Current Status and Applications of New Embryo Technologies in Dairy Herd Management

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Take Home Message

- Superovulation remains the standard for embryo transfer in Canada but commercial organizations now exist for creation of in vitro produced embryos
- In vitro produced embryos can be used to enhance genetic selection and to select calves of predetermined sex
- In crossbreeding systems, in vitro produced embryos can be used to maintain heterosis by transferring F1 embryos into F1 recipients
- As compared to AI, embryo transfer can improve fertility of lactating cows when herd fertility is very low (as shown for heat stress) but not when herd fertility is moderate or high

Current Status of Embryo Transfer in Canada

Embryo transfer has been performed commercially in cattle since the 1970's. Since that time, an impressive array of new technologies has been incorporated into systems for embryo production and transfer. These technologies have resulted in new methods for producing embryos, for improving embryo quality, for long-term storage of embryos and oocytes, and for screening of embryos for important genes.

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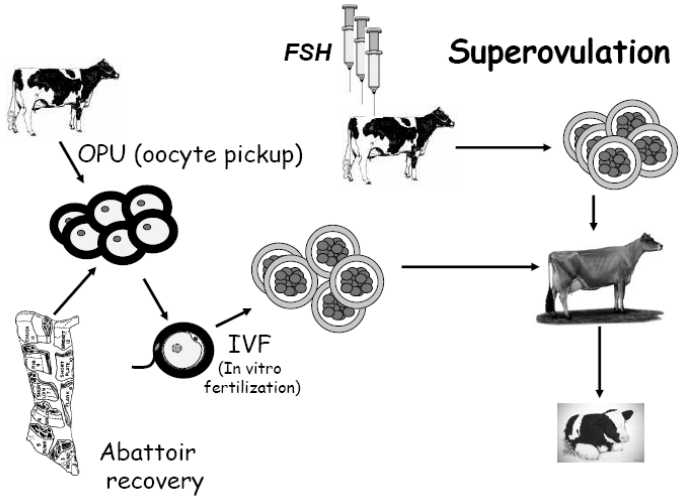

Figure 1. Different schemes for producing embryos for embryo transfer programs in cattle.

Shown in Figure 1 is a schematic illustration of common methods to generate embryos for embryo transfer programs in cattle. A fundamental goal of embryo transfer programs is to increase the number of embryos produced by one female. The typical way to achieve this increase in embryo numbers is to cause the cow to ovulate multiple follicles by injecting follicle stimulating hormone to recruit multiple follicles to grow and ovulate. An alternative method is to harvest multiple oocytes from a cow and fertilize the oocytes in vitro. Two methods of harvest exist. Oocytes can easily be removed from ovaries collected at slaughter. Commercial production of embryos produced using ovaries recovered at an abattoir is now practiced. In addition, oocytes can be harvested from the living cow by piercing the vaginal wall with a needle to allow puncture of follicles on the ovary that have been visualized by ultrasound. This procedure, called transvaginal, ultrasound-guided oocyte aspiration (oocyte pickup or OPU for short), allows for oocytes to be obtained multiple times, including during pregnancy.

The standard for embryo production remains the superovulation procedure coupled with non-surgical recovery and transfer that was first developed in the 1970s. Reuben Mapletoft from the University of Saskatchewan and Karen McDermott from the Canadian Embryo Transfer Association maintain records on the scope of embryo transfer in Canada. For 2005, a total of 46,975 embryos produced by superovulation were transferred. Of these, 39,211 or 83% were transferred to dairy cattle. In contrast, only 142 embryos produced by in vitro fertilization were recorded as being transferred.

Given that there were 1.1 million dairy cows in Canada in 2005, it is obvious that embryo transfer remains a rare event. It is not too surprising that this is the case. Embryo transfer is expensive and time-consuming and the advantages over artificial insemination not obvious in many cases. In vitro production has its own, additional problems. As compared to embryos produced by superovulation, in vitro produced (IVP) embryos don't survive freezing as well, generate lower pregnancy rates following transfer, and can be associated with larger neonatal death losses.

Most of those roughly 47,000 embryos transferred in Canada in 2005 were probably used to increase the number of offspring from elite females from purebred herds. This remains an important use of embryo transfer and embryo transfer has had a significant impact on breeding strategies for dairy cattle. As of November 2006, 39 of the 122 Canadian-proven Holstein bulls actively marketed in Canada were produced by embryo transfer.

One consequence of new technologies being incorporated into embryo production systems is embryo transfer is being used in new ways for genetic selection and for other purposes also. The purpose of this paper is to highlight some new uses of embryo transfer based on production of embryos produced in vitro.

