Gene transfer and genetic improvement

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Various new reproduction and breeding technologies will have great influences on modern pig production and will change the current production structure and breeding pyramids.

In order to paint a good picture of these influences in the future as we perceive them, it is best to first look at the current model(s) of the pig production industry. Current breeding pyramids are partly determined by the reproduction rate of swine, the usage of fresh semen with a limited shelf life of 4-5 days, and the application of quantitative genetics to estimate genetic values.

Relatively small numbers of nucleus and multiplication boars are needed to produce crossbred sows for the commercial level. A significant amount of terminal sires are placed in AI studs to provide terminal sire semen to the production systems. The differentiation between maternal and terminal sire lines leads to an efficient production of slaughter animals. Terminal sires, either crossbred or purebred/synthetic lines, are selected to steer the final products according to the needs of the market.

This has lead to a widely used and accepted conventional ABCD breeding pyramid (Fig. 1) or a BCD pyramid in cases where terminal sires are purebred.

The biggest challenges of this breeding pyramid model are the health status, generation interval, and uniformity of final products (ABCD). Every level is a health challenge when animal shipments are involved. The ultimate objective of the model is to bring genetic improvement as fast and as bit-securely as possible to the commercial level and create products of the highest quality.

Nucleus populations have to have the highest health status and at the end of the breeding pyramid the highest realisable and maintainable health status is the target.

Quite often it happens that the health status at the lower levels of the pyramid is lower (more disease challenge) than at the nucleus level. This necessitates the implementation of various quarantine, adaptation, and vaccination protocols to acclimatise the animals being moved down the pyramid to the health statuses of the target levels.

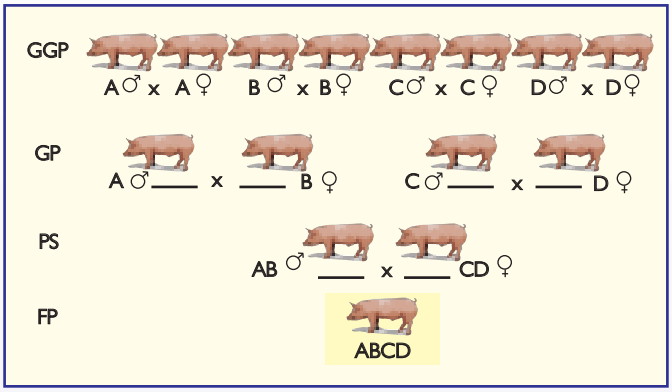


Fig. 1. Classical breeding pyramid.

In the breeding pyramid model, as shown in Fig. 1, all the genetic improvement takes place at the top (nucleus). One of the factors that determine the speed at which that improvement gets to the bottom of the pyramid (commercial producer) is the generation interval. Ideally, the generation interval to the market level should be as short as possible to get genetic improvement as soon as possible.

A shorter generation interval will most likely also improve the uniformity of parent stock animals. The correct choice of terminal sire lines and a detailed knowledge of the genetic level of the boars used finally determine the uniformity of the final product and its fit to the local market needs.

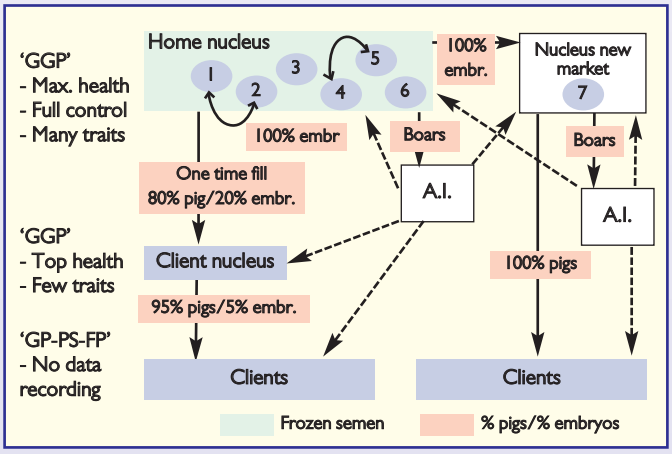
New technologies will play a big role towards providing solutions to the bottlenecks of the pyramid.

