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# TRANSGENIC ANIMALS: PRODUCTION AND APPLICATION

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## ABSTRACT

Organisms containing integrated sequences of cloned DNA (transgenes), transferred using techniques of genetic engineering (to include those of gene transfer and gene substitution) are called transgenic animals. The development of transgenic animals has been part of biotechnology research which has been expanding rapidly. Transgenic animals produced with the purpose of producing better and good quality breed, increased in milk yield, as well as to produce organs to meet the demand for organ transplantation. Genetically modified animals are proving ever more vital in the development of new treatments and cures for many serious diseases. Transgenesis is a radically new technology for altering the characteristics of animals by introducing the foreign genetic material. Now a day's numbers of methods are available to produce transgenic animals like Pronuclear micro-injection, Embryonic stem (ES) cell manipulation, Cre-lox technique, Viral vectors, Cytoplasmic injection, Primordial germ cells, Nuclear transfer and Spermatogonial manipulation among them Lentiviral vectors and Chimera generation by injecting the pluripotent cells methods are becoming important tools for transgenesis and they contributed to human welfare in many ways such as in Agriculture, medicine, food, disease model, industrial purpose, drug development and research etc. The application of transgenic animals showed that within the next five to eight years genetically modified animals will play a significant and important role in the biomedical field, in particular via the production of valuable pharmaceutical proteins and the supply of xenografts. This paper attempts to give an idea about development of transgenic animals as well as their application for human welfare.

**INTRODUCTION:** Before the advent of molecular genetics, the only practical way to study mammalian genetic regulation and function was to observe certain traits. The farmers used this technique for more production of milk. The first chimeric mouse was produced in 1970 [1]. The first transgenic animals were produced almost 20 years ago by using microinjection of foreign deoxyribonucleic acid into the pronuclei of zygotes [2, 3].

The foundation for the production of transgenic animals was started using sperm mediated gene transfer; [4] and in 1980s, the transgenic mice is produced using the most popular microinjection technique [5]. The creations of many transgenic animals were subsequently reported in 1985s [6]. There are several methodologies employed in producing transgenic animals. Microinjection method used frequently but having some drawbacks like low efficiency, variable expression patterns. So, alternative methodologies are used like sperm-mediated DNA transfer [7, 8], intracytoplasmic injection of sperm heads carrying foreign DNA [9], injection or infection of oocytes and/or embryos by different types of viral vectors [10] ribonucleic acid (RNA) interference technology [11]nd the use of nuclear transfer [12].

Organisms containing integrated sequences of cloned DNA (transgenes), transferred using techniques of genetic engineering (to include those of gene transfer and gene substitution) are called transgenic animals. There are several types of transgenic animals like transgenic sheep, birds, chickens, pigs, insects etc. Transgenic animals produced with the purpose of producing better and good quality breed, increased in milk yield, as well as to produce organs to meet the demand for organ transplantation. Genetically modified animals are proving ever more vital in the development of new treatments and cures for many serious diseases. Transgenesis is a radically new technology for altering the characteristics of animals by introducing the foreign genetic material. Some important benefits and risks of transgenic animals are shown in **table 1**.

**TABLE 1: BENEFITS AND RISKS OF TRANSGENIC ANIMALS**

| BENEFITS | RISKS |
|---|---|
| Desired characteristic may be introduced for animal that require few feed supplements as well as medical treatments. | Insertion of foreign gene may upset the expression of the genome. |
| A desired characteristic of offspring could be established in one generation. | Normal reproduction may result in a transgene being released to the wild population. |
| The characteristic required can be chosen with greater specificity and accuracy. | |

**Methods for Producing Transgenic Animals:** The main principle in the production of transgenic animals is the introduction of a foreign gene or genes into an animal (the inserted genes are called transgenes). The foreign genes must be transmitted through the germ line, so that every cell, including germ cells, of the animal contains the same modified genetic material [18]. The first transgenic animals produced in 1980, were mice [13, 14]. There are various methods for producing transgenic animals which are summarized [15] in **table 2**.