■ Large-Scale Production of In Vitro Produced Embryos

Worldwide, the number of cattle embryos produced in vitro has grown rapidly. Data complied by Michel Thibier and colleagues on behalf of the International Embryo Transfer Society indicates 265,991 recorded transfers of IVP embryos in 2005 vs. 612,178 transferred embryos produced by superovulation. In 2000, by contrast, only 41,761 IVP embryos were transferred as compared to 528,540 embryos produced by superovulation.

This growth in transfer of IVP embryos has been accompanied by the development of private and university organizations devoted to the production of embryos in vitro. Examples of commercial organizations in the United States include Trans Ova of Sioux Center, Iowa, Evergen Biotechnologies of Storrs, Connecticut, and Embogen of Gainesville, Florida. An example of a Canadian company involved in bovine in vitro fertilization is L'Alliance Boviteq of Saint-Hyacinthe, Québec. Currently, exportation of IVP embryos from the United States to Canada is not allowed but intergovernmental discussions on developing a protocol for importing IVP embryos into Canada are planned for 2007.

A simple and inexpensive way to obtain the oocytes needed to produce large numbers of embryos in vitro is to harvest oocytes from ovaries recovered at an abattoir. This approach is utilized extensively by companies like Trans Ova

and Evergen. There are two disadvantages to such a source of oocyte. The obvious one is that the donor is usually anonymous and there is little opportunity for donor selection beyond breed. On average, however, the oocyte donors are not inferior cows genetically, even though they ended up sent to slaughter. Jack Rutledge at the University of Wisconsin has estimated that the predicted merit for milk yield in cows at slaughter is nearly the same as the average cow. A second disadvantage relates to transmission of disease. There is no case report of an embryo transmitting an infectious disease but the possibility, even if remote, cannot be discounted. When ovaries used to obtain oocytes are obtained without knowledge of the donor, it is not possible to verify the health status of the donor. Moreover, ovaries and oocytes from different cows usually come in contact with each other so that there is a possibility for cross-contamination.

Use of OPU to obtain oocytes for in vitro fertilization does not have the disadvantages listed above. The identity of the female is ensured so that selection of elite females can be achieved and the health history of the donor is known. Also, cows can be subjected to the procedure frequently (sometimes twice a week) and oocytes can be harvested during pregnancy. However, OPU is an expensive process that requires trained personnel and can cost about US$150-200 per procedure in addition to costs for in vitro fertilization. Reports on the average number of oocytes from unstimulated *Bos taurus* cows harvested can vary from 0 to 5 so that one or two embryos per session would be a reasonable yield. This yield can be increased to about three embryos per session if cows are treated with follicle stimulating hormone to increase follicular development. It is likely that superovulation is superior to OPU in terms of cost-effectiveness in most cases. Exceptions may include cases where one wants to maximize embryo yield per year (because OPU can be done as frequently as twice a week) or where an individual female does not respond well to superovulation.

One problem with the IVP embryo is that it doesn't survive freezing very well. Some companies and research groups have reported acceptable pregnancy rates for cryopreserved in vitro produced embryos but others do fresh transfers only. Worldwide, 31% of transferred IVP embryos in 2005 were cryopreserved vs. 46% for embryos produced by superovulation. Transferring embryos fresh without cryopreservation means that it is necessary to synchronize production of the embryo with stage of the estrous cycle of the recipient cow. Fortunately, timed embryo transfer can be performed using similar hormonal treatments used for timed artificial insemination. An example of one effective protocol is in Table 1. Using this procedure with lactating cow recipients during cool weather in Florida, the calving rate for a group of 44 recipients was 23%.

Table 1. Recommended schedule for timed embryo transfer using in vitro produced embryos.

Day	Activity
Day 0	Inject 100 µg GnRH, i.m.
Day 7	Inject 25 mg prostaglandin $F_{2\alpha}$, i.m.
Day 9	Inject 100 µg GnRH, i.m.
Day 10	(Anticipated day of ovulation)
Day 17	Transfer fresh in vitro produced embryo fertilized on Day 10 Transfer should be performed only on cows with a corpus luteum - place embryo in the uterine horn adjacent to the corpus luteum

■ Use of In Vitro Produced Embryos for Genetic Improvement

Genetic selection can be enhanced by increasing the number of offspring per female. Doing so increases selection intensity (a smaller proportion of females are selected to produce the next generation) and also the accuracy of selection (because more records are available from individual animals and their relatives). The most common way to increase number of offspring per female is to utilize superovulation or OPU. In addition, some genetic progress can be obtained using oocytes recovered from ovaries at slaughter.

