Chapter 8

A Novel Discipline in Embryology — Animal Embryo Breeding

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61299

Abstract

The modern animal biotechnologies, such as animal cloning, transgenesis, sex determination, stem cells, designing new livestock, must be performed on animal gametes including sperm and oocytes, and embryos based on embryology theory. Currently, some key biotechnologies in embryology have become the most powerful tool for animal scientists and breeders to improve genetic construction of animal herds. Here, authors put forward a new concept of **Animal Embryo Breeding** Science to describe this discipline formation, development, and application in animal genetic improvement and breeding. The relationship of embryo breeding with other disciplines has been profiled. Thus, animal scientists and breeders can easily understand and apply embryo breeding theory and related key techniques to accelerate animal improvement speed, to modify genetic construction of animal population, and to design and create new animal individual or breed.

Keywords: Discipline, embryo breeding, biotechnology, livestock

1. Introduction

Animal breeding sciences concern the management and care of farm animals by humans for profit. Not only does it refer to the practice of selectively breeding and raising livestock to promote desirable traits in animals for utility, sport, pleasure, or research [1], but also it refers to the efficient exploitation of a species in agriculture advantageous to humans. The genetic improvement of livestock depends on defining breeding objectives and accurately identifying the right animals to be used for future breeding. Traditional breeding programs involve 1) the design of animal breeding goals including improvement traits, such as milk, wool, growth, carcass and fertility, females vs. males, progeny test and nucleus vs. commercial animal population; 2) application techniques, such as artificial insemination and embryo transfer, are

used as methods not only to guarantee that females breed regularly but also to help improve herd genetics; 3) based on quantitative genetics theory, estimation of breeding value by phenotype, pedigree, BLUP (best linear unbiased prediction) method, and genetic markers; 4) selection and culling of individuals based on genetic evaluation, balancing rate of change, and inbreeding; and 5) determining mating system. This is a long-term process for livestock genetic improvement.

As modern biotechnology develops, some new techniques can be applied to animal breeding programs 1) to accelerate genetic progression by shortening generation interval and increasing female reproduction; 2) to add new genetic trait to animal body by transgenic technology or to remove bad traits from animal body by gene knockout method [2]; and 3) to create new animal individual or breed by modern biotechnologies including nuclear transfer, cloning, and genetic modification. These new technologies will make it easier to manipulate animal genomes, but food products from genetically engineered animals face a long road to market. Examples of biotechnology applications of particular interest to the department include cell culture, genomics, molecular-marker-assisted breeding, cloning, bioprocessing, and diagnostic testing, as well as gene technology (genetic modification). Genetic modification deliberates change of an organism's genetic material by moving, introducing, or eliminating specific genes, such as taking a single gene from an animal cell and inserting it into another animal cell to give the second cell a desired characteristic. The terms "gene technology," "genetic engineering" and "genetic manipulation," "genetic enhancement," "gene splicing," "transgenics," or the use of "recombinant DNA" are used to describe genetic modification processes. Genes can be found in and moved between different plants, animals or microorganisms such as viruses or bacteria, for example, transferring worm fat-1 gene to pig to produce more omega-3 fat acid in pork meat [3]. Genes can also be changed within a specific plant or animal individual. For instance, "knocking out" an undesirable characteristic gene such as susceptibility to a particular disease can be beneficial to the plant or animal life.

In mammals, the realization of these goals must depend upon *in vitro* manipulation of animal oocytes and embryos. Thus, embryology has become a core of these biotechnologies (Figure 1). Currently, embryo biotechnology, which most people call **embryo bioengineering,** has gradually become the most powerful tool for animal scientists and breeders to improve genetic construction of their animal herds or populations. Embryo transfer in cattle has recently gained considerable popularity with seedstock dairy and beef producers. Many kinds of species have been cloned and some transgenic animals have been produced. Thus, embryology has become a core of modern biotechnologies in animal genetic modification and breeding. Combining the new advances in modern biotechnology with future application, authors put forward the new concept of **Animal Embryo Breeding Science** to describe embryology development and application in animal genetic improvement and breeding.

