Animal Biotechnology

Transgenics are genetically modified organisms with DNA from another source inserted into their genome

A large number of transgenic animals have been created

Mice Cows Pigs Sheep Goats Fish Frogs Insects

Currently, no transgenic animal or animal product is approved by the FDA or USDA for human consumption

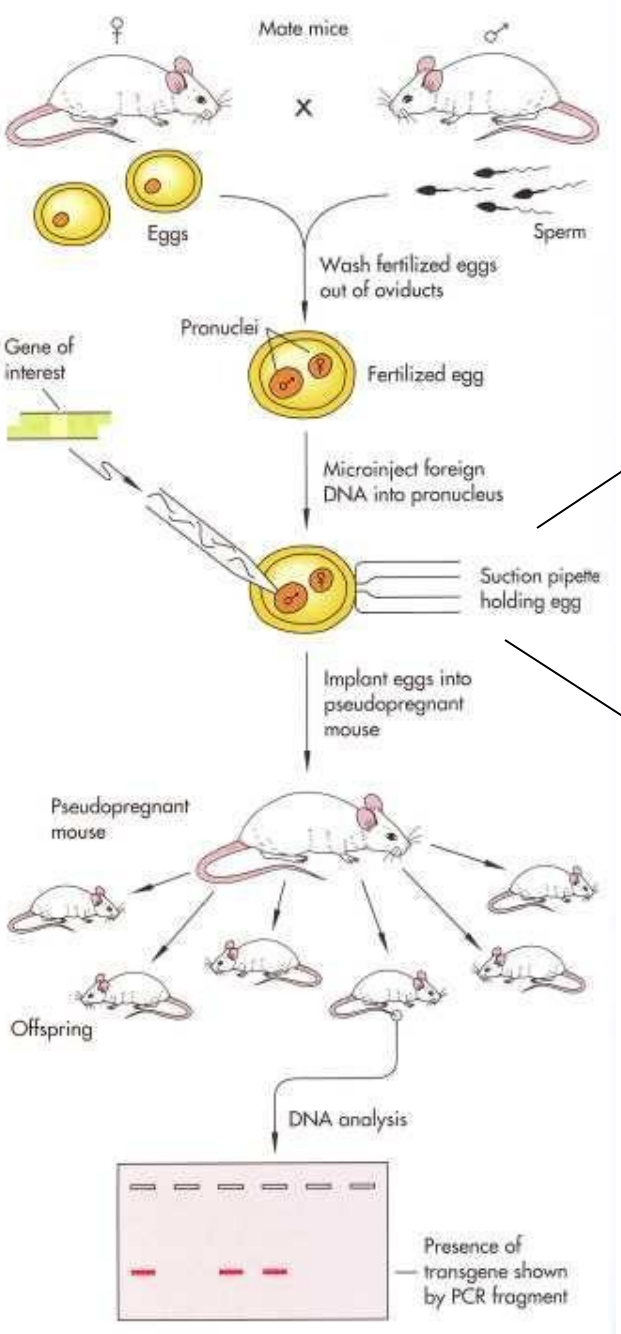
Some of the goals of transgenic animal creation are:

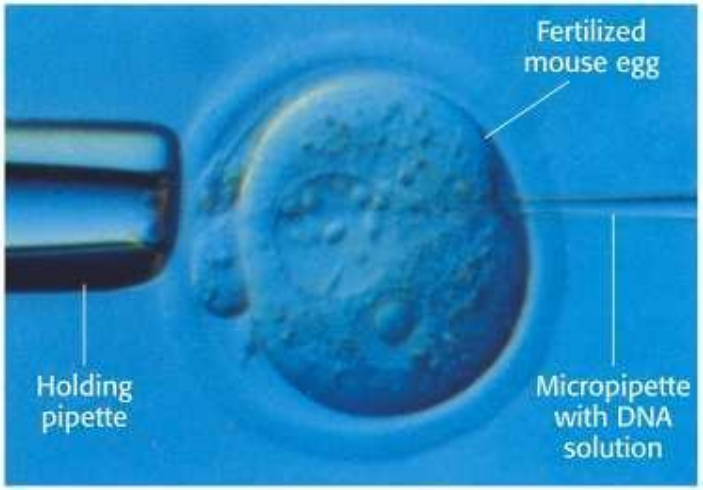
- Research into animal and human disease
- Improve livestock animals
- Use of animals as bioreactors

