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TRANSGENIC ANIMALS

An Inter Qualifying Project

Submitted to the Faculty of the

WORCESTER POLYTECHNIC INSTITUTE

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by

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ABSTRACT

Transgenic animals have come to be a potential answer to some of the problems that plaque the human race. Given this new technology, one can, in theory, cater this artificial being to produce new pharmaceuticals, serve as Disease models to research cures, or serve as alternative food sources. But with this step of forced evolution comes the rise of ethical and legal battles. And only with these questions answered should transgenic animals proceed.

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EXECUTIVE SUMMARY

Our project investiates the area of transgenic animals. A transgenic animal is an animal that has a foreign gene deliberately inserted into its genome, and is able to pass that gene to future generations through its gametes. In order for a transgenic animal to be produced it must undergo a long, extensive and difficult process. This is because all cells must have the same piece of foreign DNA incorporated into each of its cells. There are two main ways in which this can be done. One such way is through a process called microinjection into an enucleated egg, and another is through a process that uses embryonic stem cells. Due to the rapid progression of this technology, there have been many transgenic creations. Scientists are finding new uses for transgenic animals, some being a production source of medicine in their milk (transpharmers), others serve as Disease models, and even food sources.

However in spite of their medical importance, many people disagree with the creation of transgenic animals. For our purposes we used the definition of ethical issues as "...suggests a set of standards by which a particular group or community decides to regulate it's behavior – to distinguish what is legitimate or acceptable in pursuit of their aims from what is not. Hence we talk of 'business ethics' or 'medical ethics'"(Straughan, 2000). There are many reasons for investigating transgenic ethics. One of the largest arguments in favor of transgenic animals is their use in medical research. Mostly to provide critical models for disease, and with those models help develop treatments and cures for those diseases. These reasons are intended to help not only mankind but the

animal kingdom as well, when genes are inserted for providing disease resistance for that species.

We also touched upon the controversial topic of patenting life. A patent protects the exclusive right to an invention and the rights to make, use, and sell that invention for a set period of time. In addition a patent is a grant issued by the government, and used by the inventor of said property or concept, in order to recoup expenses generated in the research and development phases. The inventor also controls the invention and its exchange with society. A patent is not the right to use an invention, rather it is the right to prohibit the use of an invention by others for unauthorized gain (Houdebine, 1997). Regarding biotechnology and patent rights, there is a gray area in which the American court system has made its initial judgments. The United States Supreme Court ruled, in a case concerning genetically engineered microorganisms, that one can patent "anything under the sun made by man" (Woessner, 1999), however many current transgenic legal cases are still under appeal.

PROJECT OBJECTIVE/PURPOSE

The purpose of this inter qualifying project was to describe the area of transgenic animals so someone without a technical background could understand this new technology. In order to do this we researched how transgenic animals are made, and the types of transgenic animals that have been made to date. In order to analyze the impact of this novel technology on society, the ethical and legal issues that arise from this technology were also investigated. After different sides of transgenic arguments were presented, we gave our own opinion about transgenics and the use of transgenic animals.

CHAPTER 1: TRANSGENIC ANIMALS

The term transgenic animal refers to an animal in which there has been a deliberate modification of its genome, by inserting a gene not normally present in that species.

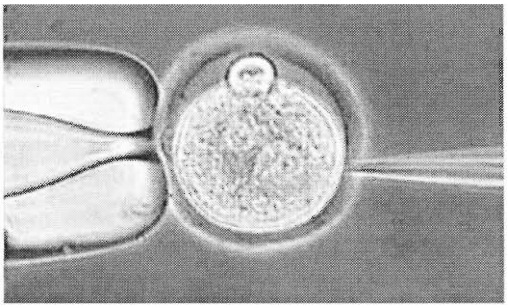


Figure 1 illustrates the microinjection of DNA into a pronucleus.

Figure is from http://www.brinkmann.com/brink2001/ECET_appl5.htm

A transgenic animal is made up of billions of cells. It is an animal that has a foreign gene deliberately inserted into its genome, and is able to pass that gene on to future generations through its gametes. In order for a transgenic animal to be produced it must undergo a long, extensive and difficult process. This is because all cells must have the same piece of DNA incorporated into each of its cells. There are two main ways in which this can be done. One such way is through a process called microinjection and another such way is through a process called embryonic stem cells. Scientists have discovered that every animal begins as a one cell organism. This cell divides over and over again until it finally produces the billions of cells that make up an animal, which we then call a transgenic animal. In order to ensure that every cell contains the same DNA, scientists add the

foreign DNA to the one-celled entity before it starts dividing. This new DNA will then become incorporated into the animal's genome. In theory these cells will become present in all the animal's cells if there is no complication. Although as we know in life everything has some type of complication as we will find out.

