

# Embryo Transfer as an Assisted Reproductive Technology in Farm Animals

Diah Tri Widayati

**Abstract**—Various assisted reproductive techniques have been developed and refined to obtain a large number of offspring from genetically superior animals or obtain offspring from infertile (or subfertile) animals. The embryo transfer is one assisted reproductive technique developed well, aimed at increased productivity of selected females, disease control, importation and exportation of livestock, rapid screening of AI sires for genetically recessive characteristics, treatment or circumvention of certain types of infertility. Embryo transfer also is a useful research tool for evaluating fetal and maternal interactions. This technique has been applied to nearly every species of domestic animal and many species of wildlife and exotic animals, including humans and non-human primates. The successful of embryo transfers have been limited to within-animal, homologous replacement of the embryos. There are several examples of interspecific and intergeneric embryo transfers in which embryos implanted but did not develop to term: sheep and goat, mouse and rat. An immunological rejections and placental incompatibility between the embryo and the surrogate mother appear to restrict interspecific embryo transfer/interspecific pregnancy. Recently, preimplantation embryo manipulation procedures have been applied, such as technique of inner cell mass transfer. This technique will possible to overcome the reproductive barrier interspecific embryo transfer/interspecific pregnancy, if there is a protective mechanism which prevents recognition of the foreign fetus by the mother of the other species

**Keywords**—Embryo Transfer, Assisted Reproductive Technology, Intraspecific-Interspecific Pregnancy, Inner cell mass.

## I. INTRODUCTION

EMBRYO transfer (ET) is a technique by which embryos are collected from a donor female and are transferred to recipient females, which serve as surrogate mothers for the remainder of pregnancy. Embryo transfer procedures have been used to increase the reproductive performance of particular females of agriculturally important species, such as cattle, horse, goat and sheep. Commercial cattle ET started in North America and elsewhere during the early 1970s, primarily as means of multiplying the number of young produced by exotic breeds of beef cattle. In the early years, embryos recoveries usually involved midventral laparotomy with the donor under anesthesia. By the mid-1970s researcher had developed non-surgical embryos recovery procedures to the point where they matched the surgical intervention [1]. Around the same time, non-surgical transfer techniques were also being developed. The availability of non-surgical recovery and transfer procedures allowed exploitation

Diah Tri Widayati. Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia, Jl. Fauna, no. 3, Bulaksumur, Yogyakarta 55281, phone: +620274513363 (e-mail: widayati@ugm.ac.id)

of cattle ET on the farm. An effective freeze-thaw method, which permitted embryos to be shipped to the furthest parts of world, completed the crucial requirements to commercial exploitation of cattle ET.

The commercial advantages of embryo transfer in domestic animal include: (i) facilitating genetic improvement in the domestic animal industry by obtaining a large number of desirable progeny from parents of high genetic quality; (ii) enabling embryos to be moved from country to country in the frozen state, thereby reducing the need for long distance domestic animal movement; (iii) permitting high quality breeding stock to be available for sale in much larger number than was previously possible; and (iv) exploiting developments in reproductive technology, such as embryo sexing and embryo splitting. It should also be remembered that ET technology is the means by which advances in techniques such as in vitro fertilization (IVF), large-scale cloning technology and genetic engineering can be exploited. Genetic screening by way of deoxyribonucleic acid (DNA) probes/polymerase chain reaction (PCR) technology is likely to be applied to embryos and assist in the selection of the most appropriate genotypes for transfer [2].

Although the basic procedures (superovulation, embryo recovery, storage/freezing and transfer) employed in the domestic animal (especially cattle) ET are now well established, there is considerable research for improvement in various areas of ET technology. Future development of ET technology will almost certainly include the recovery oocytes by ultrasound guided aspiration with subsequent in vitro fertilization (IVF)/in vitro cultured (IVC).

## II. DISCUSSION

### A. Embryo Transfer among the same Species

Most of embryo transfers have been carried out among the same species (intraspecific embryo transfer), and usually it was for the purpose of propagating an animal with desirable characteristics of the two parental species. A listing of the species includes rabbit, rat, sheep, mouse, goat, cattle, pig, hamster, ferret, mink, horse, baboon, cat, dog, and water buffalo.

Embryo transfer is now commonly used to produce artificial insemination (AI) sires from highly proven cows and bulls. Although technical costs would seem to preclude the use of ET techniques for anything but seed-stock production at this time, the commercial cattle industry can benefit by the use of bulls produced through well-designed MOET (Multiple Ovulation and Embryo Transfer) program [3]. The success of MOET program has now led to the use of

this technology to test AI sires genetically. Selected cows are super stimulated and inseminated to highly proven bulls. Male offspring are placed in waiting while female offspring are placed into production. Bulls are then proven by production records from siblings rather than offspring. With this approach, it is possible to test a bull genetically in three-and-a-half years, as opposed to five-and-a-half years using traditional progeny testing schemes.