Transgenic Animal Creation

Microinjection

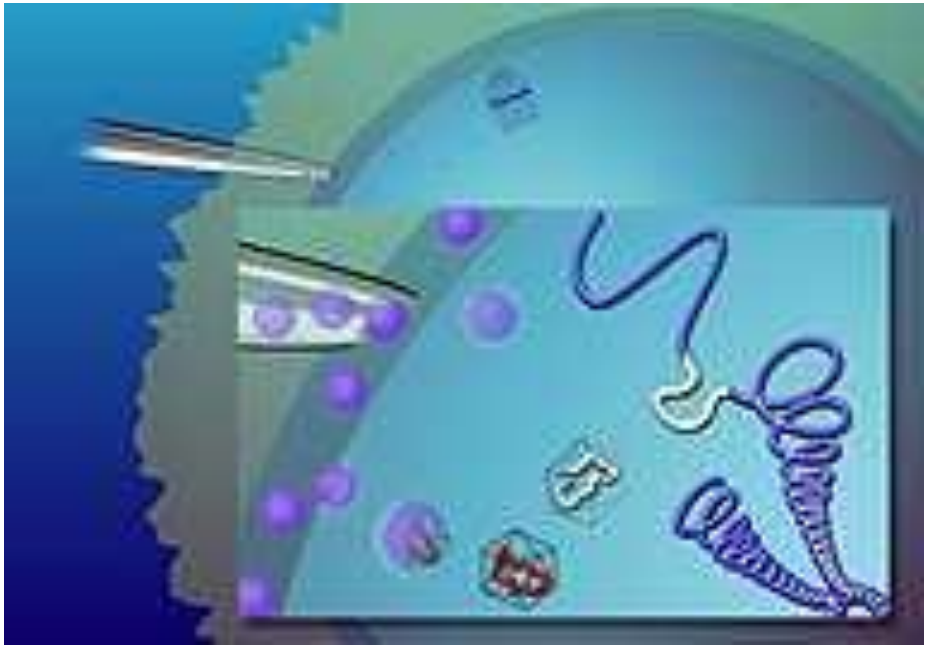
into the germ line -> transgenic animal





Gene injected into the male pronuclei

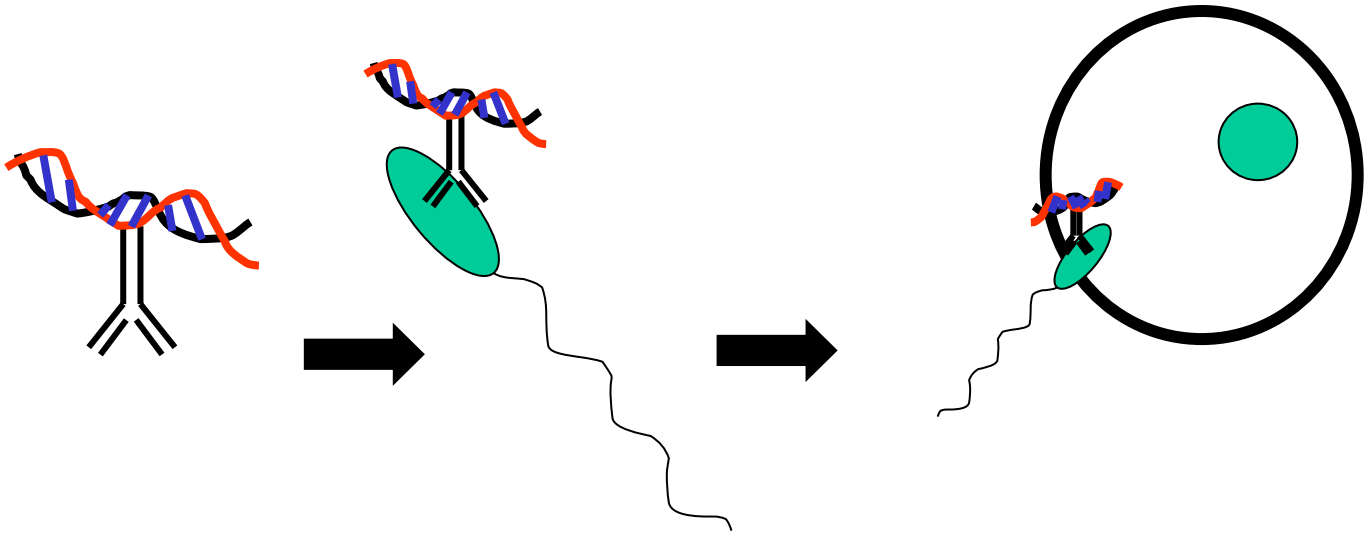
Recombinant Defective Retrovirus



Eggs are infected prior to fertilization

Virus integrates into one of the chromosomes

Linker Based Sperm-Mediated Gene Transfer (LB-SMGT)



Sperm fertilizes the egg carrying the foreign gene into the egg where it is incorporated into the genome

Transgenic Animal Generation

Some of the drawbacks of these methods are:

- The inserted DNA randomly integrates into the genome
- The eggs must be harvested & fertilized *in vitro*
- More than one copy of the gene may get into the genome

Examples of Transgenic Animals

Transgenic Cattle

Dairy cows carrying extra copies of two types of casein genes produce 13% more milk protein

Not only will this make the milk more nutritious, it would allow for less milk to make more cheese

Currently the milk from these animals is under FDA review

The important difference between this & other transgenics is that the DNA added is not foreign

EnviroPig ™

Transgenic pigs express phytase in their salivary glands

Phytic acid in the pig meal is degraded releasing phosphorus

The phosphorus is absorbed by the pig

Normally the phytic acid/phosphorus complex passes through the pig and is excreted as waste

