

Transgenic Animals

Transgenesis is the insertion of foreign (exogenous) genes into the genome of an organism.

The **Transgene** is the foreign gene that is transferred to the recipient cell/organism.

The Rationale for creation of Transgenic Animals includes:

1. Improve the commercial value of farm animals
2. Pharming. The use of farm animals as bioreactors that produce pharmaceuticals or organs for xenotransplant.
3. Create mouse models for human disease

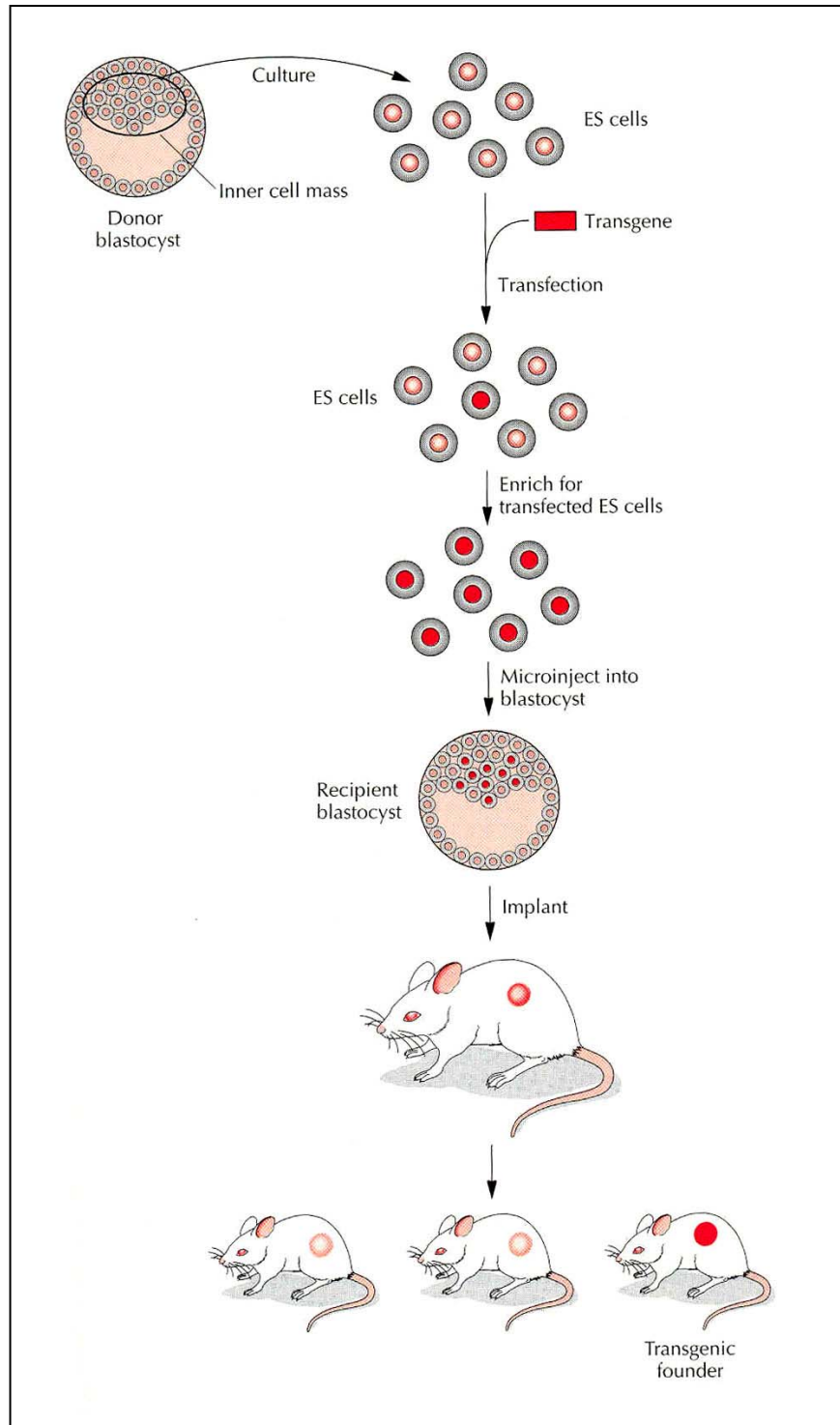
Methods for introduction of the transgene into animals include:

