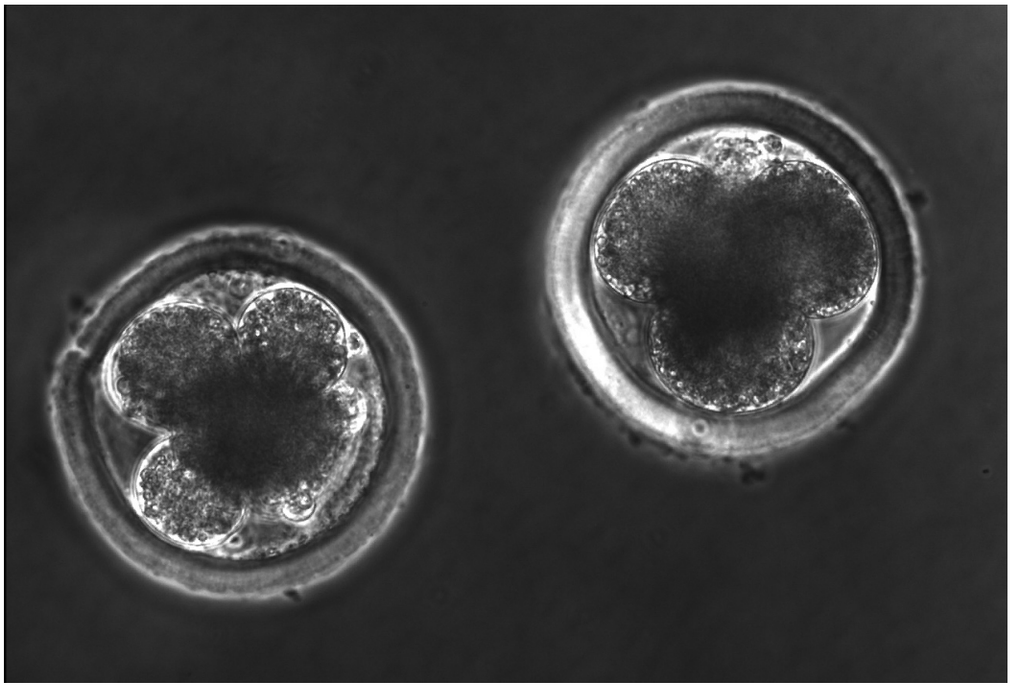
Pig embryo transfer: A glimpse into the future



Porcine embryo cells, shortly after fertilisation.

Embryo transfer is a collective term for a technology used for replacing embryos from the uterus of one female organism into another. A technique used predominantly in cattle, but also very well applicable to swine – in the future perhaps even more so.

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This article introduces the basics of swine embryo transfer, a collective term for a technology that could well be more common in the future of the swine industry. Once an oocyte or 'egg' is fertilised by a sperm cell, the resulting zygote is an embryo. The early stages of embryonic development have been the focus of embryo transfer in multiple species for many years.

What is ET?

Embryo transfer (ET) refers to the area of reproductive science involved in removing embryos from the uterus of one female (the donor) and transferring one or more of them into a host or surrogate female (the recipient). The first mammalian offspring resulting from ET was achieved in rabbits almost 119 years ago! The first ET piglets were born on March 27, 1950 in Ukraine. Since then, research on porcine reproduction including ovulation, fertilisation, embryo development, preservation, migration, implantation and survival has been the result of ET related research.

Embryo transfer is now a global industry that reports the transfer of nearly 955,000 commercial (non-research) embryos from mammalian species in 2005, 92% of which are from cattle. The remaining 8% are primarily derived from sheep, goats, pigs, and horses. It is estimated that nearly 10,000 in vivo (derived from live animals) and over 40,000 in vitro (derived from laboratory research projects) pig embryos are produced each year. The major countries reporting this activity to the International Embryo Transfer Society include the United States, Canada, Taiwan, Korea and countries in the European Union.

How is ET possible in the pig?

Commercial embryo transfer has been a practical alternative in cattle breeding for years. The reproductive tract and the size of the animal enable rectal palpation of the reproductive tract, further enabling the non-surgical rectal-vaginal transcervical deposition of an embryo into the ipsilateral (side of ovulation) uterine horn. In the pig, the smaller size of the animal limits or inhibits the use of non-surgical rectal-vaginal trans-cervical deposition of embryos. As well, the pig is a polytocous animal with characteristically long uterine horns needed to nurture a brood or litter of piglets during gestation. It is the presence of these long uterine horns and the characteristic 'corkscrew' type cervix of the pig that hinders the non-surgical transcervical collection and deposition of embryos.