Pig waste is a major pollutant & can cause eutrophication of lakes & streams

http://www.nature.com/news/2005/050221/images/nbt0305-283-I1.jpg

Transgenic Fish

Tilapia

Salmon/trout

Catfish

Can grow up to 6 times faster than wildtype fish

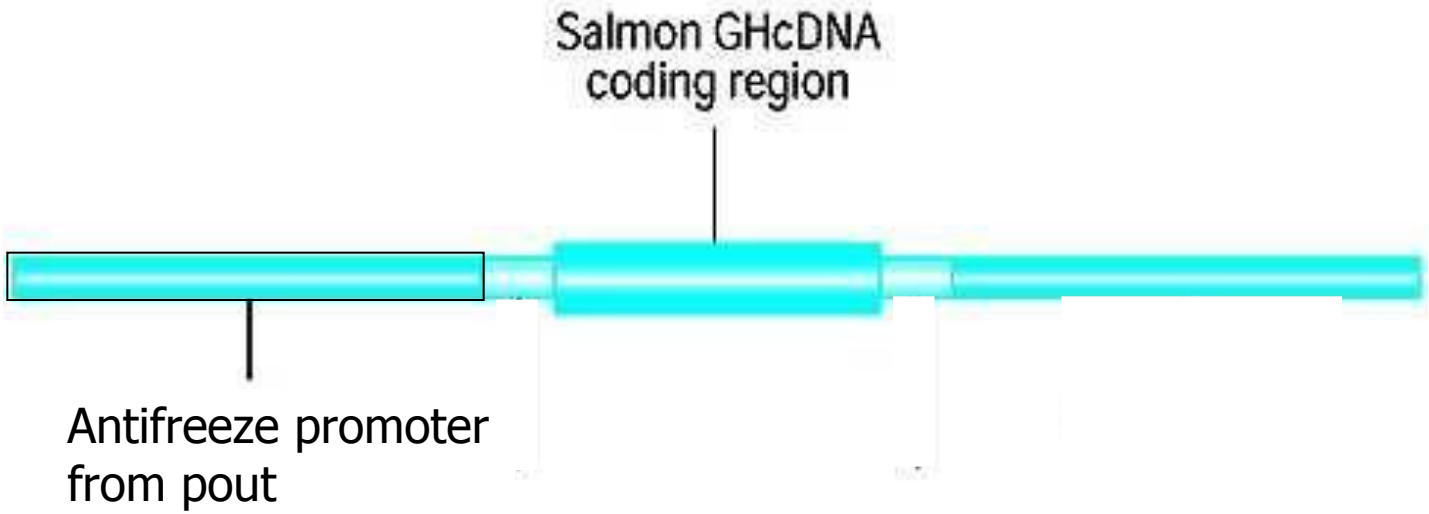
Most have extra copies of growth hormone (GH) gene

Transgenic

Wildtype

http://www.nature.com/nbt/journal/v19/n6/images/nbt0601_500a_I1.jpg

The transgene used to increase growth utilizes an antifreeze protein promoter connected to the GH cDNA



As water temperature drops the GH gene is turned on

The fish continue to grow when normally they would not

http://pubs.acs.org/hotartcl/chemtech/99/jun/fletcher.html

Concerns if these 'supersized' transgenic fish got loose

Transgenic fish are farm-raised, isolated from wild stocks

But even during farming of wildtype fish, escapes happen frequently (~14 million/yr)

What would happen if a large number of transgenic escaped & started breeding with wild fish?

http://pewagbiotech.org/buzz/index.php3?IssueID=10

In experiments, transgenic males mated 3x more frequently than the smaller wild males

Offspring of transgenic males lived <70% as long as wild males

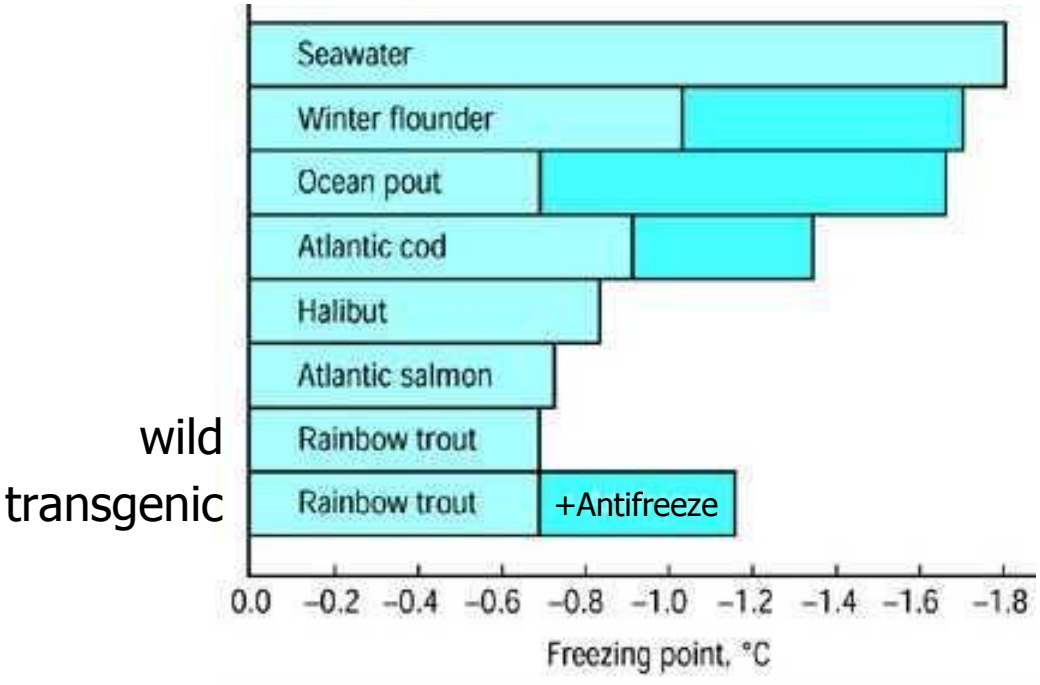
Could lead to a decline of the wild fish population & endanger a species as whole

Antifreeze Proteins (AFP)

AFPs lower the freezing temperature of blood & fluids

Trout normally do not survive in water below –0.6°C

Transgenic trout containing an AFP gene & promoter can survive in waters as cold as –1.2°C



http://pubs.acs.org/hotartcl/chemtech/99/jun/fletcher.html

Animal Bioreactors

“Pharming”

