published a technique for non-surgical embryo transfer that essentially mimics the process for artificial insemination. They have developed a PVC rod that uses a very small amount of flushing fluid (0.1 ml) to transport embryos. The application instrument has a short 1 cm hook at the tip. After moving beyond the cervix it is turned gently to reach the top of the uterine horns where the embryos are deposited. The report added that transfers done in this way have taken no more than a few minutes to complete, in sows at day 5-7 after estrus. Their data showed 16 pregnancies from transferring 28-30 embryos of one donor sow into each of 27 recipients. Slaughter records on day 35 revealed an average litter size of 10.9 that they assume can translate to 9.5 to 10 live born at farrowing.

Potential applications of embryo production and transfer technology : Movement of genetic resources in the swine industry currently relies predominantly on the shipping of live animals. The high cost of shipping plus the risk of disease transmission are some of the disadvantages of transporting genetics via live animals (Li et al., 1996). Apart from the problems associated with cryopreservation of boar semen, the use of AI can change only half the genetics of the offspring. Shipping embryos might be a more cost-effective method for disseminating genetic material, but the high cost of surgical collection and transfer of embryos in conjunction with relative inability to cryopreserve porcine embryos has limited commercial application of this technology.

When large scale ET is adopted, the consequences for swine breeding programs is that superior females could have as much influence on genetic progress as males through an increase in selection intensity. Apart from the

effect of increased selection intensity at the nucleus level, *in vitro* embryo production in conjunction with embryo preservation and transfer technology could alter the dissemination structure of the industry (Visscher et al. 2000). These authors advocated the development of a few large-scale 'embryo farms' in which matured oocytes from superior nucleus sows are recovered and artificially inseminated with semen from nucleus boars (from a different line). The embryos would be implanted into recipients who sexually mature early and have large reproductive capacity, for example purebred Meishan genotypes or other hyperprolific dam lines. The piglets born from the recipients would be transported to commercial farms. The advantage of such a scheme is that fewer animals are transported from nucleus to multiplication tiers, fewer sows are needed at the multiplier level, and there is greater control over the multiplication process for the breeding companies. They concluded that such a scheme might remove the need for a purebred multiplication tier, and reduce the crossbred tier in the industry, thereby reducing the genetic lag.

Elimination of disease by embryo transfer technology : From a biosecurity viewpoint, the health advantages of embryo transfer are enormous. PCR testing will quickly differentiate infected vs. non-infected embryos. New research shows that transferring early-stage embryos while the zona pellucida is still intact along with embryo washing techniques can assure a disease-free embryo (except for viruses incorporated into the pig genome) (Besenfelder et al., 1998). Recently the National Hog Farmer (45 (11) 2000) reported that embryo transfer technology has changed the health status of a 280 purebred Duroc herd from an all-PRRS-positive herd to a negative status herd. The report also added that Drs. John Pollard and Marie-Claire Plante of Ontario Veterinary College, University of Guelph, developed the technique and performed trials demonstrating the PRRS cycle could be stopped. In addition, the technique greatly facilitates the performance of embryo transfer programs allowing the transfer of frozen-thawed embryos at any time into appropriate recipients.

Cloning and gene transfer in pigs

Embryo splitting : A different and simpler cloning procedure, called embryo splitting, or artificial twinning, was developed in the 1980's and was adopted by animal breeders. In this procedure, an early embryo is simply split into individual cells or group of cells, as happens naturally with twins, triplets, and other multiple births. Each cell or collection of cells develops into a new embryo, which is then implanted into a surrogate animal, who carries it to term. Although this technique permits the production of multiple clones, the clones are derived from an embryo

whose genetic potential is not completely known rather than from an adult animal with known characteristics. This constitutes a serious limitation for practical applications of the procedure.

Although embryo splitting technology has been the subject of many reports in cattle, few studies have been reported on embryo bisection in pigs. Cambridge workers (Polge, 1982) described two sets of identical twin pigs. Other studies by Reichelt and Niemann (1995) were more promising, showing that pig morulae and blastocysts could be bisected relatively easily and yielded large numbers of viable embryos.

Cloning by nuclear transfer in pigs : In 1952, developmental biologists Robert Briggs and Thomas King, developed a cloning method called nuclear transfer, which was proposed in 1938 by the German scientist Hans Spemann. In this method, the nucleus is removed from an egg cell (oocyte) of an organism, a procedure known as enucleation. The nucleus from a body cell of another organism of the same species is then placed into the enucleated egg cell to grow into an embryo. Because the embryo's genes came from the body cell's nucleus, the embryo is genetically identical to the organism from which the body cell was obtained.

In 1997, scientists led by Ian Wilmut of the Roslin Institute near Edinburgh, Scotland reported they had used mammary gland cells from an adult female sheep to create a genetically identical lamb known as Dolly. This marked the first time researchers had produced a clone using a specialized cell from an adult vertebrate. Variations of this technique pioneered by the Scottish scientist have been used since 1999 to clone cattle and pigs. In March 2000, The Scotland-based PPL Therapeutics announced that five healthy pigs were born as a result of nuclear transfer (cloning) using adults cells. This is the first time cloned pigs have been successfully produced from adult cells. The method used to produce the five female pigs, named Millie, Christa, Alexis, Carrel and Dotcom, was different from that used to produce Dolly the sheep. Details are described in *Nature Biotechnology*, 2000:18 (10), 1055-1059.

