## Progress of animal embryo technology in China

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ABSTRACT: Embryo technology, including super – ovulation, embryo transfer and embryo nucleus transfer, was a well – developed technology in 20th century. It has been widely used in animal science and veterinary and boomed livestock industry in China. Chinese livestock producer could gain high grade breeds in short time through embryo technology by the reason that embryo technology could make outstanding gene spread out in herd. With the help of embryo technology, rare animals could have a chance to extend their progeny in fast changing world. In this article, we briefly introduced the process of embryo transfer and application of embryo technology in China.

Key words: Embryo transfer; Embryo nucleus transfer; Donor cow, recipient cows; Superovulation; Animal preservation

#### Introduction

Embryo Transfer was basically multiple injections of hormone to stimulate and multiply ovulations in the cow that you want to get the embryos from; and then technician removed embryo (fertilized egg) from the donor cow and placed it in a surrogate cow where the transferred embryos developed into calves. Embryo transfer techniques give cows the ability to produce multiple offspring, leading to more rapid genetic gain. Superovulation was a treatment on donors with gonadotropin (especially follicle stimulating hormone, FSH) to produce more than a single ovum.

# History of embryo transfer and embryo technology

Embryo transfer was first carried out and recorded by Walter Heape in 1890 by transferred two Angora rabbit embryos into a gestating Belgian doe. A mixed litter of Belgian and Angora bunnies was the result of this experiment<sup>[5]</sup>.

Embryo transfer in herd began in the 1930s with sheep and goats, but it was not until the 1950s that successful embryo transfers were reported in cattle and pigs by Jim Rawson at Cambridge, England. Most of the applicable embryo transfer technology was developed in the 1970s and 1980s<sup>[9]</sup>; embryo transfer in herd gained considerable popularity with seed stock dairy and beef producers in recent year<sup>[4]</sup>.

From 1991 to 2000, embryo transferred increased with the rate of 20% in China. By the end of 2000, the number of embryos (including fresh embryo and frozen straw embryo) which were used for transplanting reached to 500,000 in China<sup>[17]</sup>.

Research on embryo technology in China launched out by Tong

Dizhou, a leading science of cytology in 1960s. He was the first scientist who implanted nucleus of goldfish into bitterling ovum and cultivated zygote into a new species. During that time, P.R. China was in Culture Revolution; all academic communication was suspended for political reason. Mr Tong's achievement was less known to western scholar field. Mr Tong has also launched nucleus transfer in mammal in Chinese Academy of Science in 1970s, and then Chinese technologists have made series of achievements in nucleus transfer<sup>[1]</sup>.

### Process of embryo transfer in cattle

Since a British scientist initiated embryo transfer in 1890, cytologists have done lots of research work on embryo transfer during several decades. Embryo technology was developed into a large industry for the contribution of cytologists, formula of embryo transfer mature into a set of rules. Here is the brief introduce of embryo transfer process:

#### 1) Preparation

Donors were usually selected on the basis of superior genetic merit compared with the rest of the herd. Technicians need to choose donor cattle and surrogate cattle. The principle of choosing, depended on that donor cattle, must have super gene in one or two traits of reproduction or yielding. This guaranteed that the offspring of embryo transfer wouldn't degenerate in future.

#### 2) Superovulation of the donor cow

In this step, donor cows were injected with follicle stimulating hormone (FSH). FSH was injected twice daily for four days in the range of 8 to 14 days following estrus while a functional corpus luteum (CL) is on the ovary. The amount of FSH given to a donor will vary with the weight of the donor. According to reports of embryo transfer in cattle, donors could be injected with FSH 300IU and LH 7.5IU.

#### 3) Insemination

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Since ova released from multiple follicles on the ovaries would reach oviducts several hours later, it is needed to inseminate enough viable sperms in oviduct of the super ovulating donor cows. Many embryo transfer technicians will choose to inseminate the donor cows several times during or after estrus.

#### 4) Flushing the embryos and evaluation of the embryos

Seven or eight days after estrus, the technician could harvest embryo from uterus. For the purpose of collecting embryo without surgery, he would insert a small synthetic rubber tube into the cervix of the donor cow. A special medium was flushed into uterus. He needs to pick out low grade embryo and observed it under microscope.

