Micromanipulation of mammalian embryos

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Abstract: Micromanipulation of embryos provide a new and valuable biological tool for animal agriculture and medical fields. Current techniques for embryo manipulation are in practice but some of techniques is still way off for application. The present status in new biotechnology applying to animal science and medicine is reviewed and its future is also discussed.

Key Words: embryo, gene, manipulation

Introduction

The history of agriculture is largely a history in the progress of scientific phenomena especially concerned to genetics. Recent advances in manipulating techniques of mammalian embryos involved oocytes in oogenesis invitro have resulted in opportunities for research an mechanism of gene action and its expression during development and also have made possible the transfer of cloned genes from one organism to the genome of another (Wagner and Murray 1985, Trautwein 1985).

Current technology for embryo micromanipulation and gene transfer is now available to use for improvement and making of new bred or strain in livestock species and experimental animals (Church, R. B. et al 1985, Meinecke-Tillmann 1985, Sugawara 1986)

In medical field, the techniques had been applied to making a model animals for research and treatment of genetic disorder in human and animal health.

In this paper, I will review the progress in new biotechnology in animal agriculture and mankind, and present one of our research on cell interaction between oocytes and other ovarian tissues by using chimeric animals.

Genetic(developmental)engineering and methodology in mammals.

A. Domain of embryo micromanipulation

The purpose and research area of embryo micromanipulation are given in Fig.1 in correlation to the close related scientific fields: embryology and experimental embryology.

B. Procedures for micromanipulation of mammalian embryo

The procedures for embryo manipulation consisted of the essential and basic techniques shown in Table 1. Also, a schmatic program for embryo

Fig 1. Correlation of embryology, experimental embryology and embryo micromanipulation.

Table 1. Procedures for embryo manipulation

A. Essential techniques

- a) Embryo micromanipulation:
embryo splitting, embryo aggregation, blastomere separation, enucleation, nuclear transplant, gene transfer etc.
- b) Culture of the manipulated oocytes or embryos:
until transfer of embryos to recipient. culture for recovery and judge of manipulated embryos
- c) Transfer of manipulated embryos to recipient
- d) Preservation of manipulated embryos

B. Basic techniques

- a) Embryo production:
donor selection
superovulation, mating system, egg recovery
- b) Estrus synchronization:
recipients
- c) A. I. :
- d) Gene making (plasmid construction)
DNA→cDNA, Promoter
- e) Cryopreservation of gametes

manipulation in mammals is shown in Fig 2.

Therefore, not until each steps for the treatment are proceeding completely, before genetic engineering of mammalian embryo resulted in success.

C. First successful study of embryo manipulation

First successful application of embryo manipulation in laboratory and

Fig 2. Schematic program for embryo manipulation and gene transfer in mammals

livestock species are summarized in Table 2. The routine application of these techniques in the Table is not practicable in domestic animals yet.

Application and results of embryo manipulation.

Application of new biotechnology to animal agriculture can be expected in at least major area as follows: A)effective increase in animal production B)genetic improvement and making for new bred of livestock species C)animal health management D)manipulation of animal physiology E)improved crops and feeds.

A. Effective increase in animal production

a)Twin production. It is well known that twins or multiplets occur naturally in several mammals such as man, catle, sheep, goat and armadillo, In domestic animals, first experiments to produce artificial monozygotic twins were done by Willadsen(1979) who devided sheep embryos at the two cellstage and morla stage, respectively. At present time, monozygotic twin ortetraplets can be produced in many of domestic animals using the morula orearly blastocysts method or the 4-cell stage deviding method (Williams, et al 1982). Twins or multiplets are economic interest in monotocous species for increase the number of offspring per ovulation. In research, monozygotic twins can be used for investigation of interaction between genotype and enviroment.

Table 2. First successful experiment of micromanipulation to embryos

	Laboratory animals		Livestock species	
Embryo transfer	rabbit	Heape(1980)	sheep	Warwick et al (1934)
Monozygotic twins triplet	frog mouse, rabbit	Spemann(1901) Seidel(1959)	cattle sheep, cattle	Umhaugh(1949) Willadsen (1979, 1982) Williams(1982)
Parthenogenesis	frog mouse, rabbit	Hertwig(1911) Illemensee (1975) Pincus(1936)	- -	
Chimeras	rat rabbit, mouse	Nicholas & Hall(1842) Gardner (1968) Moustafa (1972)	sheep, goat cattle	Stranzinge et al(1983) Brem et al (1984)
Cloning	frog mouse	Briggs & King(1952) Ilmense & Hoppe(1981)	sheep	Willadsen (1986)
In vitro fertilization	rabbit	Chang(1959)	cattle	Brachett et al (1982)
Deep-freezing	mouse	Whittingham et al (1972)	cattle	Wilmut & Rowson (1973)
Sexing	rabbit	Edwards & Gardner(1967)	cattle	Hare et al (1976)
Gene transfer	mouse	Janish(1975)	swine, cattle	Hammer et al(1985)

b) In vitro fertilization. Normal offsprings born from in vitro fertilization has been reported in 8 species of mammals such as cattle, sheep, goat, monkey, human and laboratory animals(rabbit, rat and mouse). This technique is very important as any because many of embryo manipulation will utilize in vitro fertilized embryos as recipients in their application(see Fig 2). At present, normal development after in vitro fertilization of oocytes, which were collected from abattoir ovaries and matured in vitro, has been described only in cattle.

