EMBRYO TRANSFER IN PIGS: CURRENT STATUS AND PROSPECTS FOR COMMERCIAL APPLICATIONS

T.van der Lende[1] W. Hazeleger[2]

[1]Animal Breeding and Genetics Group & [2]Adaptation Physiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

1 Introduction

During the last decades of the twentieth century rapid scientific advances in embryo and reproductive technologies have been made, both in medical science and in animal sciences. Except for artificial insemination (AI), which is used on a large scale, no other embryo or reproductive technologies are currently routinely applied in commercial pig breeding or in the dissemination of superior genetics from pig breeding units to commercial herds. Surgical embryo collection and surgical embryo transfer have been used commercially for some time now (Brüssow and König, 1988; Cameron *et al*., 1989), but only occasionally and to a very limited extend. Because of the high fecundity of pigs, the need for extra offspring per breeding female is less than in cattle breeding. However, the current use of new reproduction techniques in cattle breeding and the increased need for transfer of pig genetic material around the world with minimal health risks and low costs, lead to a stronger motivation to use new reproduction technologies in pig breeding and pig production. Important for the successful application of new reproduction techniques in cattle breeding programs, e.g. superovulation, nonsurgical embryo collection and transfer, ultrasound-guided transvaginal ovum pick-up, *in vitro* maturation and fertilization (IVM-IVF) of oocytes and embryo freezing, was the early development of a reliable, efficient technique for nonsurgical embryo transfer. Likewise it is to be expected that the availability of such a technique in pigs will contribute much to the development and commercial use of other reproduction technologies too.

In this paper we will first review the state of the art as far as embryo transfer in pigs is concerned, with emphasis on the development of nonsurgical embryo transfer. Subsequently we will review the prospects for commercial applications of nonsurgical embryo transfer in combination with other reproduction techniques, including small scale-high value applications and potentially large scale applications which may have far-reaching consequences for the structure of the pig industry.

2 Surgical embryo transfer

Surgical procedures for transferring embryos into recipients have been available for several decades. Research into factors affecting the results of surgical transfer has been reviewed as early as 1982 (Polge, 1982). Surgical procedures for commercial applications have only been used to a limited extent (James *et al*., 1980; Cameron *et al*., 1989). In principle, all surgical transfers are performed under general anesthesia,

with the genital tract presented through midventral incision in the caudal abdominal region. Usually gilts are used as recipients because they are easy to handle and tolerate anesthesia and surgery better than sows. The embryos are placed in the oviduct or tip of the uterine horn, depending on the developmental stage of the embryos. They are deposited with a small volume of transfer medium, using a small pipette or a thin catheter. On average, the pregnancy rate is about 60%, and the litter size is 6.5 piglets, with a range from 17% with 2.4 piglets to 100% with 10.8 piglets (Brüssow and König, 1988).

3 Endoscopic embryo transfer

Endoscopic procedures for embryo transfer has been developed more recently than surgical procedures (Stein-Stefani and Holtz, 1987; Besenfelder *et al*., 1997). Although they can be defined as surgical procedures, their advantage is that only a few small incisions are needed for the instruments. Nevertheless, the endoscopic procedures do require anesthesia and other surgical precautions similar to those of the conventional surgical approaches, and are therefore less applicable for use on individual farms. Pregnancy rates have ranged from 14% (2/14) with 8 and 9 piglets (Stein-Stefani and Holtz, 1987) to 40% (5/12) with an average of 7 piglets per litter for transfers into the uterus, and 33% (9/27) with an average of 6 piglets per litter for tubal transfers (Besenfelder *et al*., 1997). Pregnancy rates of 90% for routine application of endoscopic embryo transfer (after endoscopic embryo collection) were reported, but detailed results were not presented (Besenfelder *et al*., 1998). Together, these results indicate that such a minimally invasive surgical method of transferring embryos can be readily used in pigs.