2. Concept of animal embryo breeding

Breeding is the reproductive process, which is producing of elite offspring in animals or plants. Animal breeding programs involve the selection or culling of parents (such as bull and cow)

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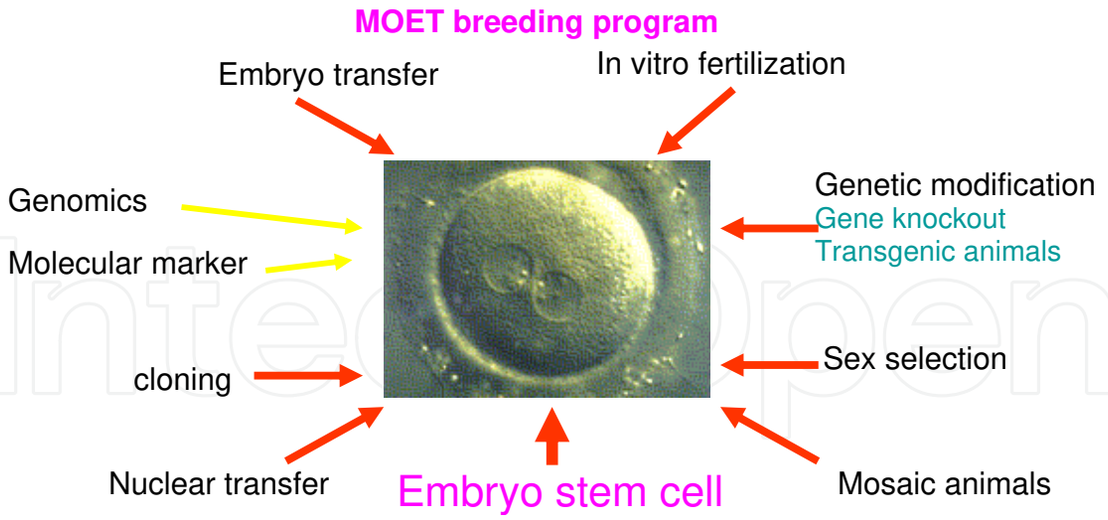

Figure 1. Embryology has become a core of modern biotechnologies in animal genetic modification and breeding. Any new developed biotechniques such as nuclear transplantation, cloning, and transgenesis, finally must be performed on animal oocytes or embryos. MOET represents multiple ovulation and embryo transfer.

and then determination of mating system. They must be female and male sex combination. However, **Animal Embryo Breeding** is an asexual reproduction of specific oocytes or embryos artificially by current developed biotechnology. The Science of Animal Embryo Breeding is to study how to use the embryo manipulation technologies to improve, create, and clone new animal individual or breed. Current developed techniques include nuclear transfer, cytoplasmic transfer or replacement, in vitro fertilization (IVF), sperm cytoplasmic injection (ICSI), parthenogenesis and androgenesis, embryo cloning, sex selection, transgenesis, gene knock out, stem cells and somatic cell cloning, etc. Although embryo breeding is a branch of traditional animal breeding discipline, the science of animal breeding is concerned with the application of the principles of population genetics and qualitative genetics to the improvement of domestic animals. However, Animal Embryo Breeding is concerned with application of the developed embryo biotechnologies to new animal individual creation, genetic cloning and preservation of animal breeds. The research main body of this discipline focuses on sperm, oocyte and embryo. After the desired animal type (genetic improved goal) has been designed, by means of a serial micromanipulation on oocyte or embryo, such as nuclear transfer, foreign DNA microinjection to egg pronucleus and stem cell technique, a modified improved embryo may be produced in vitro and then transferred into animal uterine cavity so that a new animal individual could be created. In the last couple of decades, many kinds of animals including transgenic pigs, cattle, sheep, and goat, have been produced [4].

3. The relationship of animal embryo breeding science with other disciplines

As a new developing subject, Animal Embryo Breeding Science mainly depends upon modern biotechnology development, especially molecular biology, genetics, and reproductive biology

with embryology. However, it also has a close association with other subjects such as reproductive biology and embryology, animal genetics and breeding (Figure 2).