Further developments in the area of reproduction technologies, including embryo transfer, frozen semen, semen sewing, and cloning, will logically have profound influence on the setup of the breeding structure.

The benefits of these technologies will be found in establishing higher health status, ability to shorten generation intervals and possibilities to increase the uniformity of both parent stock and final product.

Techniques of freezing semen and embryos, and embryo transfer, play an important role in the efficiency of gene flow. Semen or embryo sewing will further increase that efficiency.

Fig. 2. Geneflow 2010.



With this ability animals of a given sex will only be produced at the moment they are needed for reproduction at a certain level of the breeding pyramid.

Semen freezing and cryopreservation will mean that valuable boars can be preserved and that their genes will be available at when and where desired with minimum investment in logistics. By-products in the breeding pyramid, for example barrows at multiplication level, will be avoided.

Embryo freezing and cloning will allow intensive testing of the animal before it is produced for commercial production. This technique will further allow fine tuning of logistics and timing of production.

Nucleus barns will be highly specialised and have high health status (Specific Pathogen Free). Large amounts of traits will be measured. Genetic programmes will be run based largely on BLUP technology, and supplemented by biotechnology tools, including genetic markers and SNPs (single nucleotide polymorphisms). This means that direct linking of the DNA code to product quality characteristics will be able to explain a lot of information that is currently explained via infinitesimal gene based BLUP models.

One of the bigger headaches facing breeding companies and institutions today is the linking of different nucleus populations that could be in different countries. This headache is mostly generated by concerns for bit-security.

With the advent of these new reproduction technologies it will be possible to link different nuclei by movement of embryos or SPF-piglets. New nucleus barns will be established worldwide with the right mix of embryos after the farm has been populated with carefully health monitored recipient stock.

Client nucleus farms will be established locally to avoid too much transport of animals. They will be populated from the high health

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nucleus, while the refreshment of genes might come in by embryos and/or frozen semen (Fig. 2).

In vitro washing procedures to make contaminated embryos free of specific diseases are an additional interesting tool to make embryo transfer more reliable in the area of bit-security.

Larger production integrations will increasingly establish closed herd systems where they can manage their production and health status internally. Breeding organisations will offer services based on both exchange of performance and pedigree data and continuous influx of high level genetics via fresh or frozen semen. Continuous monitoring of genetic trends and farm performance analysis will guarantee clients that they will have up to date genetics in their barn at levels of quality close to nucleus level.

New reproduction technologies and further developments in genetics might further influence the breeding and reproduction schemes in the future (Fig. 3).

Instead of running the on-farm population as a continuous population one might regularly repopulate parent stock barns with new disease free stock.

Client nucleus barns might create a certain surplus production that is used to do a de-pop re-pop of a barn that has been infected with an undesired disease.

If techniques like cloning, sewing and embryo transfer develop further and can be applied on a large scale at the commercial level, this might change the complete breeding pyramid. The current logical differentiation of terminal and maternal lines will no longer be needed. It is not clear how fast developments will go, but conceivably in 15 years the breeding and reproduction schemes might be considerably changed (Fig. 3).

There will be a need for specialised recipient sows. Sows that combine large uterine capacity with high weaning performances. The further genetic make-up of the sows is not really important. Final products will get all their genes from a final product nucleus program. Large numbers of embryos will be produced in hatchery like operations.

The final product line will produce high genetic level animals that are exactly directed towards the local market needs.

They will be highly specialised for markets such as Italian Parma ham or Japanese high marbling segment. Most likely only one sex will be the most suitable as a market hog.

Through embryo sewing only castrates will be used in cured ham market, while only female offspring will be used in markets that prefer leaner pork.

Availability of cloning techniques will make it possible for embryos of exactly the same genetic make-up to be produced in larger quantities.

They can be pre-tested for product qualities and screened for genes of important quality characteristics and defects. Only the best embryos will be used to develop a commercial final product line. A wide variety of different genotypes can be tested and evaluated based on SNP markers. New genetic combinations could be formed out of multiple crosses of the best genotypes and multiplied for the markets.

Transfer and freezing

Porcine embryo transfer has been performed for approximately 50 years, and surgical methods have proven to be reliable for collection and transfer of embryos.