Following slaughter, ovaries typically end up as part of the offal. Rendering ovaries represents a waste of genetic resources when the cow from which those ovaries came is a genetically superior cow. There are some limited efforts being taken to utilize those genetic resources, but much more could be done. Bomed Inc. of Madison, Wisconsin performs in vitro fertilization using oocytes obtained from individual donor cows or from pooled batches of ovaries from cull cows of contracted dairies. Embryos are transferred without freezing and are typically transferred to cows from the herd in which the embryo was derived. There is also one purebred Holstein dairy in California that has organized its own in-house in vitro fertilization laboratory.

Changes in the way in which dairy cows are identified should make it possible for large-scale selection of individual donor cows at the abattoir. Theoretically, an organization producing embryos in vitro could identify individual cows electronically and query databases to obtain information on herd of origin or individual performance records. One model for such an organization would be a farmer cooperative where embryos were returned to the participating herds. The resources required to build and operate an in vitro fertilization laboratory are modest.

Regardless of the source of oocyte, in vitro fertilization should be performed using elite sires because the costs of the semen are amortized over several offspring. With artificial insemination (AI), and an assumed calving rate to a single insemination of 25%, one would need to inseminate eight cows to obtain one heifer. Using in vitro fertilization, a single straw could typically be used to inseminate 100 oocytes or more. If 100 oocytes were fertilized with a straw of semen, 25 transferable embryos or more could likely be produced. At a 25% calving rate to embryo transfer with lactating recipients, the single straw of semen would produce about 3 heifer calves.

■ Embryo Transfer as a Means of Sex Selection

It has long been possible to sex embryos. This is accomplished by taking a small biopsy of cells from the embryo and then performing a laboratory procedure called PCR to determine whether the Y chromosome is present. Embryo biopsy requires specialized training and is expensive. Now, however, the advent of sex-sorted semen makes it possible to produce embryos of the desired sex so that biopsy to identify sex is not needed.

Sex-sorted semen is prepared by using an instrument called a flow cytometer to separate X-bearing sperm from Y-bearing sperm based on the slightly larger size (i.e., higher DNA content) of the former. Straws of sex-sorted sperm are now commercially available. Unfortunately, use of sexed-sorted sperm in AI is associated with reduced fertility because of sperm damage during the sexing procedure and because the number of sperm packaged per straw is reduced. This fact, as well as the high costs, has limited the use of sex-sorted sperm for artificial insemination under commercial conditions to non-lactating heifers.

The poor fertility of sex-sorted sperm means that its use is also not recommended for superovulated donor cattle. It does, however, work with in vitro fertilization. Calves produced by in vitro fertilization with sex-sorted sperm where X-bearing sperm are retained are 90% female or greater. In the United States, there are currently at least two companies selling embryos produced with sex-sorted sperm – Evergen and Trans Ova. In Canada, L'Alliance Boviteq recently obtained a license to produce bovine embryos using sexed semen. Cost will likely vary depending upon source of oocyte and other considerations. At the time of writing, Trans Ova was selling Holstein embryos produced with sex-sorted semen and oocytes obtained at an abattoir for US$135.

Recently, the effectiveness of sex-sorted sperm was demonstrated in a large-scale study involving exportation of IVP embryos to China (Xu et al., 2006). Holstein embryos were produced in the United States using in vitro fertilization with sex-sorted sperm or conventional sperm. Embryos were then

transferred to either Chinese Native Yellow cattle or Holstein cattle in China. As a control, some commercially-purchased embryos produced by superovulation were also transferred. As shown in Table 2, 41% of the IVP embryos established a pregnancy that was maintained until at least pregnancy diagnosis at Day 70 of gestation. Pregnancy rates were the same for embryos produced with sex-sorted sperm and conventional sperm. Of the 458 calves born from the IVP embryos produced using sex-sorted sperm at the time of publication, 96.5% were female.

Table 2. Effect of embryo production system on pregnancy rates in Chinese Native Yellow and Holstein recipients receiving cryopreserved Holstein embryos (From Xu et al., 2006).

Embryo type	Number of transfers	Pregnancy rate, 70 days after transfer (%)
IVP, vitrified, sex-sorted sperm	3,627	41
IVP, vitrified, conventional sperm	481	41
In vivo, superovulation, conventional freezing	192	53

There has been only one published study in which IVP embryos produced using sex-sorted sperm have been transferred to lactating dairy cows. In that study, in Wisconsin (Wilson et al., 2005), pregnancy rates achieved following transfer of fresh embryos produced by sex-sorted sperm was 19% (n=138) vs. 36% for AI using conventional semen (n=526). There was no group in which embryos were produced with conventional sperm so it was not determined whether the lower pregnancy rates for embryo transfer was due to use of sex-sorted sperm.