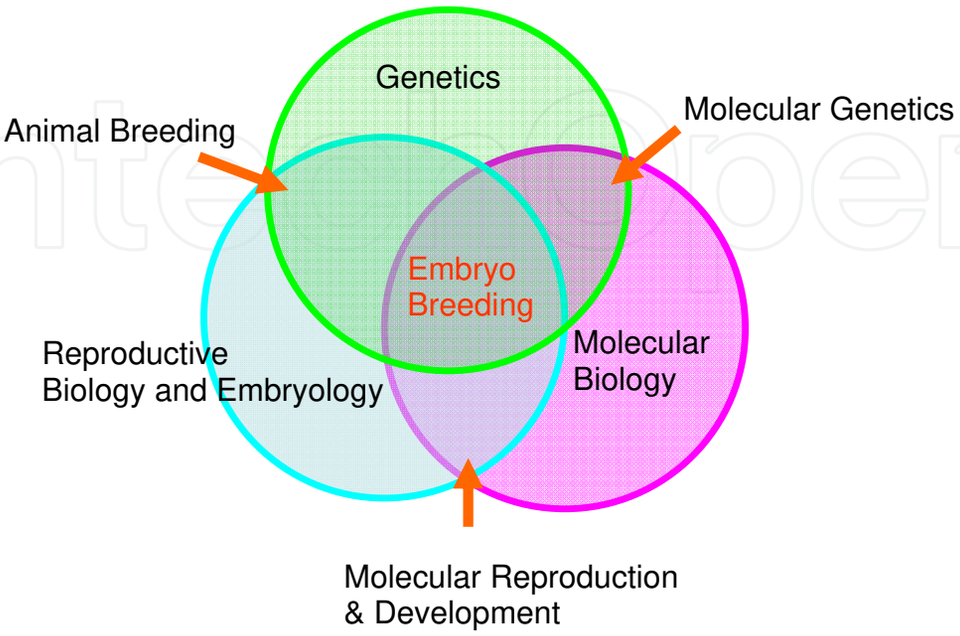

Figure 2. The designed relationship of Animal Embryo Breeding with other disciplines. The Embryo breeding is a core subject which combines molecular biology/genetics with animal genetics and breeding as well as reproductive biology and embryology.

The goal of animal breeding program can be realized by the current embryo breeding technology. Using molecular biological technique, a specific gene type for the desired animal may be designed. The new developed biotechnologies to attempt to modify animal genetic traits must be conducted on animal oocyte and embryo. The embryo in vitro production and animal individual birth must depend upon animal reproductive technology. Embryology may supply a good condition to produce many high-quality embryos. Thus, the Embryo Breeding is a core subject which combines molecular biology/genetics with animal genetics and breeding as well as reproductive biology and embryology.

4. Major research scope and content of animal embryo breeding

Animal Embryo Breeding Science is based on the current developed embryo biotechnology. The core of current embryo biotechnology is oocyte in vitro fertilization (IVF). As human IVF technique rapidly develops in infertility treatment, not only animal IVF has offered a very valuable tool to study mammalian fertilization and early embryo development, but also its commercial applications have being increased. Based on IVF research, some new developed embryo technologies consisting of nuclear transfer, transgenesis, cloning, and stem cells, etc., can be used to create new animal individual or population, and accelerate genetic progression

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of animal population during the period from early oocyte stage (oogenesis) to preimplantation embryo stage (Figure 3).