Therefore, scientists and veterinarians have long relied on surgical mid-ventral laparotomy for the recovery and transfer of embryos in the pig.

Isn't ET in the pig redundant?
Pigs are a litter-bearing species and, in contrast to a species like cattle where ET is extensively used, pigs are relatively young at puberty and have a short gestation length. As a result, aggressive mating selection in swine operations that use AI already allows for rapid genetic improvements, without the need of ET.
Therefore, what motivating factors drive the industry to continue to attempt implementing embryo transfer techniques? Here are a few:

Rapid genetic progress
Only 50% of the genetics can be affected in one generation through the use of AI, whereas 100%, or entirely new genetics, can be introduced with an embryo.

Expanded markets
Pig production continues to grow worldwide, and breeding companies that operate internationally will benefit from the cost-effective ability to transport embryos instead of live animals. This will also allow smaller operations the opportunity to become more competitive by marketing unique genetics in the form of stored/shipped embryos.

Reduced health risks
Embryo transfer can be used to bridge health differences. Pig embryos can be removed from females with diseases such as pseudorabies or Porcine Reproductive and Respiratory Syndrome (PRRS), 'washed' and transferred to a recipient to establish disease free herds. This unique characteristic of ET can also be applied to minimise or eliminate unknown disease risk between herds desiring rapid genetic progress. This use of ET has been referred to as 'genetic rescue'.

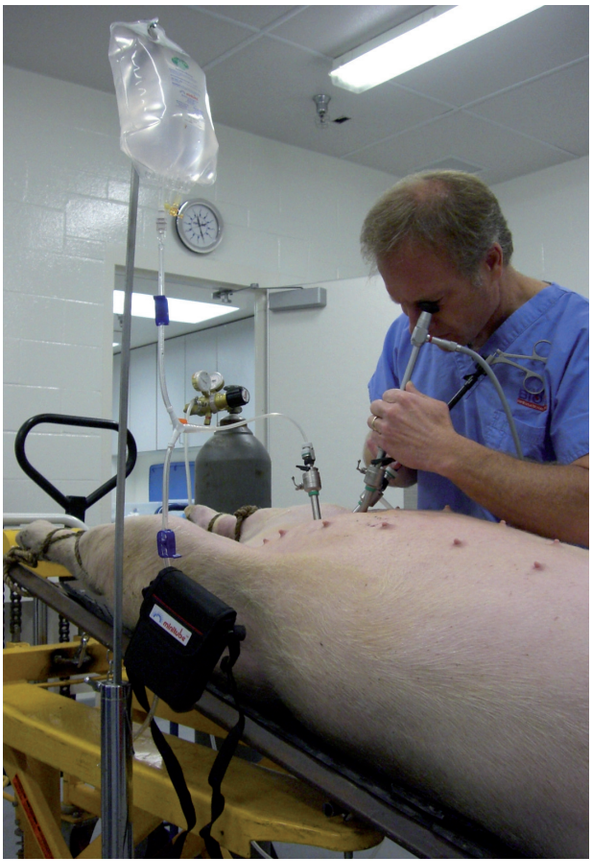
Genetic leverage
Use of other biotechnologies such as cloning and embryo cryopreservation require ET for production of live offspring.

Medical science
Pig organs are the most compatible with humans compared to other species because of similarities in anatomy, physiology and size. Ethically, they are less controversial than non-human primates. In the last decade, the number of patients waiting for organ transplant tripled leaving the US Department of Health and Human Services organ donation programme over 50,000 short. Embryo transfer procedures are a critical step involved in the development and production of gene-altered pigs suitable for supplying these organs. As well, the pig has been recognised as a more suitable animal for the basis of medical models of human disease. In the US, less than 8% of studies using rodent or other traditional models for human disease that head to clinical trials actually make it to a finished product treating the human condition. The pigs' unique physiology and anatomy make it a more suitable model for human disease, and the pig is being actively used for genetic manipulation for medical model production for diseases such as atherosclerosis, diabetes, cystic fibrosis, muscular dystrophy, cancer, and other dreadful diseases.

What are the basics?
Below is a list of management and technical factors that would be involved when integrating ET into a swine operation.