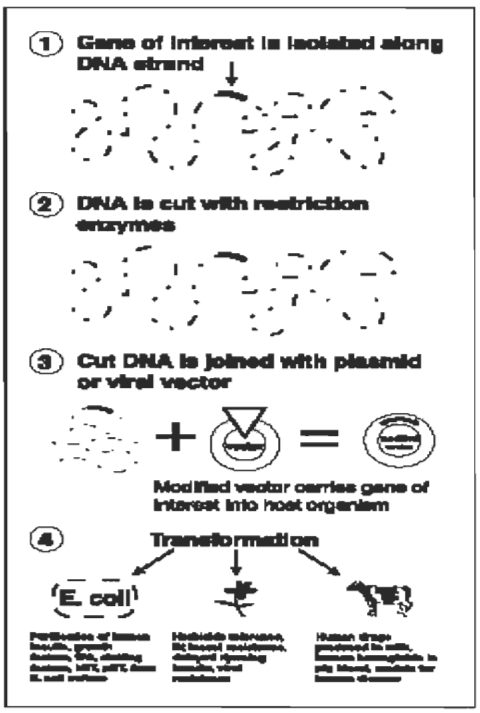


Figure 1.

Figure 2 illustrates how a transgenic animal is created starting from the DNA.

Figure is from http://www.biotech.iastate.edu/biotech_info_series/bio10.html

Altering germ cells can generate a transgenic animal. Germ cells (like egg and sperm cells) are the only animal cells capable of giving rise to a new offspring. Somatic cells, like blood, skin, brain or heart cells are not capable of giving rise to whole new animals.

In transgenic animals foreign DNA must become incorporated before the cell starts dividing. If this does not occur a mosaic transgenic animal will be produced. A mosaic animal is a transgenic animal that only contains the new gene in some, but not all of its cells. In the following picture we can see how a transgenic animal is supposed to be produced, but instead a mosaic animal is produced.

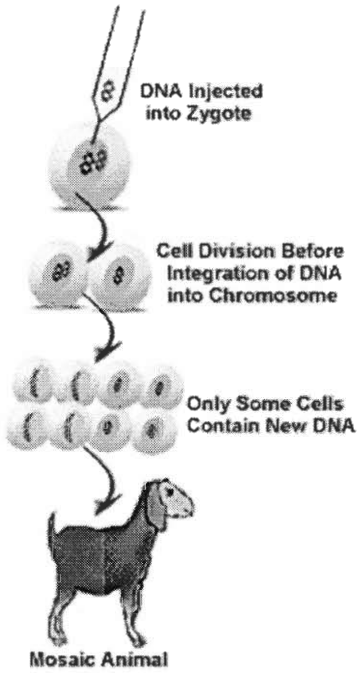


Figure 3 Production of a Mosaic Transgenic Animal.

Figure is from www.ultranet.com

This happens because once the DNA is incorporated into the nucleus there is no way to predict where or when it will be incorporated into the host genome. Scientists have found that many regions of a given chromosome are inaccessible to the enzymes responsible for

initiating gene expression. If the transgene gets incorporated into one of these inaccessible regions, the trans protein will not be produced, or may be only produced in certain cell types.

Scientists must thoroughly test these animals for whether all the cells contain the transgene, and whether the desired cell types are producing the gene's protein product.

There are four main approaches to generate a transgenic animal:

1. Microprojectile cell bombardment technique. This technique uses small gold beads coated with DNA to blast a hole in the cell membrane.
    - A major advantage is its applicability to a wide variety of species.
2. Retroviral insertion. This technique uses viruses to integrate the transgene into the genome.
    - The advantage is that it allows precise targeting of defined mutations in the gene via homologous recombination.
3. Sperm mediated gene transfer. This technique uses sperm egg fusion to deliver the transgene to the new embryo.
4. Embryonic Stem Cells. This technique uses cells capable of differentiating into a variety of all types to deliver the transgene.

All these methods allow the efficient introduction of foreign DNA sequences into the germ line. Although like many experiments the success rate in terms of live birth of animals containing the transgene is extremely low. Two of these methods will be discussed in this chapter.

1.1 Microinjection into the Pronucleus

The first successful production of transgenic mice using pronuclear microinjection was reported in the 1980's. Although the recombinant viral construct was proven to have been integrated into the mouse genome, it was rearranged and did not express itself. The first visible phenotypic change in transgenic mice was described in 1982 for animals expressing the rat growth hormone sequence.

Microinjection is the process in which DNA is introduced into a fertilized egg (*called a zygote*), before it starts dividing. This DNA is then incorporated into a chromosome in the nucleus. This process is often used to produce a large number of eggs at the same time through the use of hormones before mating.