1997, Tracy the sheep, the first transgenic animal to produce a recombinant protein drug in her milk

alpha-1-antitrypsin (AAT) treatment for emphysema & cystic fibrosis

Created by PPL Therapeutics & The Roslin Institute

http://nolswf.bbc.net.uk/science/genes/gene_safari/pharm/a_pharming.shtml

Nexia Biotechnologies transfered the silk gene from Orb spiders into goats

The resulting male goats were used to sire silk-producing female goats

Each goat produces several grams of silk protein in her milk

The silk is extracted, dried to a white powder, and spun into fibers

The fibers are stronger and more flexible than steel

Transgenic male kids carrying silk gene

GTC Biotherapeutics has received approval to sell human anti-thrombin (ATryn) purified from goat's milk in Europe

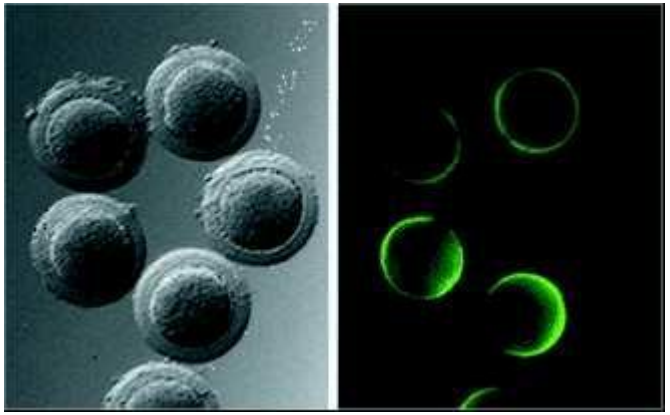
Technology is not restricted to cows, goats, & sheep

There is interest in using rabbits since housing costs are significantly less & generation time is faster

Chickens which produce recombinant drugs in their eggs have been produced by The Roslin Institute

Other Types of Transgenic Animals

Transgene -> Gene coding for a growth hormone



ANDi, the first transgenic primate born in January, 2000

224 unfertilized rhesus eggs were infected with a GFP virus

~Half of the fertilized eggs grew and divided

40 were implanted into twenty surrogate mothers

five males were born,two were stillborn

ANDi was the only live monkey carrying the GFP gene

http://www.ohsu.edu/unparchive/2001/011001andi.shtml

Alba, the EGFP (enhanced GFP) bunny

Created in 2000 as a transgenic artwork

http://www.ekac.org/gfpbunny.html#gfpbunnyanchor

Transgenic Pigs Pass on the Transgene

http://news.aol.com/story/_a/glowing-pig-passes-genes-to-piglets/20080109143909990001?ncid=NWS00010000000001

GloFish, originally developed in Singapore as a way to monitor water pollution

The normally black-and-silver zebrafish was turned green or red by inserting various versions of the GFP gene

Glofish are on sale throughout the US except in California

Glofish retail for about $5 per fish. Normal zebrafish cost around one tenth of the price

http://www.nus.edu.sg/corporate/research/gallery/research12.htm

Mouse “Knock-out” Technology

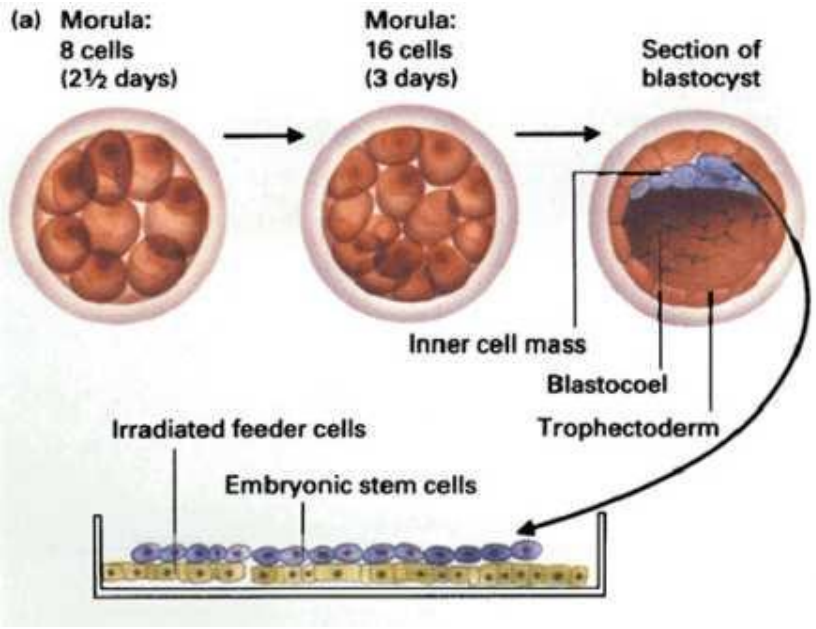
Gene Targeting

Knock-out technology allows for the specific loss of a gene in mice

Allows for the function of the KO'd gene to be deduced from the defects seen in the mice

can be used to mimick some disease

Unlike traditional transgenics the trangene is targeted to a specific site in the DNA of the mouse



Mouse Knock-outs require embryonic stem (ES) cells

These are derived from the inner cell mass (ICM) of a blastocyst (the ICM is what will become the fetus)

ES cells are pluripotent meaning they can become all the different cell types found in an adult