4 Nonsurgical embryo transfer

The first experiment with nonsurgical embryo transfer was reported by Polge and Day (1968). Of 17 transfers performed, one resulted in pregnancy: 17 days after transfer of 14 embryos, three living embryos and remnants of four regressing embryos were found at slaughter. The poor transcervical accessibility of the uterus of non-estrous sows was mentioned as a major problem. In fact, this has been considered as the main problem in the development of nonsurgical embryo transfer in pigs for a long time. Almost 20 years after the study of Polge and Day (1968), Sims and First (1987) reported successful nonsurgical transfer, but in their experiments all recipient sows had also been slaughtered during pregnancy, leaving the question about the possibility to obtain live born piglets after nonsurgical embryo transfer unanswered. In the years to follow five independent groups have reported birth of viable piglets after nonsurgical embryo transfer (Reichenbach *et al*., 1993; Galvin *et al*., 1994; Hazeleger and Kemp, 1994; Li *et al*., 1996; Yonemura *et al*., 1996; Hazeleger *et al*., 2000a). For the nonsurgical embryo transfer Reichenbach et al. (1993) used a sterile disposable AI-catheter (Spirette, Minitüb) in combination with a sterile disposable embryo transfer cannula (IMV, L'aigle). Galvin et al. (1994) and Yonemura et al. (1996) used sterile disposable AI-catheters (International Boar Semen, Eldora, IA, USA and

FA1454, Fujihira Equipments and Instruments Co. Ltd., Tokyo, Japan, respectively). Reichenbach et al. (1993) as well as Galvin *et al*. (1994) and Yonemura et al. (1996) used relatively large volumes of transfer medium. In contrast to these groups Hazeleger and Kemp (1994), Li *et al*. (1996) and Hazeleger *et al*. (2000a) used specially designed transfer equipment in combination with low volumes of transfer medium. Reichenbach et al. (1993) and Li *et al*. (1996) anesthetized the recipients and did the transfers while the animals were placed in dorsal recumbency on a modified operating table or in a V-trough, respectively. In the experiments of Reichenbach et al. (1993) the recipients furthermore had their legs secured and the hindquarters raised by mechanically raising the back of the table 90ºfrom the horizontal plane. While Galvin *et al*. (1994) only mildly sedated the recipients, Hazeleger and Kemp (1994), Yonemura et al. (1996) and Hazeleger *et al*. (2000a) performed the transfers without any pre-treatment of the recipients. In the latter studies the recipients were standing during transfer, slightly restricted in movement only.

As can be seen from the overview in Table 1, the results obtained are variable, but in general both farrowing rate and litter size are still rather low. As far as farrowing rate is concerned, the 64% obtained by Yonemura *et al*. (1996) and 59% obtained by Hazeleger *et al*. (2000a) are promising, although it should be noticed that the prenatal survival of the transferred embryos was relatively low, especially in the experiment of Yonemura *et al*. (1996).

Table 1 — Farrowing rate and litter size after nonsurgical embryo transfer in pigs

	Reichenbach *et al.*(1993)	Galvin *et al.* (1994)	Hazeleger & Kemp (1994)	Li *et al.* (1996)	Yonemura *et al.* (1996)	Hazeleger *et al.* (2000a)**
Number of recipients	58	46	21	16	25	27
Number of embryos transferred	25–40	11.8±0.5	17.2±1.9	22.5±9.5	17.8±7.9	28–30
Transfer medium (ml)	10.25–20.25	10.5–12.5	0.1	<0.3	30 or 50	<0.1
Stage of embryos transferred	8 cell to hatched blastocyst	4 cell to hatched blastocyst	Morula to blastocyst	4 cell to blastocyst	4 cell to hatched blastocyst	expanded blastocyst
Number of farrowings	5*	10	7	5	16	16
Farrowing rate (%)	<9	21.7	33	31	64	59
Litter size	2, 6, and 7	4.3±0.7 (3–6)	6.7±1.6 (4–9)	6.2±3.1 (3–10)	3.1±1.6 (1–7)	10.9±3.4 (3–15)
Prenatal survival in farrowing recipients (%)	7.4, 20.0 and 21.9	34.9±13.2 (21.4–62.5)	38.0±8.2 (22.2–46.7)	31.2±17.3 (12.9–55.6)	16.7±7.6 (7.1–33.3)	37±11 (10–50)

* 2 pregnant recipients were slaughtered between Days 35 and 45; it is assumed that these recipients would have farrowed. ** recipients were slaughtered on Day 35 of pregnancy but it is assumed that Day 35 embryos will survive until farrowing.