#### 5) Embryo implanting

With the aid of an assistant, holding and opening the vulva of the recipient cow, the transfer gun was carefully passed through the cervix. The tip of the rod was then allowed to slide into the horn on the same side of the ovary with an active corpus luteum. The embryo was gently expelled in the forward tip of that uterine horn. Be careful not to cause damage to the lining of the uterus. Such inflammation and scarring caused by operation would greatly reduce the probability of the pregnancy and antibiotic was need to inject into receptor's uterus to prevent inflammation.

During the process of embryo transfer, contamination of in vitro or in vivo produced embryos shows that the risk of transmission of pathogenic microorganisms, using infected semen, was potentially very high; so restricted sanitary rule should be carried out at every step of embryo transfer procedures<sup>[1]</sup>.

#### Advantage of embryo transfer

Compared with traditional reproduction, embryo transfer was superior to traditional way of reproduction. Generally speaking, embryo technology couldn't improve herd gene merit, but it could spread outstanding gene with a large scale. The advantage of embryo transfer was as follows: embryo transfer could make full use of high quality female livestock. Generally speaking, female livestock would produce an average of eight to ten calves in her entire lifetime under normal management programs. Like artificial insemination had done for the bull, embryo transfers could greatly multiple the numbers of offspring for super donor cattle.

Based on embryological theory, normal female cattle could yield 150,000 potential "eggs" or ova and countless billions of sperm produced by each male. Under the natural breeding process, only a small fraction of the reproductive potential of an outstanding individual could be realized. With the help of embryo transfer, female livestock could produce much more embryo than in nature process.

Furthermore, superovulating and transplanting embryos from superior donor could result in rapid progeny improvement. Under the process of commercial embryo transfer, high grade female livestock's embryo could be inseminated in vitro and preserved under liquid nitrogen. Frozen embryos were a marketable commodity and have been especially useful in international sales of beef and dairy genetics. High grade breeds could be exported to other countries and districts which from its original place. Superior genetics from donor could be attained with the use of embryo transfer without limit of time and space<sup>[7,8]</sup>.

## Commercial embryo transfer in China

Commercial embryo transfer started with cow embryo transplanting at the end of last century in China. By the end of 2001, quantity of embryo transferred to surrogate cattle reached 11,000 and rate of successful embryo transferred in buffalo was improved greatly. Commercial demand of embryo of boar goat and Dorset sheep increased sharply and embryo transferred in goat and sheep reached 20,000. Technicians from embryo transplanting company could get 6 - 8 fresh embryos for a flush in heavy milky cattle, and pregnancy rate with fresh embryo could reached 55% ~ 70%, 45% ~ 60% in frozen embryo. Up to the end of 2005, the companies which concentrated their own main business on embryo were 32<sup>[6,10,11]</sup>. Up to now, Chinese embryo industry and embryo gestation rate have reached the world level. The quantity of embryo transferred to local cattle reached 20,000-30,000 and new born calves were 10,000 ~ 12,000. Commercial embryo companies and embryo center were built in most provinces in China. This mean that china embryo industry has reached a large scale[14].

The major costs of embryo transfer were as variable as the costs of buying frozen embryo and the cost of utensil was relatively fixed. Many different options and packages were offered by embryo transfer technicians. In some small commercial company, technicians carried out embryo transfer on the farm or ranch in which the donor cow was located. In some big and famous brand company, technicians always have their own mobile laboratory and they have facilities to house and board donor and recipient cows and perform embryo transfer under hospital – like conditions. Technicians from big company always have the equipment and skill to freeze and store embryos<sup>[5,7]</sup>.

## Application of embryo technology in endangered species and breed preservation

How to preserve endangered breeds was a tough problem which has pestered animal scientists for a long time. It's well – known to us that some local breeds extinguished in accelerating speed for the peasants tend to crossbreed their local breeds with external breeds to gain short – termed yield. These short – based actions caused that some genes and excellent local breeds would lose forever, and some lost gene may weighed heavily to future breeds cultivation. Preservation of endangered breeds was long – last social actions which need government to invest it. Traditional way of breed preservation was to keep endangered breeds on a farm run by government's institution. This approach of animal preservation cost lots of money and human resources, and what's more it often caused inbreeding and lost some

precious gene in preserving process<sup>[13,16]</sup>.

