

Non surgical Embryo Transfer (ns-ET) in Pigs

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■ Introduction

Gene transfer can be done in several ways: by live animals, fresh or frozen semen, and fresh or frozen embryos. Any of these methods have their specific advantages and disadvantages.

Health is an increasingly important issue in the transfer of genetics in the swine industry and especially in the breeding pyramid. With this transfer of “gene packages” it is also possible to transfer uninvited guests, or more specifically, pathogens. For this reason transport regulations have become very strict over the last few years. The same applies to the safety precautions that pig breeding companies take to protect the health of the pigs on their own farms and on the farms of their clients. Methods for connecting breeding herds and disseminating genes around the world are becoming more and more sophisticated. The Office International des Epizooties (OIE) in France, with the International Embryo Transfer Society (IETS) as its main advisor, regard embryos as the best way to transfer genetics world-wide without pathogen transfer (Stringfellow and Seidel, IETS manual, 1998 & International Animal Health Code 2001 of the OIE). Co-financed by TOPIGS, Wouter Hazeleger developed a non-surgical Embryo Transfer (ns-ET) technique for pigs (Hazeleger and Kemp, 1994 & 1999). Over the last three years, TOPIGS further perfected the practical implementation of the method (Smits et al., 2000; Ducro-Steeverink et al., 2001).

■ Why ns-ET?

There are a lot of advantages to ET, especially when using this technique at the top of the pig breeding pyramid:

- There is minimal risk of introducing pathogens during an importation into a country or when introducing breeding animals on to a farm.

- It is possible to upgrade the health status of an existing breeding herd.
- For a simple exchange of genetic material between farms, nationally or internationally.
- Isolation is in principle not needed (although it is advisable when starting with this new method).
- ET is the perfect method of adaptation of new genetics or animals to a new farm; the uterus is the best adaptation environment.
- Intensive medication and vaccination programs are not needed, nor are blood samples for serological tests.
- Closed farms can now introduce complete genomes of a superior combination rather than only half of it through the sperm of a superior boar.
- In the near future freezing will be available and then the requested superior genes can be delivered on demand with the highest disease free guaranty available at this moment.
- Combined with the freezing technique, ET makes it possible to preserve variation in genes, an advantage for bio-diversity of the resources of nature and for pig breeding as a global activity.
- If a farm or company has an isolated frozen embryo storage bank it does not lose its genetic potential if decimated by a list A disease (e.g. FMD). With this method it is easier to comply with the (new) legislation which will very likely be focusing on minimum animal transport and closed farms.

Besides the advantages of ET in general, there are some extra advantages to the non-surgical method as compared to the surgical method. Animal welfare is becoming increasingly important within national and international legislation, due to consumer demand. This makes it advisable not to use a regular surgical method in the commercial production of animals. Also the surgical method is very expensive and can only be performed in a well equipped location and by well trained specialists.

■ Results

Since the early 60s embryo transfer in pigs has been performed mainly as a research tool (Hancock and Hovell, 1962). Good results with more than 90% pregnancy were reached with the surgical transfer of porcine embryos (Hazeleger and Kemp, 1999). But the first non-surgical procedure wasn't performed until the 90s and with much lower results (Table 1) (Reichenbach et al, 1993).

Table 1. Results of ns-ET in research.

	Sedation	# Sows	Pregnancy%	Litter size	Stage
Reichenbach	Y	58	9	5±2.6	born
Galvin	Y	46	22	4.3±0.7	born
Li	Y	16	31	6.2±3.1	born
Hazeleger	N	21	33	6.7±1.6	D35
Yonemura	N	25	64	3.1±1.6	born
Hazeleger	N	27	59	10.9±3.4	D35

At the beginning of 2000, we standardised the method and, in that year, performed ns-ET's to a total of 45 sows. For each transfer, 25 zona pellucida intact blastocysts at 120 hours of development (Picture 1) were transferred into the recipient sow with the following results (Table 2).