With the equipment as used by Li *et al*. (1996), embryos are transferred a few inches into one of the uterine horns. With the equipment used by Hazeleger and Kemp (1994) and Hazeleger *et al*. (2000a) and with the AI catheters used by the other groups, embryos are transferred into the uterine body or uterine horns, to a site close to the bifurcation. At the stage of development at which the embryos were transferred, the embryos would normally still have been in the tip of the uterine horns, very close to the utero-tubal junction. Using surgical embryo transfer, Stein-Stefani and Holtz (1987) reported that transfer to the middle of the uterine horns was as successful as transfer to the tip of the uterine horns. More recently Wallenhorst and Holtz (1995) reported that surgical embryo transfer to the uterine body gave very poor results in comparison to surgical embryo transfer to the middle of the uterine horns (pregnancy rate 12% versus 88%, embryo survival 3% versus 41%). Results after surgical embryo transfer to the caudal quarter part of the uterine horns was intermediate, with a good pregnancy rate (81%) but a poor embryo survival (28%). These results need substantiation, but if they are confirmed they illustrate the necessity to try to transfer the embryos as deep as possible into the uterine horns. For the time being the conclusion of Wallenhorst and Holtz (1995) that their results imply poor prospects for nonsurgical embryo transfer seems too pessimistic.

Based on the already mentioned studies and some subsequent studies, a number of interesting observations has been made:

- When nonsurgical transfers were classified according to developmental stage of the transferred embryos, pregnancy rate was 55% (6/11) when morulae together with blastocysts were transferred and only 10% (1/10) when only morulae were transferred ($p<0.06$) (Hazeleger and Kemp, 1994).
- When nonsurgical transfers were classified as (+,+), (+,-) or (-,-) on the basis of the combination of resistance to rotation of the spiral catheter used and resistance to infusion of the medium containing the embryos, the farrowing rates were 44% (4/9), 60% (6/10) and 100% (6/6), respectively. No differences were found in litter size or embryo survival (Yonemura *et al*., 1996).
- In contrast to what has been found for surgical embryo transfer (Polge, 1982), the chance of pregnancy after nonsurgical embryo transfer seems to be higher when the recipient ovulated at the same time or somewhat earlier than the donor (Hazeleger *et al*., 1995a; Hazeleger *et al*., 1995b; Hazeleger *et al*., 2000b.)
- Transfer of Day 5 embryos from donors with more than 25 ovulations gave better pregnancy rates (6/10) than transfer of the same number of Day 5 embryos from donors with 25 or less ovulations (2/10) (Hazeleger and Kemp, 1997).

Observations like these will contribute to a better understanding of factors influencing the success of nonsurgical embryo transfer and thereby to improvement of nonsurgical embryo transfer protocols.

5 Sources of porcine embryos

Before considering applications of nonsurgical embryo transfer, possible sources of embryos for nonsurgical embryo transfer will be briefly discussed. Embryos can

either be collected from donor sows or can be produced *in vitro*. Collection of embryos from donor sows was for a long time only possible after slaughter or by surgery. In cattle embryos can be obtained routinely by transcervical flushing of the uterus of (superovulated) cows. In the pig this is not possible, primarily due to the length and winding position of the uterine horns. By surgically shunting the top and base of the uterine horns this problem can be circumvented (Hazeleger et al., 1989; Kobayashi *et al*., 1989). This enables frequent transcervical embryo collection from the same donor sow. The embryo recovery is unfortunately highly variable, which may limit practical application (Hazeleger *et al*., 1994). Moreover, it is doubtful whether this practice will be ethically acceptable for commercial applications. In this respect the prospects for endoscopic embryo collection (Brüssow and Ratky, 1996; Besenfelder *et al*., 1997) are better. With this minimally-invasive technique embryos can be collected from the oviduct or the tip of the uterine horns. When used to obtain embryos from high merit breeding stock, a drawback of this procedure is the limited number of embryos that can be obtained.

To have large numbers of embryos available, embryos may be produced in vitro, either by IVM-IVF of oocytes or by cloning. Successful production of live born piglets from IVM-IVF has been reported, but overall success rates are still low. The occurrence of polyspermic fertilization remains a major problem (Nagai, 1996; Funahashi and Day, 1997). Once this problem can be overcome, the prospects for IVM-IVF are favorable since IVM-IVF systems are now available in which 80% of *in vitro* matured and normally fertilized oocytes do develop to the blastocyst stage (Funahashi and Day, 1997). The oocytes for IVM-IVF can be collected from ovaries of slaughtered fattening pigs or breeding females, but it is also possible to collect them in live animals by means of laparoscopical follicular puncture (Brüssow and Ratky, 1994). To our knowledge ultrasound-guided ovum pick-up, as used in cattle, has not been reported in pigs.