1. Embryonic Stem Cell approach.
2. Retroviral vector approach.
3. DNA Microinjection approach

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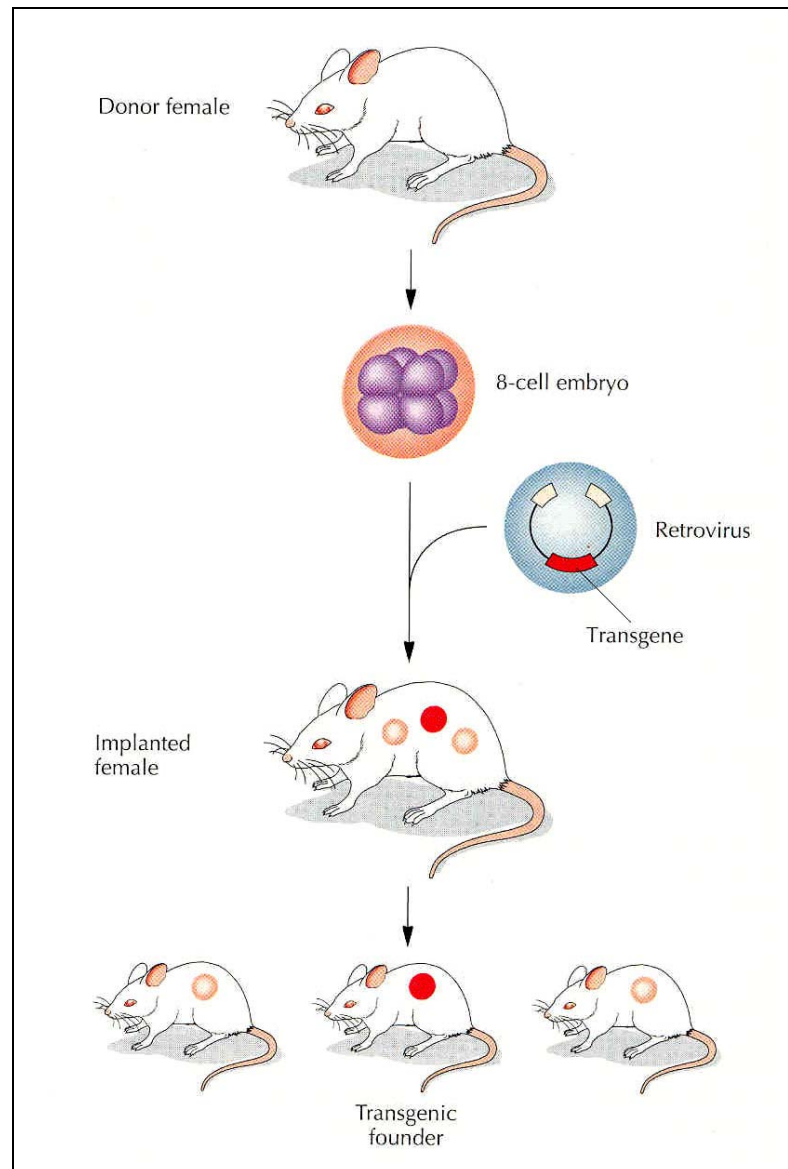
- ES cells from a donor blastocyst are transfected with the transgene (most likely on a plasmid).
- Transfected ES cells are injected into a recipient blastocyst to form a chimera.
- The chimeric blastocyst is placed into the uterus of a surrogate mother.



Methods for introduction of the transgene into animals include:

Retroviral vector approach.