**TABLE 2: VARIOUS METHODS FOR PRODUCING TRANSGENIC ANIMALS BY INTRODUCTION OF FOREIGN DNA INTO THE MAMMALIAN GENOME**

| TECHNIQUES | REMARKS |
|---|---|
| Pronuclear micro- injection (introduction of expressed gene) | Technical simplicity; low success rate; applicable to a wide range of species; most widely used; unpredictable effects due to random transgene integration |

| | |
|---|---|
| Embryonic stem (ES) cell manipulation (introduction of expressed gene, or gene inactivation by homologous recombination) | Substitution of a functional gene with an inactive gene; germ-line competent ES cells have been isolated in mice; ES-like cells identified in other species, including primates, but totipotency remains to be established |
| Cre-lox technique | Preferred method with more control over resulting phenotype; time-consuming |
| Viral vectors | Complex; largely restricted to avian species |
| Cytoplasmic injection | Less efficient than direct pronuclear microinjection |
| Primordial germ cells | Chimaeric animals result |
| Nuclear transfer | Large potential for genetically modifying livestock |
| Spermatogonial manipulation | Transplantation into recipient testes |

- **DNA Microinjection:** Pronucleus microinjection was first described by Gordon and Colleagues. Male and female pronuclei are microscopically visible several hours following the entry of the sperm into the oocyte. The transgene may be microinjected into either of these pronuclei [16]. Big Blue animals [17] and Muta Mouse [18] have been generated by using Pronucleus microinjection method. By using this technique, Transgene integration into the genome of founder animals is low i.e. only 0.5–3% of the microinjected embryos producing transgenic offspring [19].

  All the transfection techniques are applicable to cultured animal cells, but microinjection is ordinarily not used due to the tediousness of the technique and the limited number of cells that can be handled [20]. The method allows an early integration of the transgene into the host DNA, which is important to ensure that transgenic DNA is apparent in all cells of the host. Here, first isolate the piece or pieces of DNA as per requirement and clone it into a vector such as a plasmid then harvest newly fertilized eggs before the pronuclei fuse. A pronucleus is the nucleus of the sperm cell (male) or egg cell (female) before they join to become the fertilized zygote. Next the piece of DNA that has been isolated is placed in a syringe and injected into the pronucleus of the sperm cell.

  When the pronuclei have fused to become the nucleus of the new zygote, the cells are allowed to divide to form two embryonic cells after the embryo is transferred into the uterus of a pseudopregnant mouse (a female mouse that has been mated with a vasectomized male mouse) because a mouse that has had its vans deferens removed to sterilize the mouse, is to stimulate the hormones in the body to make her uterus receptive to the embryo that will be transplanted in it.

  The inclusion of the transgene DNA by microinjection is a random process, so not all of the pups born will have this gene expressed. This could happen if the gene inserts itself in an area of DNA that is not normally expressed, so a sample of the pups tissue from the tail will be taken and DNA examined.

- **Embryonic Stem Cell-Mediated Gene Transfer [21, 22, 23]:** In 1981, the term embryonic stem cells (ES cells) were used to denote a cell line isolated directly from mouse embryos while, the term embryonal carcinoma cells (EC) were derived from teratocarcinomas. Embryonic stem cells (ES cells) are harvested from the inner cell mass (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, including its gametes as shown in **figure 1**.

