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

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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 Al-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 Al-catheters (International Boar Semen, Eldora, IA, USA and
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 90° from 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**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Number of recipients</td>
<td>58</td>
<td>46</td>
<td>21</td>
<td>16</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>25–40</td>
<td>11.8±0.5</td>
<td>17.2±1.9</td>
<td>22.5±9.5</td>
<td>17.8±7.9</td>
<td>28–30</td>
</tr>
<tr>
<td>Transfer medium (ml)</td>
<td>10.25–20.25</td>
<td>10.5–12.5</td>
<td>0.1</td>
<td>&lt;0.3</td>
<td>30 or 50</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Stage of embryos transferred</td>
<td>8 cell to hatched blastocyst</td>
<td>4 cell to hatched blastocyst</td>
<td>Morula to blastocyst</td>
<td>4 cell to blastocyst</td>
<td>4 cell to hatched blastocyst</td>
<td>expanded blastocyst</td>
</tr>
<tr>
<td>Number of farrowings</td>
<td>5*</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>&lt;9</td>
<td>21.7</td>
<td>33</td>
<td>31</td>
<td>64</td>
<td>59</td>
</tr>
<tr>
<td>Litter size</td>
<td>2, 6, and 7</td>
<td>4.3±0.7</td>
<td>6.7±1.6</td>
<td>6.2±3.1</td>
<td>3.1±1.6</td>
<td>10.9±3.4</td>
</tr>
<tr>
<td>Prenatal survival in</td>
<td>7.4, 20.0</td>
<td>34.9±13.2</td>
<td>38.0±8.2</td>
<td>31.2±17.3</td>
<td>16.7±7.6</td>
<td>37±11</td>
</tr>
<tr>
<td>farrowing recipients</td>
<td>(21.4–62.5)</td>
<td>(22.2–46.7)</td>
<td>(12.9–55.6)</td>
<td>(7.1–33.3)</td>
<td>(10–50)</td>
<td></td>
</tr>
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* 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 \textit{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 \textit{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