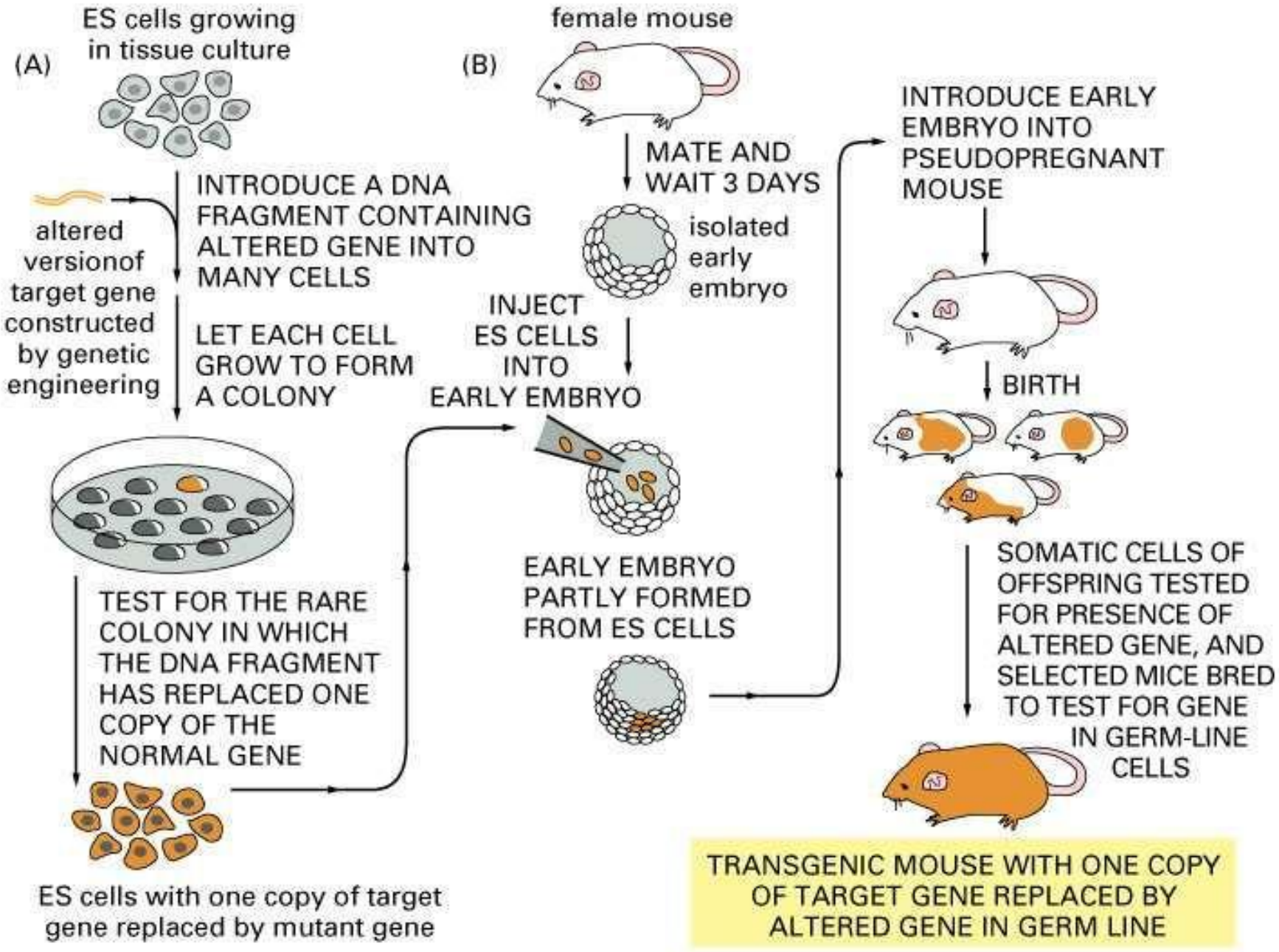
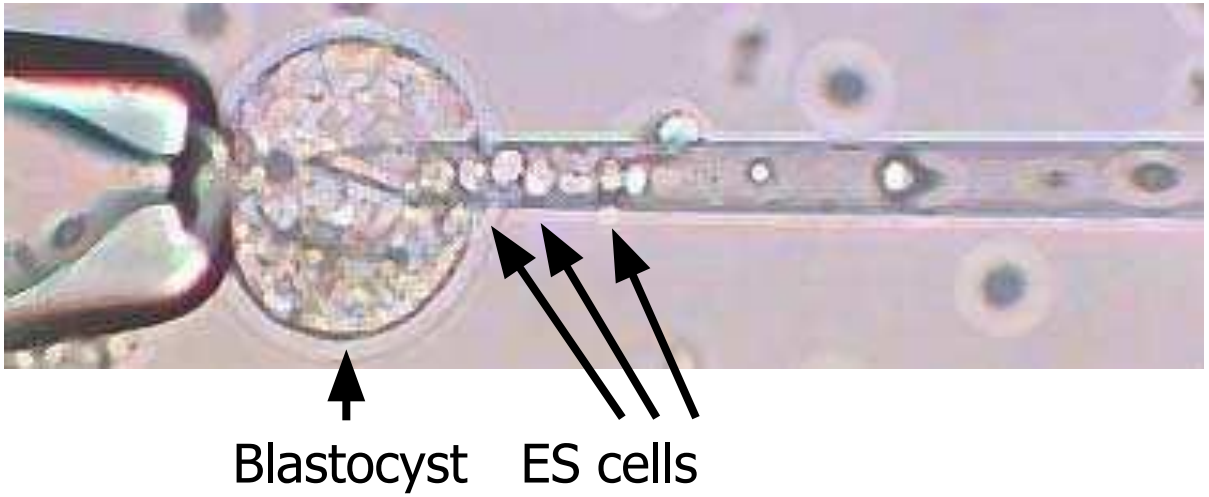