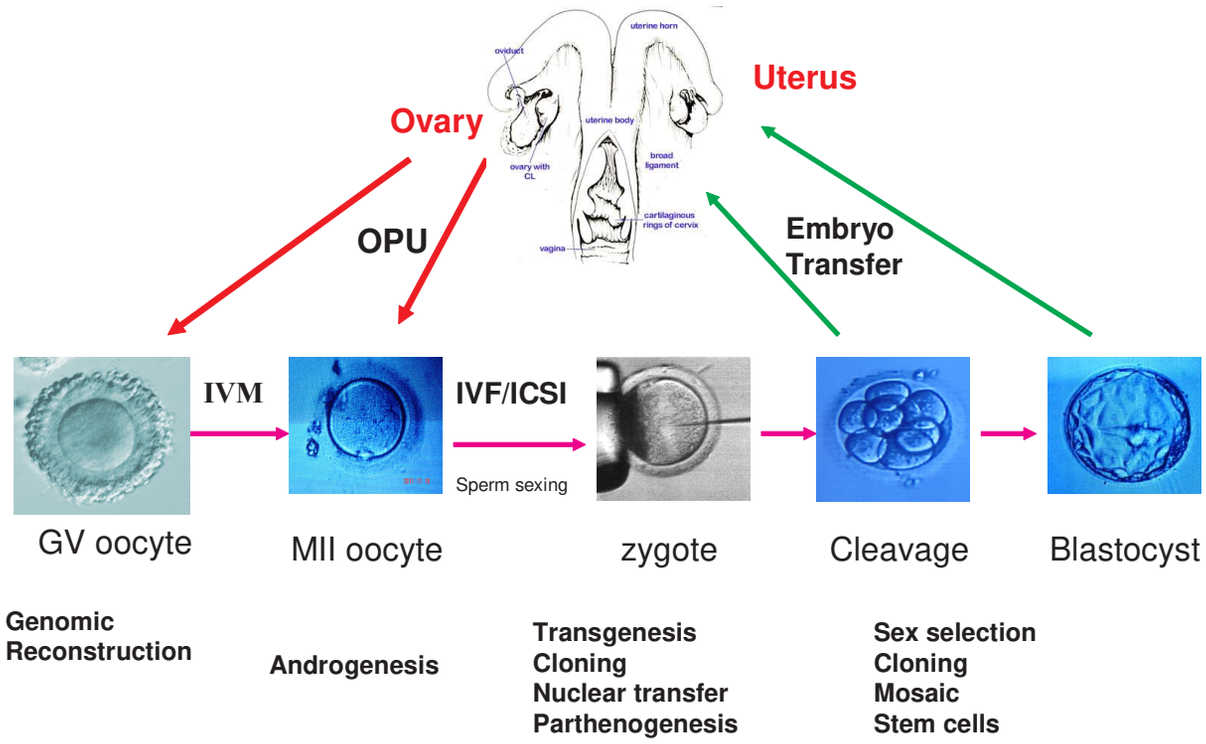

Figure 3. Schematic representation of main embryo biotechnologies which can impact on the genetic improvement programs on animal embryo breeding.

Based on this schematic picture, we may focus on several fields for Animal Embryo Breeding research. In the early stage of oogenesis and oocyte maturation, some key techniques such as genomic reconstruction, nuclear transfer, androgenesis and parthenogenesis, cytoplasm replacement, etc., may be used to change animal genetic construction [5]. At the fertilization stage, the sexing sperm may be used to produce specific-sex (female or male) animal population to achieve better economic results [6]. Using intracytoplasmic sperm injection (ICSI) technique may make an elite performance bull with a very few sperm produce a lot of offspring. At the pronuclear stage, the foreign DNA may be injected to zygote to produce transgenic animals. In the preimplantation cleavage and blastocyst stage, preimplantation genetic diagnosis (PGD) or preimplantation genetic screening (PGS), embryo cloning, mosaic animal and embryo stem cell techniques may be used to produce various different types of animals. Also, at any stage, sperm, egg and embryo, as well as somatic cells may be cryopreserved for future use [7]. Thus, we may profile the outline of Animal Embryo Breeding study as shown in Table 1 (Table 1).

Early Gamete Manipulation
Artificial Insemination
Semen collection and its storage
Sperm sexing
Ovulation control
Superovulation
Ultrasound-guided oocyte retrieval (TVOR) or nonsurgical ovum pick up (OPU)
Oocyte and egg cryopreservation
Embryo Transfer
Multiple ovulation (superovulation)
Multiple ovulation with embryo transfer (MOET)
Embryo splitting
Embryo sexing
Embryo transfer technique
In vitro embryo production (IVP) technology
In vitro maturation (IVM) of oocytes
In vitro fertilization (IVF) of oocytes
Intracytoplasm sperm injection (ICSI)
Culture of *in vitro* fertilized embryos
Preimplantation embryo diagnosis
Embryo Cloning
Embryo blastomere cloning
Somatic cell nuclear Transfer (Dolly)
Embryonic stem cell nuclear transfer
Induced pluripotent stem cells (iPS) nuclear transfer
Transgenic animals
Transfer gene construct
Inserting genes
Knockout genes
Strategies for gene transfer
a) Directly inject a gene into egg pronucleus
b) Sperm-mediated gene transfer
c) Stem-cell-mediated gene transfer (transfection)
d) Retrovirus and viruses vector for gene transfer