A Japanese team lead by Akira Onishi announced in August 2000 the birth of Xena, a black-coated piglet from a white-coated sow produced by cloned genetic material from fetal pig cells. She is named after the field of research that scientists hope her birth might advance-xenotransplantation-the use of genetically modified animal organs for transplant into humans. To create Xena, this team used a needle-like pipette to inject genetic material from fetal pigskin into oocytes or egg cells that had been stripped of their own genetic material. Next, the team stimulated the injected eggs with an electrical pulse that triggered them to develop into embryos. Those embryos were then transplanted into surrogate sows. Xena was the one successful birth of 110 transplanted embryos. When researchers cloned Dolly the

sheep, they fused entire cells into empty host eggs. Researchers on Xena's team believe their technique of injecting only genetic material will allow better flexibility for genetic manipulation.

Transgenic pigs

Animals modified to carry genes from other species are called transgenic animals. The technique producing transgenic animals is best illustrated by the recent work by Dr. Forsberg and colleagues at the University of Guelph. This group has produced the 'Enviropigs' (Wayne, Jacques and Goldie)-named after Canadian hockey players. Although outwardly they look like any other pigs of Yorkshire breed, inside the nucleus of each cell of an Enviropig is a piece of DNA that contains a snippet of mouse and a bit of bacteria genes. This composite gene was inserted into the nucleus of a one-cell pig embryo with a microscopic needle. These pigs are transgenic for the enzyme-phytase. The transgene will allow these pigs to produce phytase in the salivary gland. When swallowed with food, the phytase releases organic phosphorus in the animal's gut where it can be absorbed, thereby emitting less phosphate pollution.

Potential impact of cloning technology : The prospect of cloning offers the possibility to reduce the genetic lag between the nucleus, multiplier, and the commercial tiers, since genetically superior or performance tested animals can be cloned and supplied to the commercial farmer. A quote from the late Dr. Charlie Smith emphasizes this point, "one result would be that commercial animals could be genetically superior to the breeding animals. Thus, it can be said that cloning could turn conventional breeding on its head".

According to Van Vleck (1999) cloning, on first impression suggests a perfect way to improve performance of livestock. Such impressions imply that breeders only need to find the perfect animal so that no further effort to breed better animals is needed: when the perfect animal is found and cloned, the traditional methods of breed improvement would be obsolete. He concluded that for most important traits in farm animals, these perceptions are generally incorrect.

Finally, can this technology be made inexpensive enough to compete with a mature technology, such as AI or niche technologies such as sexed semen or embryo transfer? There are complex technical, societal, political and ethical determinants of its application that must be addressed.

Novel reproductive technologies: Likelihood of effective use : Visscher (2000) summarized the likelihood of implementation and impact of various techniques in table 3.

An overview of problems and prospects of adopting some novel reproductive technologies in commercial swine breeding programs appears in table 4.

Table 3. Predicted impact of reproductive techniques in terms of likelihood of effective use in the next 5 years and the estimation of the potential impact on genetic change (scores on scale 1-3. 1=low, 3=high)

Technique	Likelihood of effective use	Potential impact
Sex sorted-semen	3	3
Gamete cryopreservation	3	3
Non-surgical embryo transfer (ET)	2	1
<i>In vitro</i> embryo production (IVEP)	1	2
Cloning	1	2
ET+IVEP+Cloning	1	3
Molecular biology techniques	3	2
Gene transfer	0	0

CONCLUSIONS

The potential exists to improve specific sow reproduction traits, such as age at puberty, estrous symptoms, ability to become pregnant, litter size, piglet survival and weight, milk production, maternal behavior and ability to show estrus after weaning. In practice, however, considerable problems are associated with genetic selection for most of these traits as they have low heritability values. The speed at which genetic improvements can be achieved by traditional methods is slow. Many of the new reproductive technologies offer possibilities for improving the rate of genetic progress. These include *in vitro* embryo production (IVEP), non-surgical embryo transfer (ET), sperm sexing technology (SST), and molecular biology techniques. The real impact on genetic progress will come from combining new reproductive techniques with powerful molecular techniques. The new reproductive techniques will allow a

Table 4. Opportunities and challenges of adopting new reproductive technologies in commercial swine breeding programs (Adapted from Hammond and Leitch, 1998)

Techniques	Opportunities	Challenges
Reproductive techniques		
Artificial insemination (AI)	Enables dissemination of superior genetics Affords more controlled breeding and mate selection and potentially decreases inbreeding	Requires high level management for collection, processing of semen and use Technology needs further development in many areas
Embryo transfer (ET)	Potential for dissemination of genetics from superior dams, and transborder transport of genetics Passive immunity conferred on offspring by recipient female Effective for gene banking and regeneration of populations	Costly and logistically complex Technology not well refined for the pig Requires specialized training and expertise
<i>In vitro</i> embryo production (IVEP) and oocyte recovery	Potential to reduce long generation intervals by collecting ova from prepubertal females Conservation of breeds at risk Efficient use of semen <i>in vitro</i> fertilization as few sperm cells are required Enables dissemination of genetics from superior females	Technology needs further development Requires specialized equipment and training
Cloning	Mass production of crossbreed clones would enable optimum combination of adapted local with exotic genetics	Expensive Requires specialized equipment and training Technology needs further development
Transgenics	Transfer of useful genes to improve productivity	Potential consumer backlash

rapid turnover of generations, whereas the molecular techniques can provide selection, which does not need phenotypic information when the selection decisions are made.

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