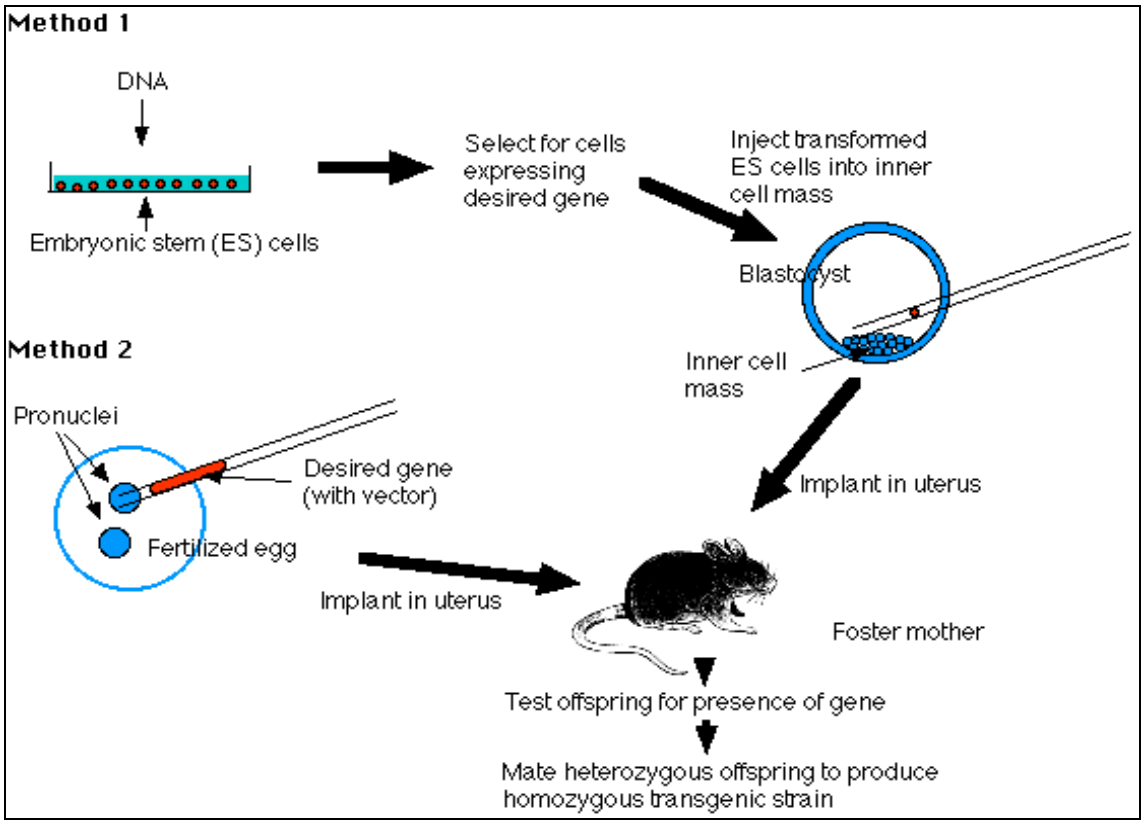


**FIGURE 1: EMBRYONIC STEM CELL-MEDIATED GENE TRANSFER**

- Using recombinant DNA methods, build molecules of DNA containing the structural gene you desire (e.g, the insulin gene), vector DNA to enable the molecules to be inserted into host DNA molecules, promoter and enhancer sequences to enable the gene to be expressed by host cells.
- Transform ES cells in culture to expose cultured cells to the DNA so that some will incorporate it.
- Select for successfully transformed cells.
- Inject these cells into the inner cell mass (ICM) of mouse blastocysts.
- Embryo transfer.
  - Prepare a pseudopregnant mouse. The stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
  - Transfer the embryos into her uterus.
  - Hope that they implant successfully and develop into healthy pups (no more than one-third will).
- Test her offspring.
  - Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10- 20% will have it, and they will be heterozygous for the gene.
- Establish a transgenic strain
  - Mate two heterozygous mice and screen their offspring for the 1:4 that will be homozygous for the transgene.

- Mating these will found the transgenic strain.

- **Retrovirus mediated gene transfer:** Transgenic mice produced by retroviral transduction of male germ line stem cells. Male germ line stem cells have ability to self-renew and genetic modification of these cells would help to study the biology of their complex self-renewal and differentiation processes and to generate wide range of transgenic animal species [24]. A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA [25]. Retroviruses used as vectors to transfer genetic material into the host cell, resulting into a generation of chimera (an organism consisting of tissues or parts of diverse genetic constitution). Chimeras are inbred for as many as 20 generations until homozygous (carrying the desired transgene in every cell) transgenic offspring are born. The method was successfully used in 1974 when a simian virus was inserted into mice embryos, resulting in mice carrying this DNA [26].

  - **Nuclear Transfer Method:** In this method, the transgenic goats were produced by nuclear transfer of fetal somatic cells. Donor karyoplasts were obtained from a primary fetal somatic cell line derived from a 40-day transgenic female fetus produced by artificial insemination of a nontransgenic adult female with semen from a transgenic male. Live offspring were produced with two nuclear transfer procedures.

    - Oocytes at the arrested metaphase II stage were enucleated, electrofused with donor somatic cells, and simultaneously activated.

    - In the second procedure, activated in vivo oocytes were enucleated at the telophase II stage, electrofused with donor somatic cells, and simultaneously activated a second time to induce genome reactivation.