Figure 10-38 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Blastocyst Injection



http://tasq.uq.edu.au/blasto.html

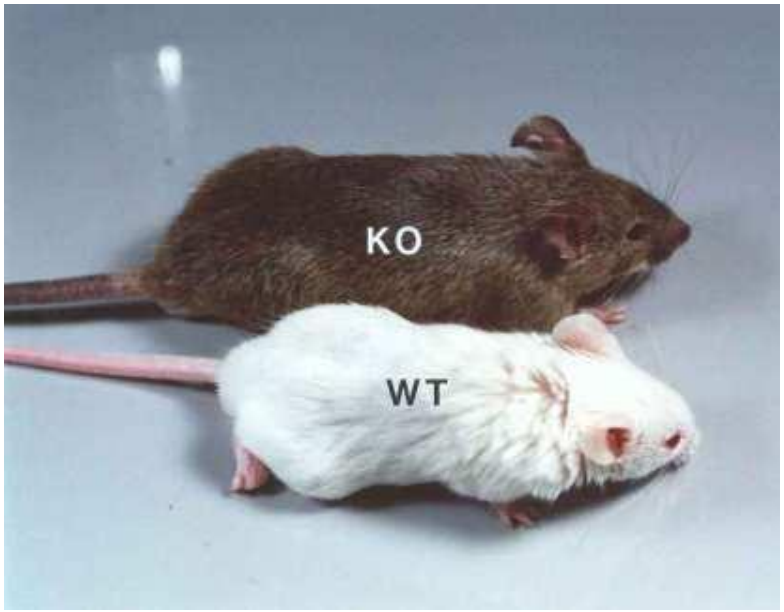
Chimeric mouse

The brown fur comes from ES cells injected into the blastocyst of an albino mouse

http://bunseiserver.pharm.hokudai.ac.jp/gihou/knockout.html

Some Examples of Knockout Mice

p27 knockout mouse

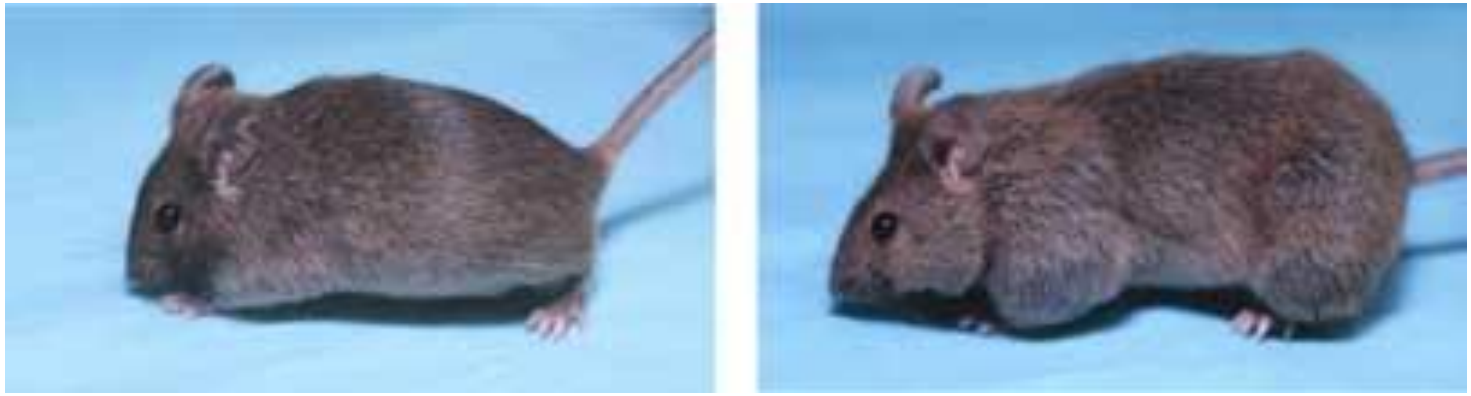


p27 knockout mouse is bigger than the control

This is not due to obesity, but the skeletal structure is increased in size (everything about the mouse is larger)

http://www.bioreg.kyushu-u.ac.jp/saibouE.html

normal knockout



GDF8 (Myostatin) knockout mouse

Over twice the muscle mass of a wildtype mouse

http://www.bbc.co.uk/science/genes/gene_safari/wild_west/bigger_and_better02.shtml

Naturally Occurring GDF8 Mutants

http://www.canada.com/victoriatimescolonist/story.html?id=67f15c17-2717-4022-bb76-1b982456e793&k=94653
http://www.bbc.co.uk/science/genes/gene_safari/wild_west/bigger_and_better02.shtml