Cloning technology enables production of large numbers of essentially genetic identical embryos. In the pig this technology is still hardly developed. As early as 1989 the birth of a single cloned piglet was reported after surgical transfer of embryos produced by nuclear transfer of 4-cell nuclei into enucleated, activated metaphase II oocytes (Prather *et al*., 1989). It took 11 years before another successful attempt to clone pigs was reported (see http://www.ppl-therapeutics.com/html/cfml/index_fullstory.cfm?StoryID=14). The production of clones by transfer of donor nuclei into enucleated oocytes is in fact still highly inefficient for all livestock species. For a long time embryonic stem cells were considered essential for large scale cloning. However, many years of research has shown that the culture of embryonic stem cells and the maintenance of embryonic stem cell lines in culture are far from uncomplicated in livestock (Wilmut *et al*., 1997a). An important breakthrough related to donor cells for nuclear transfer has been reported by Campbell et al. (1996) and Wilmut *et al*. (1997b). The latter reported the birth of viable offspring in sheep derived from fetal fibroblasts and adult mammary epithelial cells. The basis for this success was 'the induction of a state of quiescence' in the donor cells, a technique that had been reported by Campbell *et al*. (1996; see also Campbell, 1999). A synchronous population of diploid donor nuclei was produced by inducing the cells to exit the growth cycle and arrest in the G0 quiescent stage of the cell cycle. This development is generally considered as an important step in future large scale cloning of livestock embryos. It is not unthinkable that the production of clones from (certain) somatic

cells of adult livestock (and mammals in general) will be routinely possible, although probably not yet very efficient, in the near future.

6 Applications of nonsurgical embryo transfer in combination with other new technologies in female reproduction

6.1 International trade in breeding stock

With the ongoing world-wide growth of pig production, internationally operating breeding companies can largely benefit from the possibilities to transport embryos instead of live animals. Next to an enormous reduction of transportation costs, it also minimizes the risk of disease transmission around the world. The latter is especially the case if embryos can be stored frozen before transport in order to test the donor animals for seroconversion during a certain period after collection. Although both collection and transfer of embryos can be done surgically for purposes of international transport, the possibility to e.g. collect embryos with minimal-invasive techniques such as endoscopy and to transfer the embryos nonsurgically will enhance the efficiency and profitability of such an application and will be preferable from an animal welfare point of view. The latter as well as the earlier mentioned improved health status of pig populations when embryos instead of live animals are transported around the world are both important for acceptance of these procedures by society.

6.2 Improved breeding programs

Successful embryo transfer in combination with other reproductive technologies would affect the structure and efficiency of pig breeding programs drastically (Brascamp, 1998; Van der Lende *et al*., 1998; Visscher *et al*., 2000). The effect of the use of embryo transfer on the genetic gain in pig breeding programs has so far received little attention. Ollivier (1988) has shown that with about 5 transfers per donor, coupled with early breeding (about 5 months of age), the selection response in percent of that in a standard situation might exceed 150%. In combination with *in vitro* oocyte maturation and *in vitro* fertilization, embryo transfer makes it possible to breed with more similar numbers of dams and sires in a crossed or factorial mating design. At a given rate of genetic progress, this mating design gives less inbreeding than the currently used nested mating designs (Brascamp and Haley, 1994). According to Visscher *et al*. (2000) the ultimate impact of biotechnology, including non-surgical embryo transfer, will be increased rates of progress, efficient use of variation, reduced genetic lag, and the removal of one or two tiers in the pig breeding pyramid.

Implementation of embryo transfer in commercial pig breeding will have consequences for the breeding organizations. The selection of animals, synchronization of donors and recipients, timing of insemination of donors and collection and transfer of embryos have to be performed by trained people, according to strict protocols. This will require much attention and labor, but is unavoidable for successful implementation of these techniques.