There was generation of three healthy identical female offspring. Genotypic analyses confirmed that all cloned offspring were derived from the donor cell line. Analysis of the milk of one of the transgenic cloned animals showed high-level production of human antithrombin III. The nuclear transfer application may be more useful and beneficial for agricultural is the ability to efficiently produce a large number of identical offspring derived from a particular mating. Therefore, nuclear transfer using a embryonic cell lines derived from that mating maybe more attractive[27].

- **Transfection of Gametes:** The first transfection procedures occurred in the early 1960s and experiments with different cell types and tissues has now become widespread. Different transfection methods have been employed:

  a) The in vitro procedure when foreign genes are introduced into cultured cells or tissues.

  b) The in vivo method, when genes are directly introduced into the tissue (by injection, aerosol, etc);

  c) The ex-vivo system, in which cells are transfected in vitro and then introduced into a living organism.

Gametes are incubated during short time periods in a solution containing the gene constructions and then they are checked for transfection, used for inseminations or for in vitro fertilization procedures. In several cases naked DNA was employed successfully, but DNA-Liposome complexes or electroporation procedures have been also used. In the case of the female gamete in vitro transfections using liposomes or retroviruses have been applied successfully. As well as, electroporation, high velocity microprojectiles or

particle gun methods have been also employed. The localization of the foreign gene in spermatozoa has been done using fluorescent in situ hybridization, autoradiography or immunocytochemistry. After using the in vitro or in vivo transfection procedures high percentages (80%) of spermatozoa appeared transfected. These results usually showed that the foreign gene appeared into the nucleus of spermatozoa and molecular procedures (Slot-Blot, PCR, Southern Blot and gene sequences) have shown the presence of the transgene in the DNA of the gametes [28].

- **Artificial Chromosome Mediated Gene Transfer [29]:** A group of nuclei injected with transgene DNA, the eggs are transferred in medium of incubation and visual evaluation within next few hours. An individual animal develops after receiving the transgene DNA is referred as founder of a new transgenic lineage.

  Also, Yeast Artificial Chromosomes (YACs) transgenic mice are generated by using pronuclear microinjection and represents latest generation of vectors which have the great advantage of large insert size. This method succeeded in mice and rabbits.

- **Testis cell transplantation method[30]:** Testis cell transplantation method is shown in figure 2 and its steps are as follows:

  (A) A single-cell suspension is produced from a fertile donor testis.

  (B) The cells can be cultured

  (C) Microinjected into the lumen of seminiferous tubules of an infertile recipient mouse.

  (D) Only a spermatogonial stem cell can generate a colony of spermatogenesis in the recipient testis. When testis cells carry a reporter transgene that allows the cells to be stained blue, colonies of donor cell-derived spermatogenesis are identified easily in recipient testes as blue stretches of tubule.

  (E) Mating the recipient male to a wild-type female

  (F) Produces progeny, which carry donor genes.