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	e) Transfer of animal cells/embryo
	f) Targeted gene transfer
	g) Liposomes or spheroplasts as vector
	h) Other techniques such as electroporation, use of complexes, of DNA with polycations or lipids; a particle gun, DNA with polycations or lipids, etc.
	Production of various transgenic animals
	a) Cow or cattle
	b) Sheep and goat
	c) Fish
	d) Pig
	e) Other animals
	Animal bioreactor and molecular farming
	Transgenic breeding strategies
	Transgenic effect and cost
Genomic reconstruction	
	Germinal vesicle (GV) transfer
	Androgenesis
	Parthenogenesis
	Three-parent baby
	Ova-plasma transfer
Preimplantation embryo diagnosis/screening (PGD/PGS)	
	Fluorescent *in situ* hybridization (FISH)
	Polymerase chain reaction (PCR)
	Microarray
	Comparative Genomic Hybridization (CGH)
	Gene chips
Mosaic animal creation	
	Heteromorphosis
Rare animal individual or breed preservation	
	Sperm cryopreservation
	Egg cryopreservation
	Somatic cell cryopreservation

Table 1. Outline of Animal Embryo Breeding discipline

5. Research category of animal embryo breeding

As a new discipline, animal scientists and breeders can apply Animal Embryo Breeding Science theory to animal population to improve genetic traits, to add new benefit traits to animal body and to remove some harmful traits from animal body. Major research categories involve the following several aspects:

1. The objective of embryo breeding study is to create new animal individual or improve animal population. Based on the objective of the animal breeding program – what kind of animal traits you need in the breeding program – you may adopt an appropriate method of embryo biotechnique. For instance, if you want to add new genetic trait into animal body, you may use transgenic method to insert this gene into embryo. If you need to produce a complete same animal, clone method may be used as embryo cloning or nuclear transfer technique to clone this animal somatic cell.

2. The technique selection of embryo breeding: based on your breeding objective, a specific technique should be selected; for instance, in transgenic program, what gene and which method should be used to produce transgenic animals. In the animal cloning program to increase animal population homogeneity, various cloning methods should be evaluated for the best cloning technique, such as embryo cloning, stem cell, or somatic cell cloning.

3. Inserting embryo breeding into animal breeding program. In practice, embryo breeding is a trick to produce a specific animal. By the means of transgenic tactics, a given target gene vector may be constructed and transformed to chromosome in cell. Then, a given aim-gene embryo may be formed by nucleus transfer technique. By means of the genetic screening and diagnosis on cell levels, an expected embryo with a specific genotype embryo may be determined on embryonic level. Then, this expected embryo with a specific modified gene may be transferred into animal uterus to produce a specific animal. After individual level diagnosis, the ideal animal may be placed in animal population to expend its reproduction as traditional breeding program.

6. Application of Embryo Breeding in animal improvement program

1. Genomic reconstruction by somatic cloning and parthenogenesis to produce specific animal population

When a bull or cow with elite production performance in beef cattle population is discovered, the breeding aim will be to accelerate this cow or bull reproduction to propagate a new breed of cattle. By normal breeding mating, this cow may lose half its inherent genes in its offspring. However, by the means of cloning techniques, many individuals of the same genotype can be theoretically produced. Thus, the accuracy of evaluation may be greatly increased. In spite of low cloning efficiency, many scientists are still interested in animal cloning techniques, which will eventually be used to clone very valuable animals, such as breeding stock, transgenic animals, and endangered species.

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By the means of cell nucleus transfer technology, a new animal can be produced using androgenesis method [7]. Androgenesis is a male parthenogenesis in which only paternal chromosomes are kept in the embryo with the removal of the egg nucleus at the fertilization [8]. This is a reproductive pattern from two male parents. After an oocyte nucleus has been removed, a male diploid cell is transferred into this egg in which the oocyte cytoplasm will induce this diploid cell going through meiosis to become a haploid MII oocyte. After inducement, a male sperm is injected into this oocyte to produce a paternal embryo. Finally, this modified embryo will be transferred into receipt cow to produce a new individual bull with two male parents.

2. Create new genetic variation in population by genomic modification during embryogenesis

The current animal breeding strategies are mainly based on the principle of selective breeding including the morphology of animal body, the application of quantitative genetics theory, the estimation of breeding value by phenotype, pedigree, BLUP (best linear unbiased prediction) method, and genetic markers. These methods mainly add genetic improvement by increasing the frequency of advantageous alleles of many loci, but actually very few of gene loci are identified. These techniques do not change gene movement from different species or genera due to reproductive barrier, while the new developed transgenic technique can remove the breeding barriers between different species or genera.

