EMBRYO MANIPULATION AND GENE TRANSFER IN LIVESTOCK PRODUCTION

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SUMMARY:

The manipulation of reproduction through artificial insemination and embryo transfer has had a major impact on genetic strategies in animal production during the last fifteen years. The advent of estrus synchronization, non surgical embryo collection and transfer, embryo freezing and splitting have allowed the industry to move from the laboratory to the farm. Other aspects of embryo manipulation which have a major impact on breeding strategies include embryo splitting to produce monozygotic twins, in vitro fertilization, cross species fertilization, embryo sexing, chimera production of tetraparental animals and possibly cloning. Similarly, rapid advances in recombinant DNA and plasmid construction coupled with our increased knowledge of the molecular biology of domestic animal species now permits genetically engineered animals to enter into classical breeding programs. Genetically engineered animals are now appearing, however very little is known about the molecular biology and physiology required to utilize the potential for increased production efficiency. All of the technologies associated with embryo manipulation and the production of genetically engineered animals through gene transfer will demand very different genetic strategies if they are to be utilized fully in genetic improvement programs.

INTRODUCTION

The manipulation of reproduction has had a major impact on animal breeding programs for many years through the use of artificial insemination and embryo transfer. The Dairy industry adopted artificial insemination in the 1950's. Demand for continental beef breeds sparked the development of embryo transfer, as it offered increased numbers of offspring born to genetically superior mothers. Embryo transfer is now commonly used to produce sires for progeny testing in artificial insemination programs. Planned matings provide an increased opportunity to market the improved genetics of proven elite females and progeny tested superior sires. Animal breeders have identified elite females whose offspring have superior genetic potential and marketability. Since 1974 nonsurgical embryo recovery and transfer such as developed at Alberta Livestock Transplants allowed the industry to move from the laboratory to the farm. Breeders can produce increased number of offspring from specific planned matings of superior females without the fear of reproductive performance through surgical damage (Elsden, et al., 1976). The embryo transfer industry now produces thousands of pregnancies each year, world wide and has been subject to a number of reviews (Church and Shea, 1977; Betteridge, 1981; Seidel, 1981; Mapletoft, 1984 and Church et al., 1985).

Embryo manipulation is an attempt to utilize the genetics of excellent animals. In vitro fertilization in the bovine has been achieved (Brackett, 1983). Although no reliable method of sexing sperm has been developed, rabbit blastocysts were sexed by Gardner and Edwards (1968) and day 12 bovine trophoblast cells were karyotyped by Betteridge, et al. (1981). Sexed embryos or sperm would be a major advantage to the animal breeder. Most infectious diseases in the bovine species will not affect embryos with an intact zona pellucida (Singh and Hare, 1984). Embryo transfer offers the prospect to increase the population base of endangered species (Durrant and Benirschke, 1981). Embryo freezing increased the flexibility of embryo transfer programs and transport. Successful embryo culture systems are required for programs utilizing whole or part embryo freezing (Shea et al., 1983). Similarly, embryo transfer enhances progeny testing for genetic defects. Females suspected of carrying the defect are superovulated and the embryos transferred to unrelated

reciprients. Usually, pregnancies are terminated in the first trimester, and the fetus examined for environmental or genetic defects (Fisher et al., 1984).

Manipulation of reproduction includes not only artificial insemination, sex seletion, in vitro fertilization and culture, embryo freezing and transfer but micromanipulative techniques which allow production of monozygotic twins throught the bisection of morula or early blastocysts (Willadsen, 1982; Ozil 1983 and Church et al., 1985). Commercial monozygotic twin production has been described by Shea and Baker (1985). Successful cloning of domestic animals will have a major impact on genetic programs in the near future. Although, the production of cloned laboratory species has been subject to considerable debate technology is currently available which will allow successful cloning of livestock species. Blastomere nuclear cloning expanded by subsequent embryo transfer to create significant numbers of identical animals brings a new prospective to animal breeding programs.

Another powerful embryological technique is the aggregation of blastomers from a number of embryos (Mintz, 1965) or the injection of a cell into the blastocyst cavity of an embryo (Gardner, 1978) to produce chimeras. Chimeras may or may not have germ line involvement of the two genomes contributing to the animals. We have produced bovine chimeras from double muscled, chromosome translocation marked embryos and normal embryos in an attempt to modify and exploit the characteristics of the double muscled growth pattern. To date, the tetraparental animals carrying marked cells have not shown any of the double muscled characteristics. The total contribution of each parental cell type to the chimeric offspring may vary with age. The most spectacular chimeras are the 'geeps' produced by FeHilly et al, (1984). These chimeras of sheep and goat showed phenotypic characteristics of both parental genomes. These procedures provide an opportunity for the introduction of genetically engineered cells into embryos. A schematic program for embryo manipulation and subsequent gene transfer procedures is presented in Figure 1.

Recently, foreign genetic material has been injected into fertilized oocytes resulting in transgenic animals. A transgenic animal might be defined as one whose genetic composition has been altered to include selected genes from other animals or species by methods used other than those traditional in animal breeding. The most common method of producing transgenic animals is to inject DNA sequences into the fertilized oocyte (Wagner, et al., 1981; Palmiter et al., 1982). There are some limitations to microinjection since very little is known about the actual process of genome integration or the factors involved in the control of foreign gene expression. Many animals do not express genes incorporated into their genome (Gordon, 1983; Wagner, 1985). The isolation of desired gene sequences and the regulatory DNA sequences required for controlled expression in a genetically engineered animal is not a trivial task. The production of a fusion gene involving metallothionein regulatory sequences coupled to the rat growth hormone gene injected as a pBR322 construct into mice by Palmiter et al (1982) initiated considerable speculation about the possibility of introducing desirable genes into animals. Subsequent production of transgenic rabbits, pigs and sheep (Hammer et al., 1984) and cattle (Church, 1986) confirmed the speculation.