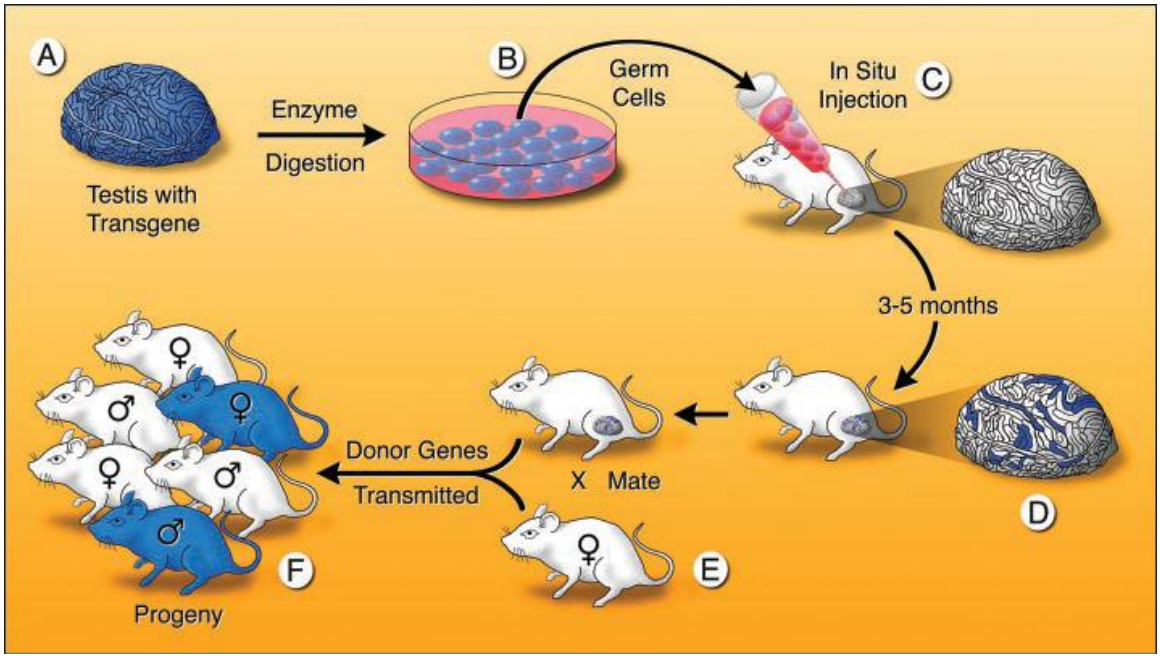


**FIGURE 2: TESTIS CELL TRANSPLANTATION METHOD**

**Recent methods for production of Transgenic Animals:**

**Lentiviral Transfer of Oocytes And Zygotes:** This method is used to overcome previous limitations of viral mediated gene transfer, containing the silencing of the transgenic locus and low expression levels [31]. Example including, generation of transgenic cattle by lentiviruses requires microinjection into the oocytes [32]. Recently H. M. Sang from Roslin Institute has reported a different approach to overcome the problem associated with retroviral vectors. This study employed lentiviral based vectors. These vectors have several advantages compared to the conventional retrovectors in that they can infect non-diving cells, can carry large amounts of transgene ~ 10kb, and can show stable expression in the tissue where they are introduced. The technique was successful in showing about 100 fold increases in the level of transgenesis [33].

**Chimera Generation by injecting the Pluripotent Cells:** Embryonic stem cells with pluripotent cells have ability to participate in organ and germ cell production after injection into the blastocysts [34]. Embryonic stem cells are important one for generating the gene knockins, large chromosomal rearrangements as well as gene knockouts [35]. As like embryonic stem cell, the another type of cells such as primordial germ cells are used for production of no. of farm animals and chimeric animals without germ line contribution have been reported in swine [36, 37].

**Applications of Transgenic Animals:**

**As disease model:** Historically, mice have been used to model human disease because of their physiological, anatomical and genomic similarities to humans. Transgenic animals are produced as disease models (animals genetically manipulated to exhibit disease symptoms so that effective treatment can be studied) such as Alzheimers, cancer, AIDS. Transgenic animals enable scientists to understand the role of genes in specific diseases. The benefits of using transgenic animals include the possibility of the replacement of higher species by lower species- through development of disease models in mice rather than in dogs or non-human primates, the extent of discomfort experienced by parent animals during the experimental procedures. Transgenic animals such as mice have been found to be valuable in investifations into gene function and for analysis of different hereditary diseases [15, 38, 39].

**As food:** The FDA suggested that cloned animals and their products were safe to eat for human being [40]. Some drawbacks are associated due to their muscle hypertrophy like difficulties in calving requiring Caesareans, poor viability of calves and poor fertility.

**Drug and Industrial production:** Transgenic animals are used for production of proteins such as alpha-1-antitrypsin, produced in liver, used in treatment of emphysema or cystic fibrosis. This process is less expensive than production of protein through culture of human cells [41]. The human lungs are constantly get affected by foreign particles such as dust, spores and bacteria. To prevent these, neutrophils releasing the elastase enzyme but this enzyme harmed the elastin in the lungs which maintains the elasticity of lungs. So, human body releases a protein α1 proteinase inhibitor which has been successfully expressed in sheep [42]. Recombinant human proteins produced in the mammary glands of transgenic animals [43, 44]. Pharmaceutical proteins are now used for commercial purpose [45, 46]. Two scientists at Nexia Biotechnologies in Canada spliced spider genes into the cells of lactating goats. The goats are used to manufacture silk, milk and secrete tiny strands from their body by the bucketful. By extracting polymer strands from the milk and weaving them into thread

which is light and tough material that could be used to prepare military uniforms, medical micro sutures and tennis racket strings. Americans are more supportive (60%) for above use of transgenic animals [47]. The mammary gland of transgenic goats is used to produce Monoclonal Antibodies. A recombinant bispecific antibody is produced by using transgenic cattles with in their blood [48].