6.3 Rapid dissemination of genetic progress

The implementation of new reproduction techniques enables new production systems. In this respect the use of nonsurgical embryo transfer technology in combination with cloning technology to produce high merit fattening pigs is an interesting possibility. In such a new production system the nucleus will consist of recipient lines and fattening lines instead of sow lines and boar lines. The recipient lines will only be selected for uterine capacity and mothering ability. The fattening lines will be selected e.g. for daily growth rate, feed intake capacity and percentage lean meat. Embryos, selected on the basis of pedigree, will be cloned for implantation in recipient sows in the multiplier units. These recipient sows will be clones as well, cloned from the very best individuals in the recipient lines. Cloned female embryos (F1's) from the recipient lines will be transferred to produce replacement gilts. Cloned embryos from the fattening lines (also F1's) will be transferred to produce fattening piglets.

The genetic and economic effects of a production system as outlined in the previous section, have been studied by Beeren *et al*. (1994). In comparison to the current production system, the new system largely increases the returns per slaughter pig, indicating that substantial costs for the *in vitro* production of embryos are acceptable. This study showed furthermore that the testing of clones before commercial use is not profitable unless four individuals are tested per clone and only one clone is selected. It seems that clones can best be selected on the basis of their pedigree only.

In addition to its potential for dissemination of genetic gain, cloning can have some additional benefits in commercial pig populations. Brascamp and Haley (1994) suggested the use of cloning to propagate specific genotypes for particular markets. According to these authors it is likely to be possible to derive these specific genotypes from the available genetic variability at the nucleus level if it is possible to develop methods to detect individual animals as a source for cloning for these markets. Often the increased uniformity of product offered by embryo transfer of cloned (identical) individuals in the commercial population is mentioned as an additional advantage. However, as indicated by Brascamp and Haley (1994), the degree of increased uniformity is quite small. In the case that 40 per cent of the variance in a trait is genetic, about 75 per cent of the population standard deviation will still be present in a population consisting of individuals of identical genotype.

Apart from potential benefits of the use of clones, there are also potential risks. The oocytes needed for nuclear transfer have to be collected from slaughterhouse material, which may be risky from a disease transmission point of view, unless extreme precaution is taken. With the use of clones, the between-animal genetic diversity in commercial herds will decrease. The animals will not only be more uniform in e.g. productivity and behavior, but also in disease resistance and susceptibility (Wilmut *et al*., 1992; Brascamp and Haley, 1994). Knowledge to quantify this problem is currently still not available. Wilmut *et al*. (1992) justly stated that models are needed to improve our understanding in this respect before breeding structures are changed.

7 Concluding remarks

Small scale-high value application of new techniques in female reproduction are already possible. Nonsurgical embryo transfer can now be applied, but farrowing rate

and litter size are still relatively low in comparison to what can be achieved with AI. In a field experiment in the Netherlands in which the method developed by Hazeleger (see e.g. Hazeleger and Kemp, 1994 and Hazeleger and Kemp 1999) had been used, pregnancy rate was 60% and litter size 2–14 piglets (Smits *et al*., 2000). With more research and increasing experience with the various aspects of the technique, results will undoubtedly improve. The time needed to get to farrowing rates and litter sizes as obtained after AI is difficult to predict, but there is no reason to assume that it should take more than a few years, provided that financial support for research remains available. With the current level of success some of the small scale-high value applications may already be feasible. Through these small scale applications experience will be gained, which will also contribute to further improvement and application of nonsurgical embryo transfer in pigs in general. The further development of nonsurgical embryo transfer will lead to more widespread application. Together with the further development of embryo technologies such as in vitro production of embryos (IVM-IVF, cloning), it may lead to completely new, more efficient production systems. The possibility to store embryos frozen will largely facilitate applications of nonsurgical embryo transfer. Fortunately, over the last years good progress has been made in the field of cryopreservation of pig embryos (Dobrinsky, 1997; Dobrinsky *et al*., 2000).