The most efficient method of transgenesis in mammals is the genetic manipulation of the pronuclear stage embryo [9]. By injecting foreign DNA into one of the two pronuclei of the zygote, the birth offspring may contain a functional foreign gene in the genome. In the last 20 years, many kinds of transgenic species have been produced for agriculture and medicine application [10]. For example, the transgenic technology in beef cattle industry may improve animals for faster growth, higher quality beef products, or disease resistance [11-13].

The transgenesis first starts with identification of the genes of interest. Current molecular biotechnology may help us to search for some interesting markers used as reference points for mapping relevant genes. These molecular markers can also be used for identification of the animals carrying the transgenes. Most of the quantitative genetic loci (QTL) are polygenic in nature but the manipulation of transgenesis is a single gene trait [14,15]. The technology holds promises in the future in moving polygenic QTL across the breeding barriers of animals. However, it is expected that molecular markers will serve as a potential tool to geneticists and breeders to evaluate the existing germplasm, and to manipulate it to create animals of desired traits [16].

3. Shorten generation interval by embryo in vitro production

As the oocyte in vitro maturation (IVM) and in vitro fertilization (IVF) techniques rapidly develop, the ultrasound-guided oocyte retrieval (TVOR) or nonsurgical ovum pick up (OPU) technique can retrieve many oocytes repeatedly from a cow or a heifer. As many as 1000 oocytes have been collected from one female cattle in a year [17-19]. Thus, the embryo in vitro production (IVP) technology has been able to promote a cow to produce more than one hundred

offspring in a year and greatly accelerate herd genetic improvement speed [20]. In order to improve ordinary cattle herd, slaughterhouse ovaries also may be used as in vitro embryo production. A lot of oocytes could be obtained from slaughter house cow ovaries. After maturation, these oocytes may be inseminated with elite bull semen for in vitro fertilization [21]. Although the detail genetic backgrounds of these slaughterhouse animals are not known, these embryos have a very high genetic merit from elite bulls. Using these embryos, an ordinary cow herd could obtain at least 50% genetic improvement.

The multiple ovulation and embryo transfer (MOET) was used initially to produce more embryos from genetic elite cows in shorter time periods. Currently, the MOET breeding schemes have widely established in many countries and their use accounts for about 80% of cattle embryos transferred commercially [22]. Currently, the application of transvaginal ultrasonically guided OPU technique may significantly improve MOET scheme efficiency because about 1000 oocytes may be collected and 300 embryos may be produced *in vitro* from a cow in a year at frequent intervals using IVF technology [19]. Also, oocytes may be collected from prepubertal heifers and cattle generation interval may be shorted for 2-3 years. The combination of MOET program with OPU/IVF technique is providing a more efficient way to produce more embryos from an individual donor donor than superovulation stimulation program [23]. Thus, OPU/IVF technique greatly increases MOET breeding scheme efficiency in milk and beef industry.

4. Increased economy from animal population by sex selection

Animal sex selection may increase animal economical value for humans. Embryo breeding theory may provide several ways for animal sex selection, including sperm sex selection and preimplantation embryo sex selection. Sperm sex selection is to try to separate semen into X- or Y-bearing chromosome sperm by flow cytometry [24, 25]. Current sorted sperm has been successfully used in IVF for in vitro embryo production and artificial insemination in cattle [6, 26].

Another sexing pathway is to determine the sex of an embryo prior to transfer. Preimplantation genetic diagnosis (PGD) technique has become an efficient method for sex selection. Y-specific chromosome probe for polymerase chain reaction (PCR) and Fluorescent *In Situ* Hybridization (FISH) are two common methods in animal sex determination. On the ordinary farm, cattle embryos may be sexed by complete cell biopsy and PCR technique. Our clinic farm practice [7] showed that a few of trophoectoderm cells could be microbiopsied from blastocyst embryos by transzonal incision using a microsurgical blade. The mini-tube PCR was carried out for 30 minutes and the gel electrophoresis was run for 20 minutes. The sexing result could be obtained in 2 hours. These results clearly demonstrate that the microsurgical technique and subsequent PCR sex analysis allow the rapid commercial exchange of genetic resources on the basis of fresh or frozen sex-desired embryos in embryo transfer programs.