DISCUSSION

The objectives of embryo manipulation and gene transfer experiments carried out in our laboratory have been to develop methodology for the propagation of genetically elite animals. We aim to create transgenic cattle capable of producing unique biologically important peptides. Such animals could be viewed as production systems for biologically important peptides which require posttranslational modification prior to secretion. A transgenic cow with a fusion gene for biologically important protein and regulation sequences for a milk protein would secrete the biologically important protein into the

milk.

The first attempt at alteration of the animal genome with exogenous DNA was carried out by Munro (1968) with chickens. He injected DNA isolated from bantam fowl, which are colored and have a different claw structure into the ovaries and testis of white leghorn chickens. He obtained some offspring which had patches of dark feathers and the bantam type claw. Molecular technology was not available to assess the gene transfer. In our laboratory we attempted to develop a "heterosis index" based on observed differences in the numbers of copies of repetitive DNA sequence families between inbred lines of chickens (Schultz and Church, 1972). Studies of preimplantation embryo molecular biology (Church 1974) led to the application of genetic engineering technology to livestock production. A number of structural gene-promoter-enhancer combinations have been incorporated into mouse and bovine oocytes. Cloned DNA sequences are injected either into the pronucleus or ooplasm of fertilized oocytes in one pL of buffer. Incorporation of injected sequences is confirmed by Southern DNA probe hybridization. DNA can be introduced into over 100 embryos per hour. About 25% of all injected mouse embryos develop as transgenic offspring. Typical data form a transgenic mouse experiment are presented in Table 1. We have noted a high frequency of sterility and other physiological problems associated with transgenic animals (Church et al. 1984).

Hammer et al. (1985) reported that of 1032 sheep and 2035 pig ova injected and transferred, the integration frequency for the MT-hGH fusion gene was 1.3% and 10.4% respectively. Only 10% of the injected sheep eggs developed to blastocysts while 23% of the injected pig eggs developed to blastocysts. Only 1 of 73 newborn sheep incorporated the fusion gene.

Utilizing superovulation, natural mating and subsequent flushing of the fertilized oocytes yielded 852 bovine fertilized oocytes for microinjection with alphafetoprotein fusion gene. Only 4 out of 111 bovine embryos incorporated the fusion gene into their genome (Table 1).

Work with large domestic animals in any transgenic program requires a considerable investment in the number of animals required for such experimentation. The logistics of recovering sufficient newly fertilized bovine embryos, their microinjection or transfusion, culture in the laboratory to select those which are developing normally and their subsequent transfer to recipients is a major management task. The successful development of technolgy to allow genetic engineering and embryo manipulation of domestic animals has great importance to animal breeding strategies. Such programs will be long term programs, limited, in the case of cattle, by the number of embryos available. The potential gains include improved efficiencies of reproductive performance, growth, disease resistance and changes in milk and wool production and composition. The introduction of growth or growth hormone related genes may increase growth but an understanding of the molecular physiology of growth to insure such animals are capable of a normal reproductively fit lifespan is needed. The field of animal genetic engineering and embryo manipulation including cloning will have significant impact on animal breeding strategies and programs in the future.

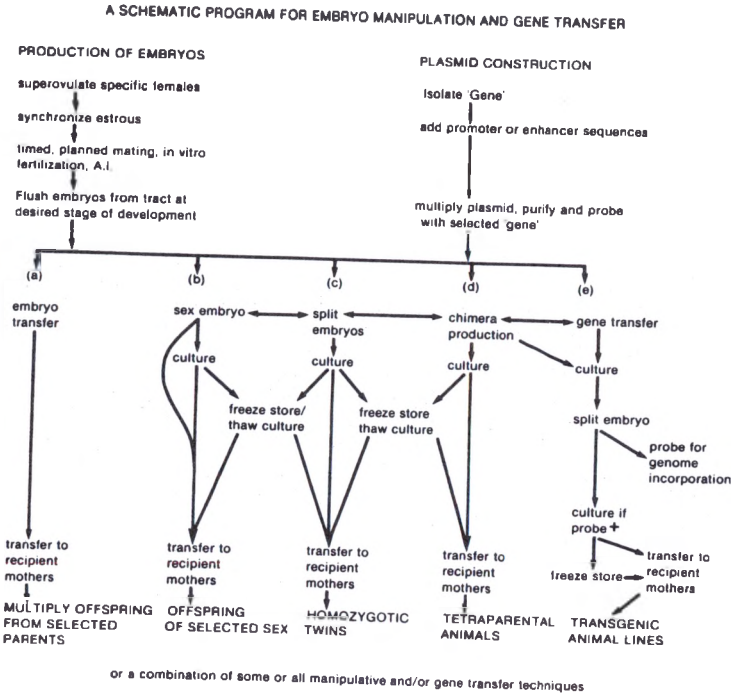

TABLE 1

COMPARISON OF MOUSE AND BOVINE GENE TRANSFER

SPECIES	number injected ova	number embryos transferred	number embryos	number with integration
MOUSE	1742	563	376	106
CATTLE	852	237	111	4

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