The technique was well known by its application in the certain types of infertility of humans.

c)Sex selection. For many years experiments were made to determine the sex of spermatozoa and embryos. In animal sciences, the breeder is

Table 3. Sexing by several techniques in mammals

Methods	Species
A. Sex chromatin a) trophoblast (blastocysts)	rabbit, cattle
B. Sex Chromosome a) blastomere (beyond 4-cell stage) b) trophoblast	rabbit, cattle
C. H-Y antigen a) Rabbit antibody vs spleen or antigen b) autoantibody vs spleen or testis antigen	mouse, cattle, swine rat, mouse
D. Separation of XY spermatozoa a) Serial density gradient of percoll (i) F-body (ii) Hamster egg: male pronucleus b) Electrophoresis	human cattle cattle Swine

interested in shifting the sex ratio according to the need. There has been success in separating x and Y-bearing spermatozoa of a number of mammals, for example, human, cattle, pig, mouse and rats. However, at present the technology (percoll method) is not practical for collecting the number of sperm used with routine artificial insemination and in vitro fertilization. Sex selection of embryos can be done by biopsying embryos and examining sex chromatin or chromosome or by identifying male embryos with an antibody to the male-specific molecule (H-Y antigen) (White et al, 1987).

d) cryopreservation of gamete and embryos: In vitro storage of sperm has been extensively used for artificial insemination program in a number of species, for example cattle, sheep, goat and humans. Freeze storage of female gametes and embryos is less well advanced, but the embryos developed to compact morula and early blastocysts beyond can be stored at low temperature in liquid nitrogen. This technique was first successfully demonstrated by Whittengham, et al (1972) in mouse and established by him and other groups for domestic and laboratory animals. Therefore, frozen embryos circumvent the problem of synchronization of recipients for they can be used at any time. Currently, this method was applied to storage of human embryo according to the program of in vitro fertilization. In animal agriculture, valuable genotypes may be stored permanently and used for animal production.

e) Chimera. Chimeric embryos and individuals can be produced either by fusing two or more embryos at 8-cell stage to make an aggregation chimera or by injecting one or more totipotent cells into the inner cell mass of a blastocyst (Papaioannou & Dietherlen-Lievre, 1984).

In current techniques, the injected cells should be from another embryos and from a cell line derived from a teratocarcinoma or a stem cells maintained in cell culture in laboratory. Usually, a genome of the injected cells have partially expressed in host embryos, but, frequently the introduced cells become incorporated fully into all tissue of the developing chimeric embryo including the germ line. As yet it is not possible to combine various genotypes in relative contributions as desired. But, this technique could be used for the making of a model animal and the treatment of genetic disease in human and livestock.

B. Genetic improvement of livestock species

Selection or phenotype of animals including sire has resulted in significant improvement of domestic animals over the past decades. The use of A.I. and other breeding techniques have allowed to increase markedly in refinement of evaluating individual animals.

Recombinant DNA technology in combination with new biotechnology should allow further refinements in evaluation perhaps by the use of restriction fragment length polymorphisms desired. A necessary prerequisite for this should be to identify linkage between restriction fragment of gene and production traits. Because production traits are controlled primarily by multiple genes, this may be a difficult task (Rando and Masina 1985).

The possibility of improving livestock species by the direct gene transfer to early embryos suggested in the experiments that is the first reports of successful gene transfer to mice.

Metablothionine-human growth hormone (MThGH) fusion gene have been transferred to male pronucleus of the fertilized mouse ovum. This cloned DNA sequences have been shown to be integrated into the chromosomes, and are inherited as Mendelian traits. Offspring that inherit the donor DNA have also been shown to grow larger. Hammer et al (1986) reported the production of transgenic rabbit, sheep and pig which retained MThGH fusion gene and shown that hGH levels in plasma of several pigs increased between birth and 90 d of age, but did not stimulate postnatal somatic growth rates.

C. Applications in animal health management

In animal industry, economic losses due to animal morbidity and death are significant. In the developed countries, total economic losses due to decreased production being estimated to about 20% of the direct gross income. Advances in immunobiotechnology can be expected to have a significant impact in the area of animal health management. Improved kits using monoclonal antibodies are being developed to detect and treat disease conditions. In future, it is possible to make a bred or strain inserted gene for resistance in the germ line of viral disease (Kobbe, 1985).

D. Manipulation of animal physiology

Materials produced by eukaryotic genes in bacteria will apply to research in determination of physiological mechanisms and controls involved

in such processes as milk production and reproduction.

Recent studies with recombinantly derived bovine and porcine growth hormone have been accumulating and indicating that these hormone can stimulate milk production or growth rate in livestock animals.

E. Improved crops and feeds for animal production

Significant advances in plant breeding and crop improvement are expected by applying biotechnology. Application of engineered microbes also are predicted to results in significant utilization of feeds and feed technology. Such advances will impact significantly on animal agriculture (Coffman, 1983).

Future in new biotechnology

Technical problems with gene transfer to livestock species can be expected to be overcome within next 10 years. Progress in the area of recombinant DNA and gene transfer technology has been more rapid than originally expected. Animal and medicals science research and application of the future can be expected to incorporate the new biotechnology of recombinant gene and related technologies.

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