Oestrous cycle control
If the process of ET is to be successful, the donors and recipient females must be synchronised (for fresh embryo transfer) so that the recipients are in oestrus near the same time as the donors.

Super-ovulation
Even though the pig naturally releases or 'ovulates' 10 to 25 oocytes per oestrous cycle, the donor can be hormonally stimulated to produce more, making her even more efficient, since the process of ET involves removing those embryos from the donor female. However, super-ovulation is not necessary for successful ET.



Conventional embryo transfer in sows as it has been practised for a long time, using surgical mid-ventral laparotomy.

Oestrus detection
A reliable and consistent method of oestrus detection and record keeping must be employed for both donors and recipients so that the recipients can be precisely matched with individual donors and their embryos.

Artificial insemination
In most species, ET is typically coupled with an AI programme because producers want to take advantage of mating genetically-superior, donor-quality females with genetically-superior males.

Embryo recovery & transfer
Present technology is rapidly advancing, but several surgical, semi-surgical, and non-surgical techniques are currently being evaluated to determine the most effective and practical approaches in the pig. Currently, surgical recovery and transfer yield the most consistent results.

Donors and recipients
Donor females supply embryos to be transferred into recipients. Donors can be maternal or paternal lines but maternal lines are preferred for recipients. Donors and recipients can be either gilts or sows but gilts are much easier to handle for surgical procedures.

Embryo handling and expertise
Acceptable ET results are only obtained when embryos are properly managed from recovery to transfer. Trained personal use sterile techniques and media designed for embryo growth to handle embryos from recovery to transfer in controlled environments.

Embryo transport
Embryos must be properly identified and packaged to be transported to the recipient location. Transportation can vary from walking, driving or flying from one location to another. Embryos can be cultured for up to 72 hours with diminishing results.

Embryo preservation
Once recovered, embryos may be cultured during transport, especially if ET is to occur within 24 hours of recovery. Alternative methods of embryo preservation have been developed, including hypothermic embryo storage for up to 48-72 hours and embryo vitrification for long-term cryopreservation. While not as routine as embryo culture, these technologies will be instrumental for the logistical implementation of a global embryo transfer service in the future.

What is the future?

At present, pregnancy and farrowing rates up to 85% and embryonic survival rates (within pregnant recipients) of 50-55% are typical. This means that combined, up to 50% of all transferred embryos will result in the birth of piglets. Results are dependent on the reason for ET. Using ET to rescue genotypes from health compromised donors may be less efficient than using ET for genetic transfer in healthy herds (see *Table 1*). Length of time in culture may impact international transfer results. The success of pig ET in a wide range of applications shows that it is a robust procedure worthy of consideration. In our experience, greater risk is taken with donors and embryo quality to accomplish genetic rescue resulting in reduced efficiency. The practical implementation of embryo transfer requires specialised facilities, equipment and skilled surgical teams.

Non-surgical embryo transfer is a relatively new technique, developed by scientists at the University of Murcia.

Non-surgical ET?

In the last decade, much research has been devoted to more feasible and repeatable non-surgical techniques in swine. Borrowing techniques from other species had limitations, due to the smaller size and unique nature of the female porcine reproductive system. Research continues to make progress towards making more practical use of non-surgical ET in the pig. The most promising results have come from a catheter developed at the University of Murcia by Dr Emilio Martinez. A 70.8% farrowing rate and 6.9 pigs born was achieved using the catheter that is now available from Minitube. Continued research efforts such as these could make non-surgical ET a viable technology, making the benefits of ET even more cost effective and practical for pig producers.

The future of the swine industry is going to find, however, that embryo transfer procedures will become commonplace, it's just a matter of time. Many global market forces and technological breakthroughs are contributing to this inevitable fact. It is an exciting time to be involved in this endeavour, both, from the standpoint of on-going integrated research efforts and new possibilities for producers. **PP**

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Table 1. Embryo transfer experience: Comparing genetic transfer to genetic rescue.

ET group	Embryos/ Recipient	Transfers	Recipients farrowed	Farrow rate	Born live/ Recipient	Pigs/Embryo (farrowed recipients)	Pigs/Embryo (all recipients)
Genetic transfer	15.7	63	52	82.5	8.25	0.52	0.43
Genetic rescue	15.7	54	30	55.6	8.17	0.52	0.29