FGF5 knockout mouse has long, angora-like hair

http://www.med.uni-jena.de/ivm/deutsch/method/method_7.htm

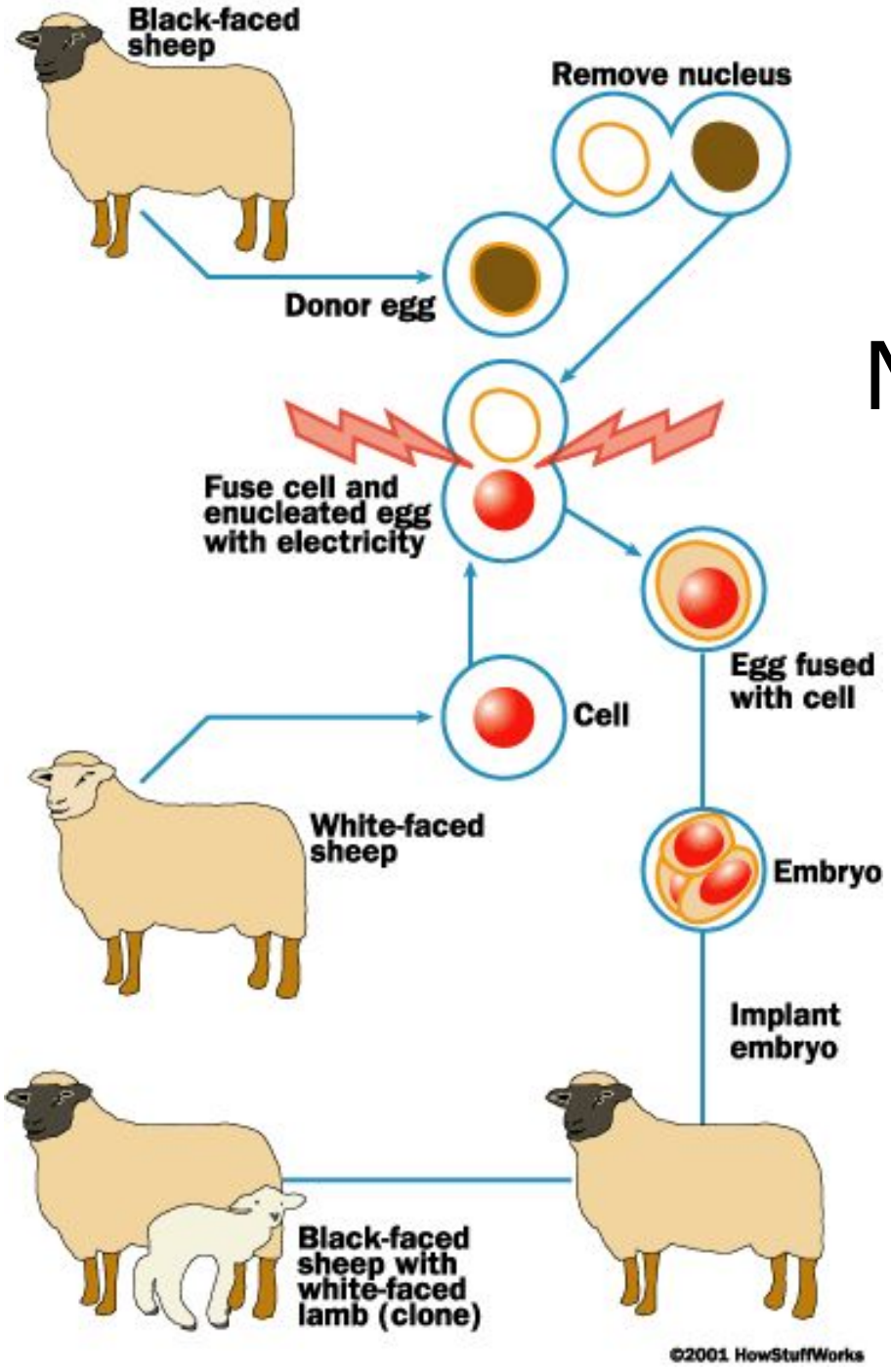
Clones and Cloning

Dolly, First Mammal Cloned From an Adult Cell

Dolly as a lamb with her surrogate mother

Dolly, as an adult

http://www.harlemlive.org/community/health-science/scientificcommunity/index2.html



Somatic Cell Nuclear Transfer

http://www.howstuffworks.com/cloning3.htm

What Has Been Cloned So Far?

Somatic Cell Nuclear Transfer

Sheep, Goat, Mouse, Rabbit, Cattle (domestic & wild), Pig, Horse, Mule, Dog, Cat (domestic & wild), Deer

Embryo Splitting (Twinning)

Sheep, Cattle, Primate (Rhesus)

Cat Clone

Donor Surrogate mother with clone (CC)

Out of 87 implants only CC survived to birth

Donor & Clone

Rainbow & CC

http://www.usatoday.com/news/science/2003-01-21-cloned-cats_x.htm

Transgenic Clones

Cloned transgenic cat containing red fluorescent protein

http://news.aol.com/story/_a/glowing-pig-passes-genes-to-piglets/20080109143909990001?ncid=NWS00010000000001

Idaho Gem, first cloned mule

Surrogate mother (horse)

1st try 134 implants 2 pregnancies, both failed

2nd try 113 implantations 14 pregnancies, one birth

http://news.nationalgeographic.com/news/2003/05/0529_030529_muleclone.html

In addition to cloning pets or prized livestock, researchers are looking to clone transgenic animals

This would allow for more uniform expression of transgenic genes

Not all transgenic animals express their transgenic genes at equal levels

Also allows for the rapid expansion to large flocks or herds of transgenic animals

Piglets clones created by PPL Therapeutics in 2000

The piglets carry a silenced copy of alpha 1,3 galactosyl transferase, or GT, an enzyme involved in organ rejection

In order to guarantee compatibility a second GT gene must also be silenced

http://www.newrivervalley.com/biotech/day1.html

Conservation Cloning

Many endangered or extinct animals are being cloned or considered for cloning

Gaur

Bucardo mountain goat

Mammoth

Quagga

Banteng

Mammoth

Quagga

Bucardo

Gaur

http://www.personal.psu.edu/faculty/t/r/trp2/mammoth.jpeg
http://www.serragaucha.com.br/rocky/zoo.html
http://www.riosmith.net/Gaur004.jpg
http://www1.ceit.es/Asignaturas/Ecologia/EspNaturales/Ordesa/mamiferos.htm#Bucardo

Noah, a Banteng clone created by Advanced Cell Technologies

Banteng are endangered wild bovine from Southeast Asian

This clone was created from frozen tissue of an animal that died in 1980

http://www.advancedcell.com/images/Banteng002-sm.jpg

Problems with Cloning

3 Pig clones, born in 2002, died of heart attacks due to "adult clone sudden death syndrome" within days of each other by the time they were 6 months old.