More recently, successful non-surgical transfer procedures have been developed which facilitate practical applications of porcine embryo transfer for pig breeding organisations. As demonstrated in cattle embryo transfer, this technique has important additional benefits besides the production of extra offspring for breeding purposes.

The use of embryo transfer for the introduction of new genetic material on a farm reduces the risk of disease transmission in comparison with transport of live animals.

Embryo transfer is also more advantageous than AI because sanitary aspects are more stringent with embryos than with semen and, in addition, 100% of a new genome is introduced into a herd using embryo transfer compared with only 50% new genome when using AI.

These aspects are important for pig breeding organisations because they are operating worldwide and, therefore to deliver disease free genomes is of utmost importance.

Additional advantages of embryo transfer are reduced transportation costs and enhanced animal welfare.

Table 1 gives a synopsis of various reported studies on non-surgical ET. It is obvious that factors affecting the success rate of non-surgical transfers have not yet been investigated fully.

Effects of embryonic development, asynchrony between donors and recipients, and location of the embryos in the uterus after transfer need to be studied in detail.

Other aspects such as age, parity and pubertal status of donors and recipients, synchronisation methods for donors and recipients, and embryo handling methods (storage or culture, medium composition, time and temperature) need to be investigated in the future.

Culturing embryos for three days has negative effects on success of embryo transfer. However, successful synchronous surgical transfers with large geographical distances between donors and recipients have been published.

This indicates that it is possible to perform successful transfers with fresh embryos after storage periods of 1-2 days.

Recent research on freezing (vitrification) of embryos indicates that this might become a valuable tool in the near future.

Different groups have shown that frozen (vitrified) embryos can be transferred successfully by surgical procedures. If this technique can be successfully applied in non-surgical procedures it will be a valuable tool for further application of embryo transfer in pigs. Ducro-Steverink et al., conclude that the quality of embryos after vitrification varies per breed. The quality of embryos they obtained might be sufficient for use in surgical embryo transfer, but non-surgical embryo transfer will give very poor results. If this technique can be applied successful frozen litters seem a logical alternative to purchasing semen to breed pigs.

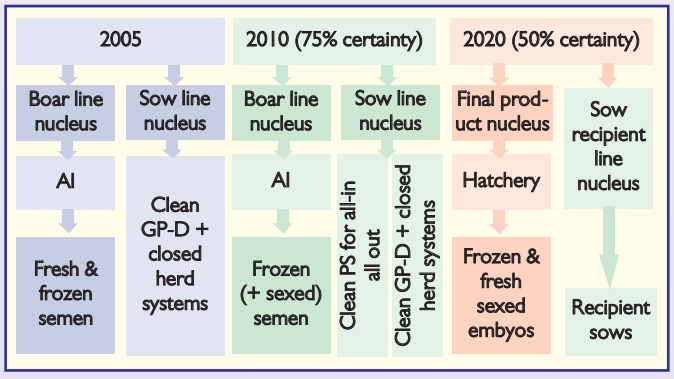
The missing link in this picture is in vitro production of embryos. Fertilisation of eggs under laboratory conditions and growing embryos in laboratory culture systems are important steps that need to be further improved. The resulting embryos could then be frozen or transferred to recipient females.

Reference	Technology	Farrowing	Litter size
Yonemura et al	AI system	16/25 (64%)	3.1±1.6
Hazeleger et al 1999[a]	Swinlet	28/47 (60%)	10.2±0.9
Ducro et al 2001	Swinlet	20/45 (44%)	7.4±3.2
Martinez et al 2001	Firflex – AI	8/11 (73%)	approx 6.5
Martinez et al 2003	Firflex – AI	17/24 (71%)	6.9±0.7
Dyck et al 2005	Swinlet	7/19 (36%)	6.8

[a] *Slaughter at day 35 of pregnancy*

Table 1. Non-surgical procedures without sedation/anaesthesia (adap-ted from Hazeleger 2004).

Fig. 3. Future breeding and reproduction schemes.



Semen freezing

A lot of research has been done on long term preservation of semen in pigs, however application of frozen semen seems to still have considerable limitations. One of the reasons is that in pigs smaller litter sizes will have a certain cost and that makes it hard for frozen semen to get accepted unless results get close to fresh semen results.