More recently, Thibier [4] reported that in 2002, 538,312 bovine embryos were transferred worldwide, of which 52% were transferred after on-farm freezing and thawing and 15% were produced by *in vitro* techniques. North America has continued to be the centre of commercial embryo transfer activity, with more than 42,000 donor cows superstimulated and more than 190,000 embryos transferred (35% of all reported embryo transfer in the world). However, commercial embryo transfer in North America is static or declining. In South America, by comparison, commercial embryo transfer is expanding, accounting for 22% of embryo transfers throughout the world in 2002. Europe and Asia each reported about 17% of the total number of bovine embryo transfers in 2002.

#### *B. Embryo Transfer among Different Species*

Embryo transfers among different species (interspecific embryo transfer) procedures allow the establishment of true interspecific pregnancy [5]. Interspecific or cross-species pregnancy is the condition of carrying the embryo of one species in the uterus of different species. In natural breeding, interspecific hybridization was able to occur in some species combinations. Adam [6] reviewed interspecific embryo transfer that had been reported up to that time and noted that mammalian embryos usually are tolerant of a foreign species during development to the blastocyst stage, thereafter, embryonic mortality is observed. More recently, several interspecific combinations have been intensively studied in various species.

Fernández-Arias *et al.* [7] reported that the transfer of embryos of a given species to the uterus of a different species is utilized in animal conservation for endangered species. It suggests that interspecific pregnancy will be a key procedure in the preservation of endangered species if the accompanying problems are overcome. Moreover, interspecific pregnancy presents useful experimental models for study of fetal-maternal interaction.

So far, interspecific pregnancies have been studied in several species combinations, such as *Mus caroli*–*Mus musculus* [8]–[9], rat–mouse [10], donkey–horse [11]–[12], goat–sheep [13], Spanish ibex–domestic goat [7], and common vole–laboratory mouse [14]–[15]. Except horse–donkey combination, the transferred embryos do not develop to full term. A few interspecific pregnancies were successfully delivered in carnivorous animals. Pope [16] reported that embryos of wild cat (*Felis sylvestris ornata*) were developed to full term in the uterus of domestic cat (*Felis catus*) and tiger (*Panthera tigris*).

The pregnancy between *Mus caroli*–*Mus musculus* was intensively studied as a model for interspecific pregnancy [17]–[19]. Histological examination of developing *M. caroli* embryos in the *M. musculus* uterus revealed that the *M. caroli* embryos developed successfully for the first 9.5 days of gestation. Thereafter, the trophoblast giant cell area became infiltrated with lymphocytes and hemorrhaged. While adjacent *M. musculus* embryos in the same uterus were unaffected. Croy *et al.* [8] reported that cytotoxic T lymphocytes accumulated in resorption sites of *M. caroli* embryos, suggesting immunological involvement in the failure of *M. caroli* development in the *M. musculus* uterus. The infiltration of maternal lymphoid cells appeared to pass through the trophoblast as if there was collapse of the physiological barrier to maternal invasion [20]. The invasion of maternal lymphoid cells into the placental tissue has been considered as an immunological response to interspecies incompatibility. Tachi and Tachi [10] experimentally transferred rat blastocysts into the uteri of laboratory mice and examined ultrastructurally. They observed that rat blastocysts can successfully undergo the stages of ovum implantation in the mouse uterus from the early attachment to the initial phase of the trophoblast invasion into the endometrium. Thereafter, trophoblast cells of xenogeneic implants were destroyed soon by the penetration of the basement membrane of the luminal epithelium of the host endometrium, leading to the degeneration and sloughing off the rat embryos. Tachi and Tachi [10] concluded that the failure of the development of rat embryos in the mouse uterus was caused by incompatibility of tissue interaction between rat trophoblast and mouse uterus, not by immunological rejection.