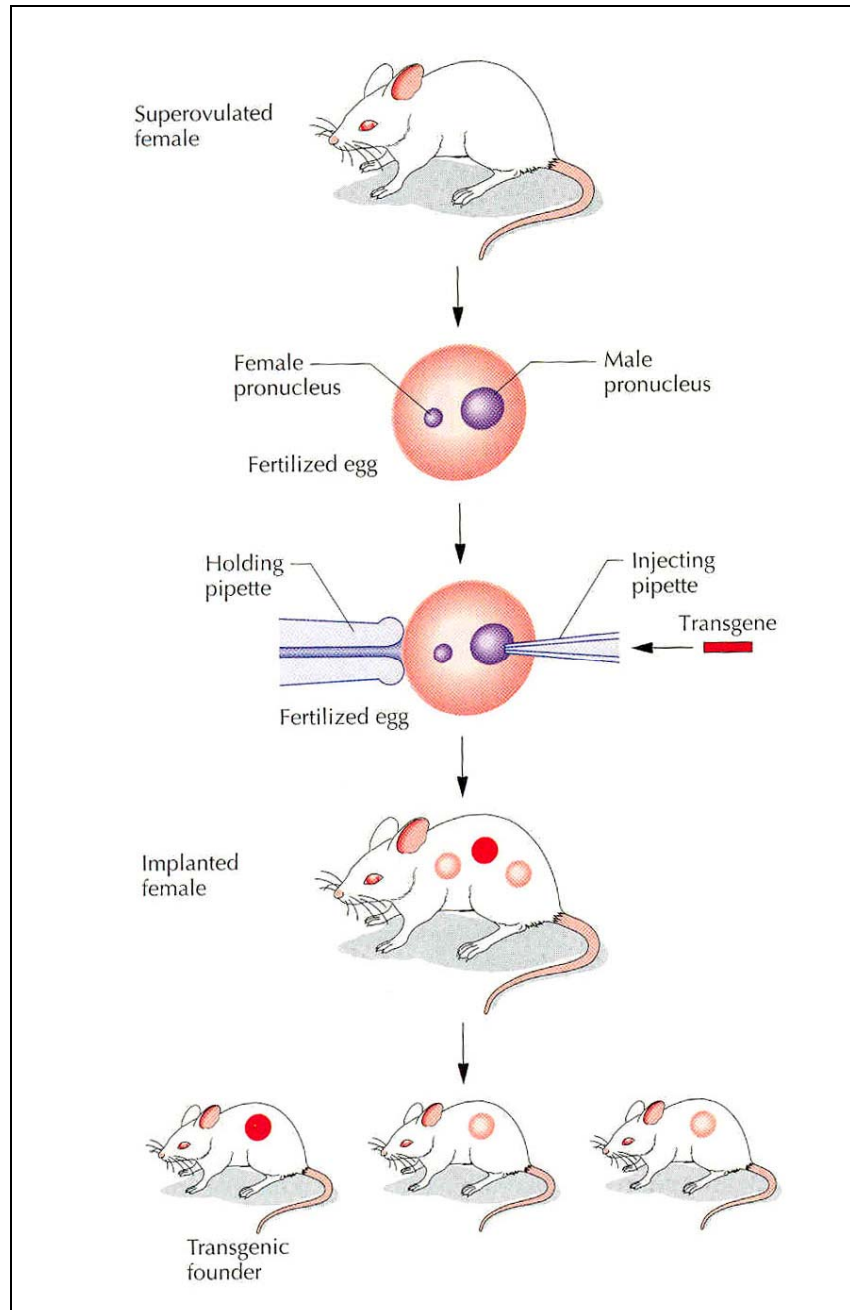
- The transgene is intergrated into the genome of a retrovirus (disabled virus does not cause disease), similar to HIV.
- Embryos are infected with the retrovirus.
- The retrovirus mediates integration of the transgene into the host genome.
- The modified embryo is placed into the uterus of a surrogate mother.



Methods for introduction of the transgene into animals include:

DNA Microinjection approach

- Eggs are fertilized in vitro
- The transgene is microinjected into the male pronucleus
- The transgene integrates into host chromosome
- The zygote grows in culture to the blastocyst stage
- The modified embryo is placed into the uterus of a surrogate mother.