Most centres of artificial insemination use fresh semen in their day to day shipments. Research progress made in long term extenders and the fact that most of the semen is sold within a relatively short distance makes fresh semen remain the normal choice for commercial parent stock semen.

For nucleus and multiplication semen as well as international linkage between breeding populations longer distance transportation is usually necessary. Frozen semen would be a logical solution here.

Use of frozen semen would also make it possible to plan and optimise pure line matings. Techniques like TGRM (Total Genetic Resource Management) could be applied to optimise inbreeding and genetic progress at the same time.

TGRM is able to provide solutions to the best boar-sow combinations and following those recommendations would not be restricted by availability of semen.

Furthermore, it would make the best boars available for a longer time and it would make it possible to use the better boars on a larger scale after they have been proven to have the best genetic values. It can also play a role in the conservation of breeds or interesting genes.

One could add some production advantages, namely the fact that the boars could start producing frozen semen while they are still in quarantine. Additionally, feed and maintenance costs per dose of semen can be a lot lower when all surplus semen can be used for freezing and boars could be culled after a certain

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number of doses have been collected.

There have been various research projects done on freezing boar semen. Freezing and thawing large quantities of sperm in a controlled way appears still to be a challenge. The pre-selection step on morphology of the semen of candidates appears to be a very critical factor. Not all boars are good for freezing and sometimes boars that are good for fresh semen appear to be worthless after freezing.

A minimum of 85% spermatozoa with normal morphology is a prerequisite for boar semen used for freezing. Various types of containers are investigated from flatpack to 5ml, 0.5ml and 0.25ml straws.

Thilmant, 1998, mentioned good results with the 0.5ml IMV-straws (Table 2). Thilmant, 2001, reported even better results with the 0.25ml IMV-straws. In a trial with 44 sows he reported 86.9% farrowing rate and an average litter size of 12.0 ± 2.8 piglets per litter. Thilmant concluded that the smaller straws give better results since they are better suited to the freezing thawing process, as the temperature of the medium can remain more homogeneous during cooling and re-heating periods.

It seems that frozen semen is getting to the point where results can be at a sufficient level to apply it at larger scale in long distance shipments to link nucleus barns.

An additional advantage of frozen semen will be that the batch of semen can be intensively tested for disease without generating any time pressure and timing issues for using the semen.

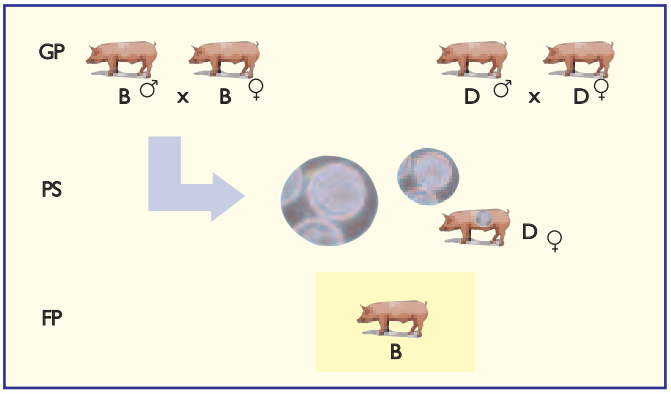

Fig. 4. Future breeding pyramid (embryo donors and recipients).

Semen sewing

In most species semen sewing is proving to be easier than in pigs. The Beltsville sperm sewing technology has been until now the most successful technology in this field. It is licensed by XY Inc for use in non-human mammals.

In pigs the technique increases the gilt to boar ratio significantly from 50:50 to 85:15 distribution.

Other techniques like flow cytometry claim to deliver similar results but the routine is far too slow for commercial application, and certainly for wider use in swine breeding.

Recently, a new technique developed by the University of Guelph in Canada was announced. The patent is owned by Microbix and the technology should be commercially available by 2008. This might be the real breakthrough that would make semen sewing widely available for commercial application.

Genetics and biotechnology

Differentiation through genetics is a logical choice. It is a way to decrease the variation in the source population. It is well known and broadly accepted that the choice of terminal sire line has a major impact on the quality of the final product.

Most genetic programmes are based on one female product (F1-cross between Large White and Landrace) and a differentiation on terminal sire lines.