Equids possess an unusual ability to interbreed freely among the phenotypically and karyotypically diverse species within the genus to produce viable hybrids, such as Mongolian wild horse (*E. przewalskii*)–domestic horse [21], reciprocal donkey–horse combination [11]–[12]. Although most interspecific hybrids are sterile [22], the viability of the equine species are considered having special features as models for interspecific pregnancy study [5]. Interspecific pregnancies between horse and donkey were carried out by Allen [11]. They reported that donkeys were able to carry horse fetuses to term, and that the endometrial cup developed normally and secreted equine chorionic gonadotrophin (eCG) and progesterone at high rate. On the other hand, the endometrial cup failed to develop in horse carrying donkey fetuses. The donkey fetuses grew normally to day 70 of gestation, but thereafter succumbed to a vigorous maternal cell-mediated reaction directed against the donkey allantochorion. Antczak and Allen [23] reported that immunization by parental donkey lymphocytes in horse carrying donkey fetuses dramatically improved the survival rate of the fetuses. The finding suggested that the development failure of donkey fetuses in the horse uteri was due to an immunological rejection.

Interspecific and intergeneric pregnancies among Bovidae have been carried out by embryo transfer in a few cases, such as bovine (*Bos Taurus*)–water buffalo (*Bubalus bubalis*). A

bovine embryo was aborted between 2.5 and 3 months of gestation. Dresser *et al.* [24] reported that a embryo of bongo antelope (*Tragelaphus euryceros*) developed to term in African eland (*Tragelaphus oryx*). The interspecific hybrids among Bovidae were produced for the purpose of propagation of animals with desirable characteristics. For example, *Bos taurus* and *Bos indicus*, i.e., European and Asian domestic cattles, readily produce fertile hybrids with heat tolerance and disease resistance more than *Bos taurus*.

Interspecific hybrids between domestic sheep (*Ovis aries*) and domestic goat (*Capra hircus*) usually do not develop to full term. Histological and ultrastructural studies suggested implication of a maternal immune response in failure of most these hybrid pregnancies [25]–[26]. Fernández-Arias [7] reported possibility to achieve interspecific pregnancy after transfer of ibex (*Capra pyrenaica*) embryo into domestic goat (*Capra hircus*), but this requires a great change of pregnancy-associated glycoprotein (PAG) profiles.

So far, embryo manipulation procedures have been applied to preimplantation embryos in order to overcome the failure of interspecific pregnancy. Sheep ↔ goat chimaeras were allowed successful interspecific embryo transplantation in sheep and goats [27]–[28]. They produced chimeric embryos with goat ICM and sheep trophectoderm. When these chimeric blastocysts were transferred into recipients of the same species as the host blastocyst, the interspecific chimeras were not rejected. Thus, embryo manipulation is possible to overcome the reproductive barrier between sheep and goats, if there is a protective mechanism which prevents recognition of the foreign fetus by the mother of the other species [28].

Successful production of live interspecific chimeras between two murine species, *Mus musculus* and *Mus caroli*, has been reported [29]. *Mus caroli* ↔ *Mus musculus* chimeras thus appear to be very similar to *Mus musculus* ↔ *Mus musculus* chimeras in their somatic tissue organization, and there is no evidence for discrimination against *Mus caroli* cells during development. The chimeras were made by injection of *M. musculus* inner cell masses (ICMs) into *M. caroli* blastocysts, and by aggregation of 8-cell embryos of both species [19]. It was reported that the presence of trophoblast cell of maternal uterine genotype allows *Mus caroli* ↔ *Mus musculus* chimeras to survive in the *M. musculus* uterus. Thus, to protect foreign embryos from maternal immune rejection, the trophoblast must be the same genotype with the maternal uterine tissue.

The mechanisms that prevent the successful outcome in interspecific pregnancies are far from being completely understood. Widayati [30] suggested that the failure of interspecific pregnancy was due to immunological rejections and placental incompatibility between the embryo and the surrogate mother. If the problems in interspecific pregnancy can be overcome, the interspecific pregnancy will play a big contribution in preservation of endangered species. And the embryos of endangered species will develop to term in the uteri of related females from non endangered species. Furthermore, interspecific pregnancies are useful model for

the study of fetal-maternal interaction.

### III. CONCLUSION

Since the placental incompatibility between the embryo and the surrogate mother can be overcome, embryo transfers possible to improve reproductive farm animals.

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**Diah Tri Widayati** was born in Yogyakarta, 7 December 1967. The occupation now as an educatif staff of The Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia. Master degree in Veterinary Medicine Science, at Gadjah Mada University, Yogyakarta was earned in 1999 and Ph.D in Bioagriculture Science at the University of Nagoya, Japan in 2004.

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Dr. Widayati, Diah Tri as a member of several professional societies, such as; (1) Indonesian Animal Science Association, 1999-Now, (2) Japanese Animal Science Association, 2002-2005 (3) Nagoya University Alumni Association, 2004-Now, (4) Indonesian Technology Reproduction Association, 2006-Now.