■ Embryo Transfer in Crossbreeding Systems

Crossbreeding has been put forward as a method for improving fertility and health traits of dairy cattle and for reducing the inbreeding present in all major North American dairy breeds. One limitation of crossbreeding is the loss of heterosis and increased variation that occurs for the offspring of F1 females. In a two-breed rotational breeding system, the percent heterosis falls from 100% in the F1 generation to 50% in the F2 and 75% in the F3 before stabilizing at 63-69% in subsequent generations. Loss of heterosis can be prevented in large part by using a three or four breed rotational breeding system involving unrelated breeds although variation in type may make management more difficult.

Another method to prevent loss of heterosis is to maintain the F1 generation indefinitely using embryo transfer. Embryos can be produced in vitro or in vivo through appropriate matings (for example use Swedish Red semen and Holstein oocytes to produce F1 embryos for a herd of Swedish Red x Holstein cows). With such a scheme, both the F1 female and her offspring experience 100% heterosis.

■ Can Embryo Transfer Be Used to Improve Fertility?

Conception rates in lactating cows are much lower than for non-lactating heifers and have been on the decline for 30 years of more. Theoretically, embryo transfer should improve fertility in the lactating cow because pregnancy failure brought about by defects in the oocyte, ovulation, fertilization, or early embryonic development can be bypassed. Typically, embryo transfer is performed at Day 7 after estrus and only those embryos that have successfully completed development to that point are transferred.

Whether or not embryo transfer can improve fertility in lactating cows at current levels of technology is not certain. Only a very few embryo transfer studies have been performed with lactating cows. An illustration of the effectiveness of embryo transfer for improving fertility of lactating cows is provided in Figure 2. These data come from an experiment by Rodriques et al. (2004) in Brazil using lactating Holstein cows. Pregnancy rates were greater for embryo transfer recipients than inseminated cows in the summer, when fertility in the inseminated group was compromised by heat stress. In the winter, when fertility in the inseminated group was high, there was no improvement in pregnancy rate caused by embryo transfer.

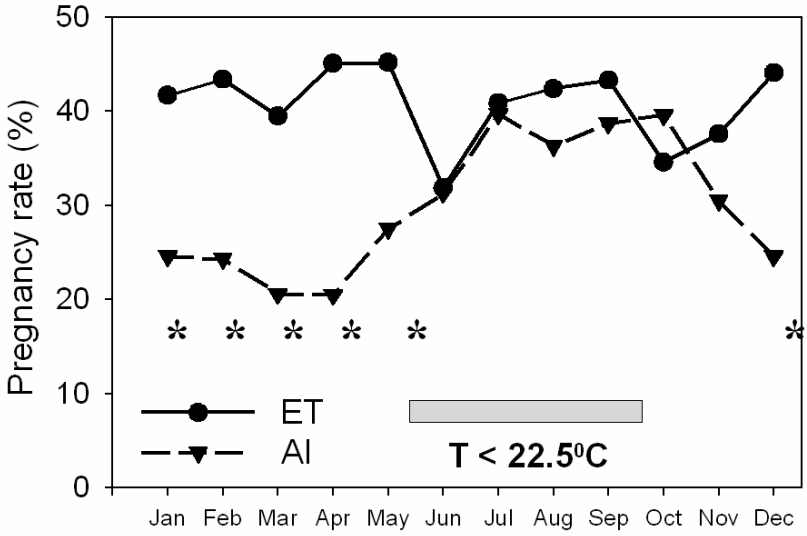

Figure 2. Differences in pregnancy rate between embryo transfer recipients and inseminated animals in lactating cows in Brazil. Lactating Holstein cows were either inseminated or received a fresh or frozen-thawed embryo produced by superovulation. Data are either the number pregnant/number inseminated or number pregnant/number receiving an embryo. Months in which the average ambient air temperature was less than 22.5°C are shown with the gray bar. Note that embryo transfer increased pregnancy rate in hot weather but not in cool weather. Data are redrawn from Rodrigues et al. (2004).

The benefits of embryo transfer for lactating cows during heat stress have been shown several times for herds in Florida and Brazil. Perhaps embryo transfer will also improve fertility in herds with low fertility for reasons other than heat stress. This hasn't yet been proven.

In a recent study with lactating cows in Wisconsin (Sartori et al., 2006) live calving rate was 26% (47 cows pregnant of 184 cows total) for cows receiving an embryo produced by superovulation vs. 28% (56 cows pregnant of 203 cows total) for inseminated cows. It is likely that further improvements in the process for production, selection, and transfer of embryos as well as in recipient management will be needed before embryo transfer can be an effective and economical method to improve fertility in herds with reasonable fertility.

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