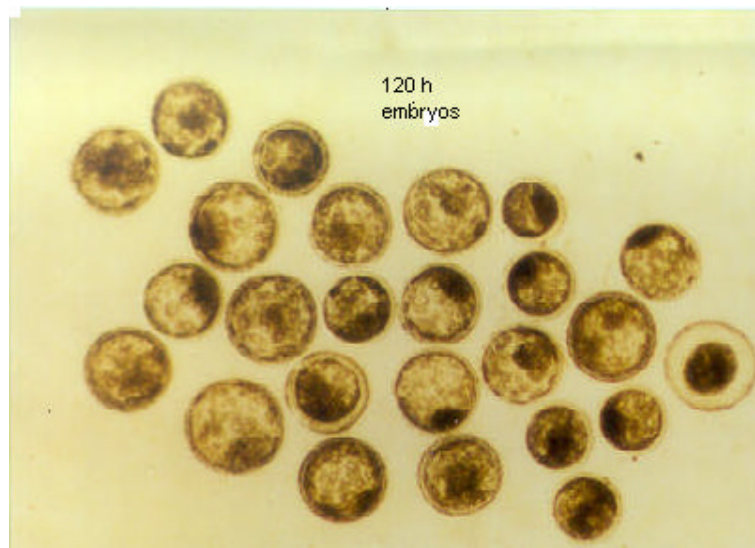
**Picture 1. An “ET-litter” of porcine embryos at 120 hours of development.**

Table 2. Results of the 45 ns-ET's performed in 2000 by IPG¹.

Days after ovulation	# recipients not returned to heat	% pregnant
5	45	100%
21	35	78%
30	24	53%
40	22	49%
115	20	44%

¹ Institute of Pig Genetics

170 piglets were born with an average birth weight of 1.7 ± 0.4 kg; compared to 788 control piglets on the experimental farm with an average birth weight of 1.5 ± 0.3 kg. The vitality of the ET piglets was normal or above normal. No anatomical nor physiological abnormalities were observed and the first sows are now producing with good results.

■ Ongoing Research Needed

Pathogen free

With in-vitro and in-vivo experiments information must be collected to prove porcine embryos are free from pathogens after the washing procedures advised by the IETS (Smits et al., 2001). (In-vitro means embryos in the laboratory contaminated with pathogens, washed and tested; in-vivo means embryo donors infected, embryos collected, washed, transferred in recipients and tested during pregnancy and after birth).

Preservation

At this moment cryo-preservation is not sufficiently reliable for routine use and the embryos have to be transferred fresh. From the initial results, 24 hours from collection to transfer seems to be no problem, and even 48 hours looks promising, depending on the medium used to culture embryos between recovery and transfer to the recipient sow. However, the logistics are very difficult when working with fresh embryos.

Cryo-preservation

The whole logistical problem will be gone when porcine embryos can be frozen. The first results are very promising (Berthelot et al., 2000; Dobrinsky et al., 2000).

Sexing Embryos

Early in development the zona pellucida protects embryos against pathogens and even extreme external influences. At day 6 – 7 the porcine embryo “hatches” from the zona pellucida and from that moment the disease free guarantee is less reliable and washing and transport will be more harmful or even lethal to the embryos.

Because of the need to transfer embryos with an intact zona pellucida the embryos can not be sexed. However, it is possible to use sexed sperm for insemination. Sexing a normal dose of semen takes far too much time to produce, but with low dose technique and deep intra-uterine insemination it will be possible in the future.

Embryo Collection

At the moment the donors are operated on or slaughtered, to collect embryos, which is not the ideal way for the future. Workable alternatives investigated are deep intra-uterine embryo collection, shunting (shortening of both uterus horns) and trans-abdominal (laparoscopic) embryo collection.

In-vitro Production (IVP)

An alternative to collection of embryos derived from in vivo fertilization is to collect oocytes from ovaries of prepubertal gilts sent to commercial abattoirs. The eggs are then matured and fertilised in vitro and the embryos are cultivated up to blastocyst stage before non-surgical transfer. This method had the first positive results (Kidson, 2001). However, this method will take many years to reach practical applicable results.