Using traditional breeding enforced with modern technologies like BLUP and biotechnology, Hypor has developed a series of synthetic and purebred sire lines that are focused on uniformity in growth, carcase and meat quality characteristics.

Tailoring goes one step further, it divides a terminal sire line in spe-

Table 2. Results from frozen semen trials executed by Thilmant

Year	Pregnancy rate (%)	Litter size mean ± SD
1995	79.9 (199)	10.1 ± 3.3 (149)
1996	76.2 (298)	9.4 ± 3.2 (188)

cialised subgroups based on selection on one or two traits of interest.

Tailoring gives you higher uniformity in your final product. Fig. 5 shows an example of a Duroc population tailored for high back fat. The tailored group normally has a standard deviation that is reduced to 50% of its original value.

In practice this will mean the difference between emptying a finishing room twotimes instead of three. In so doing, finishing batches can be managed much more easily to hit the optimum part of payment grid of the packing plant. To be able to make tailoring feasible a certain boar population size is required.

Studs with 100 boars of a single line can do effective tailoring. For clients it gives you the advantage that you can keep working with a proven product and still force some additional attention to the most important trait, getting you closer to your target.

In the example of back fat tailoring, around 15-20% in a 'back fat-plus'-segment creates a difference in EBV of around +2mm. Phenotypic performance of tested pure line animals will be around 4mm higher.

Expected back fat differences in final product can vary between +1.6 and +2.0mm. In practice tailoring has already proven to be effective in the Hypor breeding programme.

It can be the answer to specific customer needs and will push a top performing system a couple of notches higher.

For a genetic programme the field results of various tailored boar groups give valuable information on how a top selection performs in the market.

In that way screening of the future levels is possible. It provides information about the potential of a certain line as well as about the product getting out of balance at certain levels. In that way it provides information that can be used to fine tune the breeding objectives.

Designer genetics

Today's tailoring can already include several DNA markers. Terminal sire line boars can be selected on favourable functional genes or gene markers.

The elimination of genes that have undesired effects, for example Halothane and RN genes that influence meat quality and animal behaviour negatively, is commonly done.

Other genes will be implemented in the future, for example guaranteed homozygous status for the IGF-2 gene. IGF-2 influences both muscle depth and back fat and leads to leaner carcases. FABP genes are known to be good markers for meat quality, especially intramuscular fat.

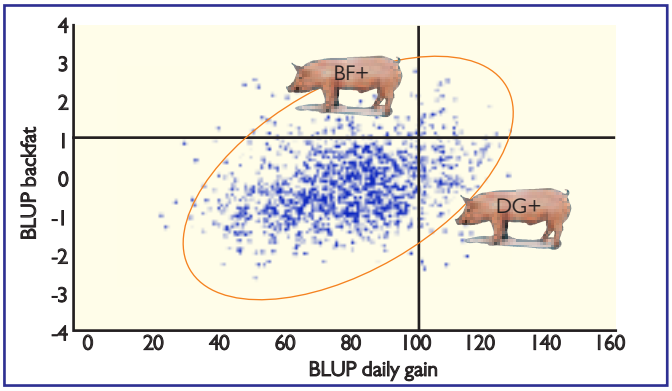

Fig. 5. Tailor made selection example.

Adding more and more genes to that list is a matter of time and the most important benefit will be more uniformity in final product quality. This will be an early version of 'designer genetics'.

In the near future SNP technology could bring an infinitesimal number of genes and gene markers to that list. SNP-markers could be linked to favourable growth and carcase parameters or a SNP marker index could be generated or combined with a BLUP index.

Also the DNA of final products at packing plants can be screened for a large amount of SNP genotypes and could reveal undesired combinations that might be avoided in future breeding material.

Further developments of techniques like embryo transfer and cloning might reduce the numbers of animals needed to produce the next generation. This by itself will already lead to higher uniformity in final products.

Lower number of more intensively selected parent stock will produce the generation of final products.

If several of the developments mentioned in this article take place, it will drastically change the current role of genetics and biotechnology. Both of which will be increasingly directed towards 'designer genetics'.

The result will me a more uniform and predictable final product, better adapted to the quality needs of defined target markets. ■