Fluorescent *in situ* hybridization (FISH) technique has also been used as embryo chromosome set (karyotype) diagnosis. A blastomere is removed from an embryo by micromanipulation, and then used to examine the embryo X/Y chromosomes by FISH. Recently, new developed technologies in PGD allow examining of all chromosomes and identifying certain genes or

genetic mutations, such as the competitive genomic hybridization (CGH) and microarray analysis. More recently, novel developed Next Generation Sequencing (NGS) for preimplantation genetic screen (PGS) is now being offered clinically to provide comprehensive, accurate screening of all 24 chromosomes for selections of euploid embryos. PGS results generated are comparable to those achieved with the CGH technology, with improved accuracy, sensitivity, and resolution for more accurate detection of euploid embryos, aneuploidies, chromosome imbalances (translocations), and embryo mosaicism. NGS is a superior technology because it looks at close to 1.1 million data points on the genome compared to around 3,000 with CGH.

5. Preservation breeding

Many animal breeders are interested in preserving bloodlines of animals, either of a rare breed, or of rare pedigrees within a breed. Therefore, Rahbek [27] put forward a preservation breeding concept to describe the purpose of preservation breeding, which is to protect genetic diversity within a species, and to preserve valuable genetic traits that may not be popular or in fashion in the present, but may be of great value in the future. In the animal embryo breeding program, two kinds of cells including reproductive cells and somatic cells may be cryopreserved in liquid nitrogen for future use. Reproductive cell cryopreservation is an important branch of embryo breeding science because it involves the preservation of gametes (sperm and oocytes), embryos, and reproductive tissues (ovarian and testicular tissues) for future use in the assisted reproductive technology. Practically, animal embryo breeding program may provide a sperm and embryo bank with the objective of avoiding genetic dilution and irreplaceable gene losses of the valuable "naturalized breeds" germplasm. It is much lower in cost than normal animal breeding, preserving rare native animal breed plan. At present, many countries have set up gene banks to store frozen embryos and semen of various animal species including native cattle, pig, and some endangered animals.

The development of embryo freezing technologies has revolutionized cattle breeding. Since then, advancements in cryobiology, cell biology, and domestic animal embryology have enabled the development of embryo preservation methodologies for our other domestic animal species, including sheep and goats. Currently, use of preserved embryos has become a routine breeding alternative for all domestic animal species. This freezing and storage methodology may provide for maternal germplasm, global genetic transport, increased selection pressure of herd genetics, and genetic resource rescue.

In the conventional breeding program, an outstanding bull may maintain normal mating for 5 years. However, if this bull semen is cryopreserved, it will extend the bull's breeding time. In embryo breeding program, when some elite bulls leave very few sperm, we may use intracytoplasmic sperm injection (ICSI) technique to inject a single sperm to an oocyte so that genetic merit embryos are obtained [28]. Also, sperm cell genome cloning technique may be used to produce many copies of a specific sperm [8]. The application of this technique to beef and dairy cattle industry has greatly increased merit bull spread in animal herd [29].

Like normal reproduction, somatic cell nuclear transfer (SCNT) starts with an egg or oocyte, but here the nucleus of the egg needs to be removed. Then the nucleus from a somatic (skin) cell is transferred into the enucleated egg which would be analogous to the sperm entering the

oocyte. As this develops into a blastocyst, cells from the inner cell mass can be isolated and purified to serve as a source for pluripotent stem cells. In animal embryo breeding, somatic cell is also an important genetic resource. Therefore, the somatic cells, such as skin, hair, and other cells from rare and endangered animals may be collected and cryopreserved so that they can be used in the future.

7. Conclusions

Currently, the following biotechnologies in embryology have been applied or will be applied in animal genetic improvement [9]: 1) Genomic reconstruction by somatic cloning and parthenogenesis can produce specific animal population; 2) new genetic variation in population can be created by genomic modification during embryogenesis, such as transgenic breeding strategies; 3) animal generation interval may be shortened by embryo in *in vitro* production; 4) economy efficiency from animal population may be significantly increased by embryo sex selection; and 5) a rare breed, or of rare pedigrees within a breed, may be efficiently preserved at low cost in liquid nitrogen. Thus, the development of modern biotechnology has brought into being the concept and theory of **Animal Embryo Breeding Science.** Understanding and applying its theory and technology will be helpful to animal scientists and students as well as animal breeders to accelerate animal improvement speed, to modify genetic construction of animal population, and to create new animal breeds.

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