8 Referências bibliográficas

1. Beeren, R.J.H.G., De Vries, A.G., Brascamp, E.W. and Van der Lende, T. (1994) Genetic and economic effects of the application of cloned embryos and nonsurgical embryo transfer in pigs. Proceedings of the 5th World Conference on Genetics Ap-plied to Livestock Production, 18, 290–293.
2. Besenfelder, U., Mödl, J., Müller, M. and Brem, G. (1997) Endoscopic embryo collection and embryo transfer into the oviduct and the uterus of pigs. Theriogenology, 47, 1051–1060.
3. Besenfelder, U., Müller, M. and Brem, G. (1998) Transgenics and modern reproductive technologies. In The Genetics of the Pig, pp. 345–374. Edited by M.F. Rothschild and A. Ruvinsky. CAB International, Wallingford, UK.
4. Brascamp, E.W. (1998) The development of animal breeding enterprises in the next decades: reasons to change the structure of breeding programmes. Acta Agricultura Scandinavica, Section A, Animal Science, Supplement 28, 61–67.
5. Brascamp, E.W. and Haley, C.S. (1994) Biotechnology in modern commercial pig breeding. In Principles in Pig Science - 1994. pp 43-53. Edited by D.J.A. Cole, J. Wiseman and M.A. Varley. Nottingham University Press, Nottingham.
6. Brüssow, K.–P. and König, I. (1988) Embryotransfer beim Schwein - Stand und Nutzungsperspektiven in der DDR. Tag.-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin, 273, 153–170.
7. Brüssow, K.–P. and Ratky, J. (1994) Repeated laparoscopical follicular puncture and oocyte aspiration in swine. Reproduction in Domestic Animals, 29, 494–502.
8. Brüssow, K.–P. and Ratky, J. (1996) Endoscopic collection of porcine embryos. Reproduction in Domestic Animals, 31, 711–715.

9. Cameron, R.D.A., Durack, M., Fogarty, R., Putra, D.K.H. and McVeigh, J. (1989) Practical experience with commercial embryo transfer in pigs. Australian Veterinary Journal, 66, 314–318.
10. Campbell, K.H.S. (1999) Nuclear equivalence, nuclear transfer, and the cell cycle. Cloning, 1, 3–15.
11. Campbell, K.H.S., McWhir, J., Ritchie, W.A. and Wilmut, I. (1996) Sheep cloned by nuclear transfer from a cultured cell line. Nature, 380, 64–66.
12. Dobrinsky, J.R. (1997) Cryopreservation of pig embryos. Journal of Reproduction and Fertility, suppl.52, 301–312.
13. Dobrinsky, J.R., Pursel, V.G., Long, C.R. and Johnson, L.A. (2000) Birth of piglets after transfer of embryos cryopreserved by cytoskeletal stabilization and vitrification. Biology of Reproduction, 62, 564–570.
14. Funahashi, H. and Day, B.N. (1997) Advances in in vitro production of pig embryos. Journal of Reproduction and Fertility, suppl.52, 271–283.
15. Galvin, J.M., Killian, D.B. and Stewart, A.N.V. (1994) A procedure for successful nonsurgical embryo transfer in swine. The-riogenology, 41, 1279-1289.
16. Hazeleger, W. and Kemp, B. (1994) Farrowing rate and litter size after transcervical embryo transfer in sows. Reproduction in Domestic Animals, 29, 481–487.
17. Hazeleger, W. and Kemp, B. (1997) Effect of number of ovulations of donors on pregnancy rate after non-surgical transfer of porcine blastocysts. Theriogenology, 47, 365.
18. Hazeleger, W. and Kemp, B. (1999) State of the art in pig embryo transfer. Theriogenology, 51, 81–90.
19. Hazeleger, W., Bouwman, E.G., Noordhuizen, J.P.T.M. and Kemp, B. (2000a) Effect of superovulation induction on embryonic development on day 5 and subsequent development and survival after nonsurgical embryo transfer in pigs. Theriogenology, 53, 1063–1070.
20. Hazeleger, W., Jenneskens, P. and Kemp, B. (1995a) Non-surgical transfer of porcine blastocysts. Theriogenology, 43, 232.
21. Hazeleger, W., Kemp, B., Soede, N.M., Noordhuizen, J.P.T.M. and Van der Lende, T. (1994) Transcervical embryo collection and reproductive performance of sows with resectioned uteri. Reproduction in Domestic Animals, 29, 38–45.
22. Hazeleger, W., Noordhuizen, J.P.T.M. and Kemp, B. (2000b) Effect of asynchronous non-surgical transfer of porcine embryos on pregnancy rate and embryonic survival. Livestock Production Science, 64, 281–284.
23. Hazeleger, W., Peeters, I. and Kemp, B. (1995b) Asynchronous non-surgical transfer of porcine blastocysts. Proc. 11e Réunion A.E.T.E., Hannover, p188.
24. Hazeleger, W., Van der Meulen, J. and Van der Lende, T. (1989) A method for transcervical embryo collection in the pig. Theriogenology, 31, 727–734.
25. James, J.E., Reeser, P.D., Davis, D.L., Straiton, E.C., Talbot, A.C. and Polge, C. (1980) Culture and long-distance shipment of swine embryos. Theriogenology, 14, 463–469.
26. Kobayashi, K., Hayashi, S., Ohtubo, Y., Honda, A., Mizuno, J. and Hirano, S. (1989) Nonsurgical embryo collection from sows with surgically shortened uteri. Theriogenology, 32, 123–129.
27. Li, J., Rieke, A., Day, B.N. and Prather, R.S. (1996) Technical note: Porcine non-surgical embryo transfer. Journal of Animal Science, 74, 2263–2268.