Transgenic Mice as Models for Human Disease

A Mouse Model for Poliomyelitis

Poliovirus infects humans and other primates. Poliovirus is tropic for the nervous system. Replication of poliovirus in the nervous system causes a neurodegenerative disease called poliomyelitis. This disease is characterized by destruction of motor neurons and muscle paralysis.

Mice are not susceptible to polio infection because they lack the cell surface protein (polio receptor) to which polio binds.

In order to create a mouse model for poliomyelitis, researchers created a transgenic mouse that expresses the human polio receptor (done by DNA microinjection approach).

Draw from the board

Pharming

The use of farm animals as bioreactors that produce pharmaceuticals or organs for xenotransplant.

Example: Human Clotting Factor VIII

- Formation of blood clots requires factor VIII
- Type A hemophilia is caused by a genetic defect in factor VIII. These individuals are at risk for bleeding to death because of their inability to efficiently form blood clots
- Treatment is injection of purified factor VIII
- Sources of factor VIII include
 - Human blood (expensive)
 - Recombinant factor VIII made in mammalian cell culture (very expensive)
 - From transgenic animals that secrete human factor VIII into their milk (potentially cheap)
- Procedure:
 - The transgene DNA construct includes the human clotting factor VIII gene fused to a mammary-specific promoter that is only expressed in mammary tissue.
 - Microinject the transgene DNA construct into pig zygotes.
 - Place transgenic embryos in the uterus of a surrogate mother pig
 - Grow and breed transgenic pigs
 - Milk the pig (100-300 liters per year)
 - Purify factor VIII from the milk.

Pharming

Xenotransplants

- Xeno means “stranger”. Xenotransplants involve the transplantation of animal (non-human) organs into humans.
- The rationale for xenotransplants is that there is a serious shortage of transplantable human organs. (20,000 transplants occur each year, 50,000 people need transplants each year, 4000 people die waiting each year.) Pig organs could make up the difference.

Why is the pig the donor of choice?

- Organs are the right size
- Cheap to raise.
- They have large litters.
- Quick to mature.
- Carry few diseases that affect humans (compared to primates)

Problem: Xenotransplants are aggressively rejected by the immune system?

Solution: Make pig organs less “piggish” by removing cell surface markers that are seen by the human immune system as foreign.

Example: A company called PPL (they made Dolly the sheep) has created a knockout pig that lacks the enzyme 1,3 galactosyl transferase. This enzyme adds sugar groups to the surface proteins of pig cells. This sugar group (α 1,3 galactose) is highly antigenic and induces hyperacute rejection of pig tissue from a human recipient.

Draw from the board

Gene Therapy

Gene therapy refers to the insertion of a gene into a human in order to cure disease.

Diseases that are candidates for successful gene therapy include:

1. Recessive, monogenic, genetic disorders.
2. Cancer
3. Heart disease
4. Infectious disease

The transgene must be delivered to the cells of the body via some vector.

Gene Delivery Systems include:

1. Viral Vectors
 - Retrovirus (integrates its DNA directly into the host chromosome)
 - Adenovirus
 - Herpes Virus
2. Naked DNA (plasmid or linear DNA)

Example of Gene Therapy: ADA deficiency and SCIDS

Adenosine Deaminase (ADA) is an enzyme in the nucleotide degradation pathway. Functional ADA converts adenosine to inosine, which is then further degraded by other enzymes. In the absence of functional ADA, adenosine enters a default pathway and is converted to dATP. The accumulation of dATP inhibits the function of many enzymes involved in the DNA Repair System. Consequently DNA damage accumulates, which then stimulates T lymphocytes to die via apoptosis (programmed cell death). The death of T lymphocytes causes Severe Combined Immunodeficiency Syndrome (SCIDS). These individual are extremely susceptible to microbial infections and generally die in the 1st year of life. SCIDS is a recessive genetic disorder.

Treatment includes:

1. Bone marrow transplant from a matched donor
2. Exogenous injections of ADA
3. Gene Therapy to restore functional ADA to T lymphocytes

Gene therapy procedure should be drawn from the board.