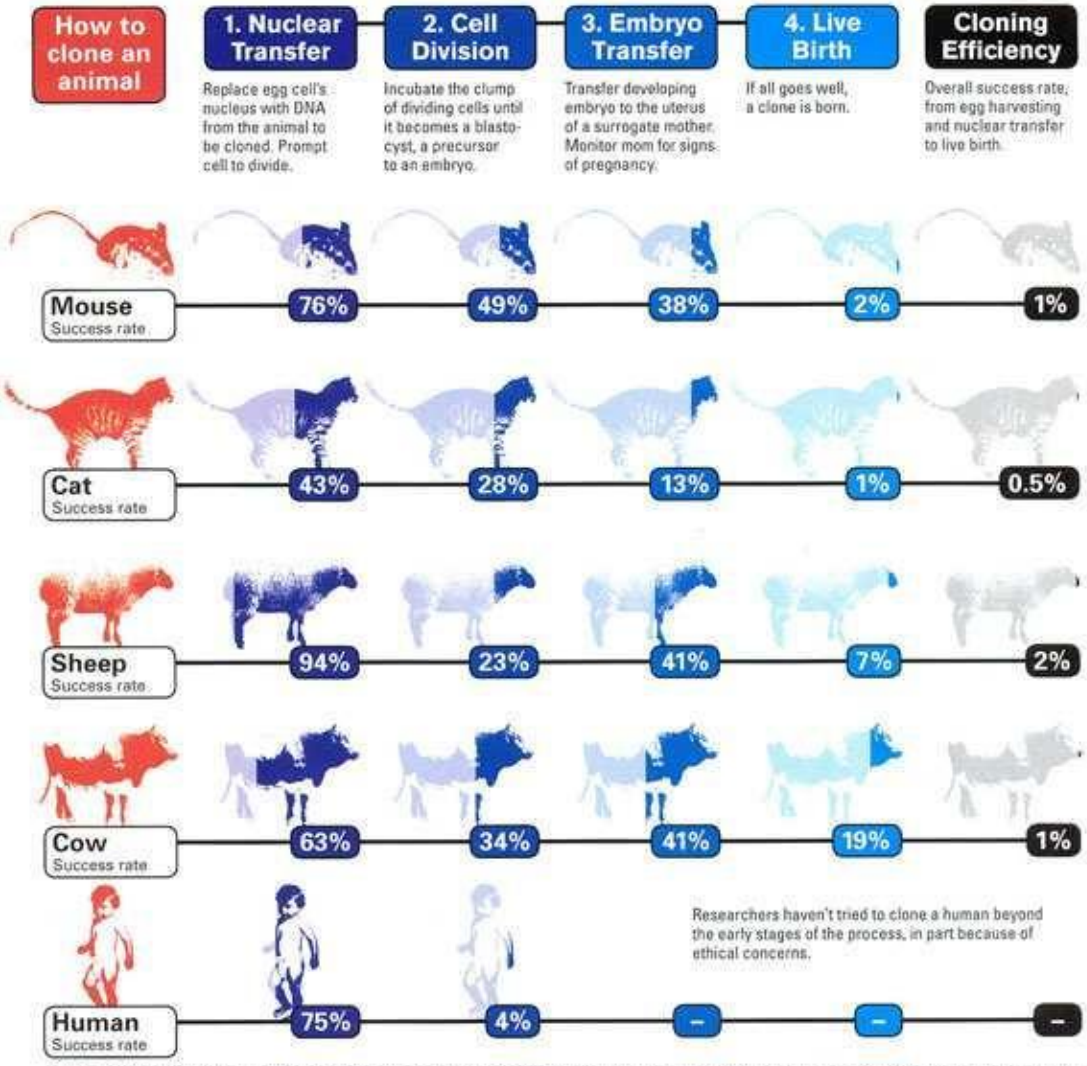
Dolly had a weight problem, telomeres 20% shorter than normal, she suffered from arthritis, and finally lung cancer due to an infection for which she was finally euthanized at age 6yrs.

The success rate ranges from 1 to 3% this contrasts to in vitro fertilization which has a success rate of 50 to 20%

The Killer Task of Cloning

Copying mammals fails 98 percent of the time.

Fears surrounding cloning are based more in hysteria than in science. After all, producing a genetic duplicate isn't exactly a trip to the Xerox machine; cloning is really hard. Researchers face significant drop-offs in success rates at each step of the process, and less than 2 percent of their efforts produce a live animal. Dolly the sheep arrived after some 250 attempts, and she lived only half as long as the average ewe. Until the science improves, there's not much to be afraid of. – **Greta Lorge**



Sources: *Biology of Reproduction; Cloning and Stem Cells; Genetics and Molecular Research; Journal of Reproduction and Development; Journal of Reproduction and Fertility; Lancet; Molecular Reproduction and Development; Nature; Nature Biotechnology; Nature Genetics; Proceedings of the National Academy of Sciences; Reproduction; Reproduction, Fertility and Development; Reproductive BioMedicine; Science; Theriogenology*

MOUSE: C.SURVIVAL DEF/STRELMAN/ANIMALS ANIMALS; CAT: RENEE STOCKDALE/ANIMALS ANIMALS; SHEEP: MIRIAM SILVERSTEIN/ANIMALS ANIMALS; COW: PETER WEIMANN/ANIMALS ANIMALS; HUMAN: ROBYN TWOMEY

http://fig.cox.miami.edu/~cmallery/150/gene/cloning.success.jpg

Nearly all clones show some genetic anomalies

Some suffer from placental defects others cardiac defects

Many suffer from large offspring syndrome (LOS)

Normal mouse pup Cloned mouse pup suffering from LOS