Embryo transfer offered a new way to breeds preservation. Cattle's embryo could be frozen and kept in liquid nitrogen; it could be used for embryo transplant when it is needed in future. Traditional way of animal preservation was to zone a large area for extinction animals to live in. Those zones for animal protection were named as nature protection zone, which was an efficient way to animal protection. With emergence of embryo transfer, scientists proposed a new way to keep those animals out of danger. They could freeze those animals' embryos firstly and seal those frozen embryo into plastic straws to preserve it for future. Those frozen embryo would be transplanted into surrogate mother's uterus when it is needed.

Giant Panda, a famous mammal's species in China, has been on the verge of extinction for reason that the circumstance which Giant Panda to living in shrunk with a large scale. China government tried to take effective measures to save this living fossil. Giant panda has a low fertility to give birth and the giant pandas which were kept in zoo even lost its ability to give birth. Chen Dayuan, a leading cytologist in animal institution in Chinese Academy of Science proposed to preserve giant panda through nuclear transfer. They planned to implant nuclear of giant panda ovum into surrogate animals ovum removed unclear; and the rest of this they have got a living fertilized embryo in vitro, the real linchpin of giant panda embryo clone was how the living embryo is embedded into uterus of surrogate mother and made it grow into fetus<sup>[12]</sup>.

#### Conclusion

As a new well - development technology, embryo technology could spread out the excellent gene in cattle, and change the genetic material of local breeds instantly. Embryo transfer offered Chinese peasants a chance to obtain excellent breeds with a rather low cost. Embryo technology will boom in china for three factors in the coming years:

Firstly, China dairies were in rather low level in cattle breeds for high grade breeds were importance to animal industry. Farmers in dairies were in urgent need to high grade breeds and routine breeding technique could improve cattle breeds in short time. Experts of animal breeding said that Chinese cattle breeds couldn't match with cattle breeds in advanced country within fifty years if they were cultivated with routine breeding technique. This means that Chinese dairy industry needs to import high grade cattle breeds in a large scale and the cost of importing alien breeds will soar up. Maybe embryo transfer was a practical choice to improve cattle breeds in modern China.

Secondly, government of P.R.China has a large burden of nurturing population and it has a strong desire to boost its industry into advanced level. This meanS that Chinese government would pay lots of attention on agriculture and livestock industry and the sequence of this effort would make embryo industry grow into a large industry<sup>[15]</sup>. Thirdly, China has unequilibrium development on social and economic development in different provinces. Generally speaking, peasants from eastern China were more superior to those from western China in social and economic development. Eastern peasants began to merge into global economic market and they had ability to apply new technique to their farm in short time. But peasants from west part of China were still living in lower social and economic condition. This unequilibrium development in livestock industry provided embryo technology a large space to grow up.

Considering the above mentioned, embryo transfer and embryo technology would boom in China several years later and it will create lots of work opportunity and commercial profit in China.

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## 中国的动物胚胎技术研究进展:

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摘要:胚胎移植技术最早是由英国的 Walter Heape 率先研究的,随后经过了胚胎学家几十年的努力才在上个世纪末期逐渐开始广泛应用于医学生殖,高牧业,动物保种等领域。本文集中论述中国的胚胎移植技术发展以及它在富牧业领域的应用。

在中国商业性的动物胚胎移植正在蓬勃发展,其成功率已经和国外同行相差不大。通过胚胎移植工作者的努力,一些从事 商业性胚胎移植的公司已经能够做到鲜胚移植后奶牛的妊娠率 55%~70%,冷冻胚胎的妊娠率达到 45%~60%。不仅如此,在 各个省还建立了自己的专门的冷冻胚胎中心,为本省的富牧产业提供胚胎或者相关的服务。

在动物保种方面,胚胎技术给人们提供了一种全断的思路;通过胚胎技术将活的胚胎冷冻在液氮罐以实现永久保存。例如中国科学院动物所陈大元先生提出通过胚胎移植技术实现对大熊猫的物种保存,并取得了一定的成果。

随着经济和社会的进一步发展,人民生活水平的提高对于肉和奶的需求会进一步提高。依靠常规的选育技术来改良牲畜是无法满足人民对于畜牧产品急切要求,要实现畜牧业的跨越式发展,就必须依靠科技的力量来实现畜牧业的大发展,满足人民生活需要。胚胎技术等新技术会为我国的畜牧业的产业提高提供了一个新的机遇,可以相信,在不远的将来,动物胚胎技术必将在中国有一个较大发展。

关键词: 胚胎移植技术; 动物保种; 物种保存; 动物胚胎技术中围分类号: Q954.4 文献标识码: A

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