■ Practical implementation

Since the beginning of this year TOPIGS has an official ET team actively working on the implementation of the ns-ET within it's breeding structure. Foot and mouth disease caused some delay, but now we are going full speed ahead. We are preparing several ET projects within Europe and from Europe to other continents.

At the moment we only want to use ET for transferring complete packages of superior genetics at the nucleus level while using the highest health security possible. However there are also already possibilities of transferring production boars into difficult markets.

In the future most likely the genetic linking of the pig breeding farms will be by frozen sperm, and ns-ET will be the best way of creating new breeding farms at the nucleus or sub-nucleus level world-wide and for the quick upgrading of breeding farms.

In a few years, with better results the costs will decrease by more than 75%. By then the technique will even be interesting at the commercial farm level. Farms who do not want to introduce gilts from other farms due to disease risks can introduce top genetics once or twice a year with ET, without having to bother with the production of their own nucleus sows,. This way they can easily produce with the best genetics and even change quickly between lines if they choose.

After a big delay, as compared to ns-ET in cattle, the ns-ET method in swine has progressed substantially over the last few years in the porcine industry by large steps. It is my opinion that it will now only take a few more years until it will be implemented and accepted as the best way of transferring genes within the porcine breeding pyramid, while providing the highest health security possible.



Picture 2. Non-surgical embryo transfer (ns-ET) in practice with the Swinlet®-catheter

■ References

- Berthelot, F., F. Martinat-Botté, A. Locatelli, C. Perreau and M. Terqui.** (2000) Piglets born after the use of vitrification of embryos using the open pulled straw method. *Cryobiology* 41: 116-124
- Dobrinsky, J.R., V.G. Pursel, C.R. Long and L.A. Johnson** (2000) Birth of piglets after transfer of embryos cryopreserved by cytoskeletal stabilisation and vitrification. *Biology of Reprod.* 62: 564-570
- Ducro-Steeverink, D.W.B., J.M. Smits, W. Hazeleger and J.W.M. Merks.** (2001) Reproduction results after non-surgical embryo transfer in pigs. *Theriogenology* 55; 1: 361
- Galvin, J.M., D.B. Killian and A.N.V. Stewart.** (1994) A procedure for successful non surgical embryo transfer in swine. *Theriogenology* 41: 1279-1289
- Hancock, J.L. and G.J.R. Hovell.** (1962) Egg transfer in the sow. *J. Reprod. Fert.* 4: 195-201
- Hazeleger, W. and B. Kemp.** (1994) Farrowing rate and litter size after transcervical embryo transfer in sows. *Reprod. Dom. Anim.* 29: 481-487
- Hazeleger, W. and B. Kemp.** (1999) State of the art in pig embryo transfer. *Theriogenology* 51: 81-90
- Kidson,** (2001) personal communication
- Li, J., A. Rieke, B.N. Day and R.S. Prather.** (1996) Technical note: porcine non-surgical embryo transfer. *J. Anim. Sci.* 74: 2263-2268
- Office International des Epizooties (OIE)** International Animal Health Code 2001
- Reichenbach, H.-D., J. Mödl, and G. Brem.** (1993) Piglets born after transcervical transfer of embryos into recipient gilts. *Vet. Rec.* 133: 36-39
- Smits, J.M., W. Hazeleger and J.W.M. Merks.** (2000) Farrowing rate and litter characteristics after non-surgical embryo transfer in pigs in a field experiment. *Theriogenology* 53; 1: 317
- Smits, J.M., D.W.B. Ducro-Steeverink, P.J.G.M. Steeverink and J.W.M. Merks.** (2001) Risk assessment of pathogen transmission by porcine embryos with embryo transfer. Presentation European Association of Animal Production, Budapest, Hungary, 26-29 August 2001
- Stringfellow D.A. and S.M. Seidel.** (1998) Manual of the International Embryo Transfer Society. third edition, 173 pp, ISBN 0-9662386-0-5
- Yonemura, I., Y. Fujino, S. Irie and Y. Miura.** (1996) Transcervical transfer of porcine embryos under practical conditions. *J. Reprod. Dev.* 42: 89-94