28. Nagai, T. (1996) In vitro maturation and fertilization of pig oocytes. Animal Reproduction Science, 42, 153–163.
29. Ollivier, L. (1988) Future breeding programs in pigs. In Advances in Animal Breeding – 1988. pp 90–106. Compiled by S. Korver, H.A.M. van der Steen, J.A.M. van Arendonk, H. Bakker, E.W. Brascamp and J. Dommerholt. Pudoc, Wageningen
30. Polge, C. (1982) Embryo transplantation and preservation. In Control of Pig Reproduction, pp 277–291. Edited by D.J.A. Cole and G.R. Foxcroft. Butterworth Scientific, London.
31. Polge, C. and Day, B.N. (1968) Pregnancy following non-surgical egg transfer in pigs. Veterinary Record, 82, 712.
32. Prather, R.S., Sims, M.M. and First, N.L. (1989) Nuclear transplantation in early pig embryos. Biology of Reproduction, 41, 414–418.
33. Reichenbach, H.-D., Mödl, J. and Brem, G. (1993) Piglets born after transcervical transfer of embryos into recipient gilts. Veterinary Record, 133, 36–39.
34. Sims, M.M. and First, N.L. (1987) Nonsurgical embryo transfer in swine. Journal of Animal Science, 65(suppl.1), 386.
35. Smits, J.M., Hazeleger, W. and Merks, J.W.M. (2000) Farrowing rate and litter characteristics after non-surgical embryo transfer in pigs in a field experiment. Theriogenology, 53, 317.
36. Stein-Stefani, J. and Holtz, W. (1987) Surgical and endoscopic transfer of porcine embryos to different uterine sites. Theriogenology, 27, 278.
37. Van der Lende, T., Komen, J. and Brascamp, E.W. (1998) The role of reproductive techniques in genetic improvement and dissemination of genetic gain in livestock production and aquaculture. Acta Agricultura Scandinavica, Section A, Animal Science, Supplement 29, 90–97.
38. Visscher, P., Pong-Wong, R., Whittemore, C. and Haley, C. (2000) Impact of biotechnology on (cross)breeding programmes in pigs. Livestock Production Science, 65, 57–70.
39. Wallenhorst, S. and Holtz, W. (1995) Transfer of pig embryos to different uterine sites. Proc. 11e Réunion A.E.T.E., Hannover, p 256.
40. Wilmut, I., Haley, C.S. and Wooliams, J.A. (1992) Impact of biotechnology on animal breeding. Animal Reproduction Science, 28, 149–162.
41. Wilmut, I., McWhir, J. and Campbell, K. (1997a) Nuclear transfer from cultured cells: a new opportunity in animal breeding? In: Milk Composition, Production and Biotechnology – 1997. pp 389–396. Edited by R.A.S. Welch, D.J.W. Burns, S.R. Davis, A.I. Popay and C.G. Prosser. CAB International, Wallingford.
42. Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H.S. (1997b) Viable offspring derived from fetal and adult mammalian cells. Nature, 385, 810–813.
43. Yonemura, I., Fujino, Y., Irie, S. and Miura, Y. (1996) Transcervical transfer of porcine embryos under practical conditi-ons. Journal of Reproduction and Development, 42, 89–94.