Another application includes newly generation of trans-chromosomal animals in which a human artificial chromosome containing the complete sequences of the human immunoglobulin heavy and light chain loci was introduced into bovine fibroblasts, which were then used in nuclear transfer. Transchromosomal bovine offspring were obtained that expressed human immunoglobulin in their blood. This could be a significant step forward in the generation of human therapeutic polyclonal antibodies [49].

**Disease control:** Scientist developed the mice by altering the genes of the mousepox virus in Australia [50]. Some scientist also thought to develop genetically modify mosquitoes so they cannot produce malaria but other scientist worry about these mosquitoes that they could have unforeseen possibly risk if, they are released into the environment [51].

**Xenotransplantation:** Now a day approximately about 250000 people are alive due to the successful transplantation of an appropriate allotransplantation. Sometimes there is limitation of appropriate organs or rejection of live organ donation. So, to rectify this problem porcine xenografts from domesticated pigs are considered to be the best choice [52, 53]. Pigs which are genetically modified can be used as a source animal for tissues and organs in human beings for transplantation purpose by delete the gene responsible for the human rapid immune rejection response [54]. In Canada, a National survey on xenotransplantation showed that only 48% found acceptable for 'the use of animals as a source of living cells, tissues or organs to prolong human life [55]. To overcome the Hyperacute rejection & acute vascular rejection, synthesis of human regulators of complement activity are produced in transgenic pigs [53, 56]. Survival rates, after the transplantation of porcine hearts or kidneys expressing transgenic regulators of complement activity proteins to immunosuppressed nonhuman primates, reached near about 23 to 135 days. So, the Hyperacute rejection can be overcome in a clinically acceptable manner [57]. For long term graft tolerance induction of permanent chimerism via intraportal injection of embryonic stem (ES) cells [58] or the co-transplantation of vascularised thymic tissue [59].

**Blood replacement** Transgenic swine are used to produce human haemoglobin. The protein obtained from transgenesis could be purified by using procine blood which is similar to human haemoglobin [60].

**Agriculture** Transgenic pigs containing a human metallothionein promoter or porcine growth-hormone gene construct referred significant improvements in economically traits including growth rate, body fat/muscle ratio[61,62]. Transgenic pigs are used to produce pork by using spinach desaturase gene which produce large amount of non-saturated fatty acids, used for diet purpose and was advantageous to reduce the risk of stroke and coronary disease [63, 64]. Transgenic animals are used for milk production. Generally, there is an improvement in milk composition. For this purpose transgenic mice have been developed, at the same time some unwanted side effects can occur [65, 66]. Transgenic pigs are use to increase milk production by altering the composition of lactose [67]. In the pig, transgenic expression of a bovine lactalbumin construct in sow milk has been resulting in higher lactose contents and greater milk yields, correlated with improved survival and development of piglets

[68]. Transgenic sheep are used for wool production in which transgenic sheep carrying a keratin-IGF-I construct showed that expression in the skin and the amount of clear fleece was about 6.2% greater in transgenic as compared to nontransgenic animals [69, 70]. Scientists are attempting to produce disease-resistant animals, such as influenza-resistant pigs, but a very limited number of genes are currently known to be responsible for resistance to diseases in farm animals [71].

**Transgenic animals are used in toxicity testing.**

**Transgenic animals are used for vaccine testing.**

**CONCLUSION:** Throughout history, transgenic animal has made significant contributions to human health and well-being. The recent advances in reproductive technologies (in vitro production of embryos, sperm sexing, somatic nuclear transfer, Lentiviral transfer of oocytes and zygotes, Chimera generation by injecting the pluripotent cells) adds a new dimension to animal breeding. The application of transgenic animals showed that within the next five to eight years genetically modified animals will play a significant and important role in the biomedical field, in particular via the production of valuable pharmaceutical proteins and the supply of xenografts. New and exciting techniques being developed will continue to expand this important and useful area of experimentation.

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