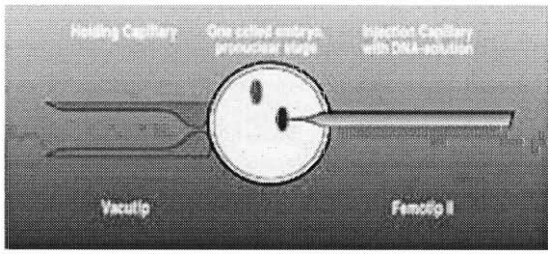


Fig. 4 Transgenic animals created by DNA-Injection into the cell pronucleus.

Figure is from http://www.brinkmann.com/brink2001/ECET_appl5.htm

Though microinjection is the most effective way to create a transgenic animal, there is a very low rate of efficiency for gene integration. Below we can see some of the percentages of some of the transgenic animals that have been able to be produced using the microinjection process.

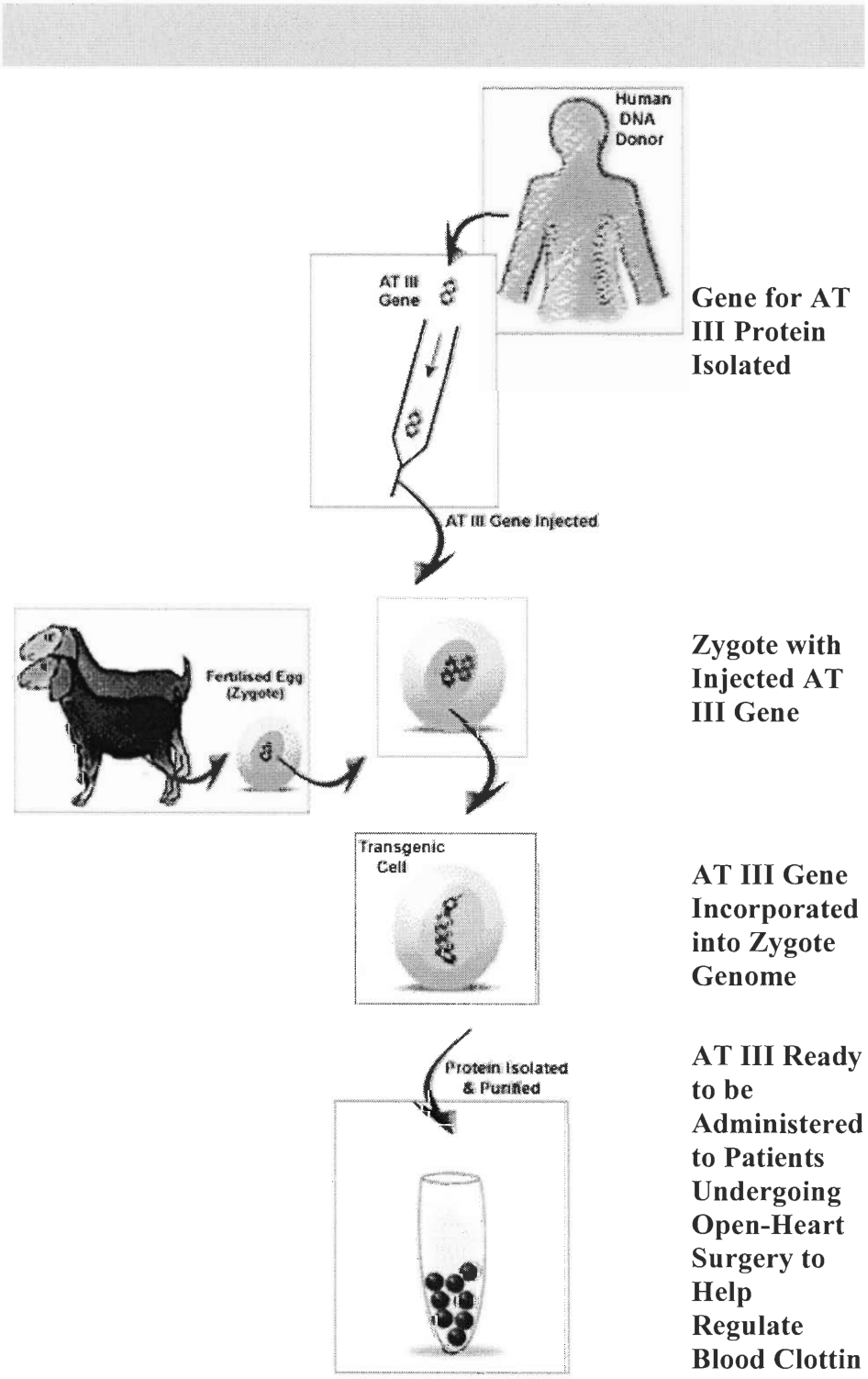
Sheep 0.1% to 4.4%
Pigs 0.3% to 4.0%
Cattle 0.7% to 3.2%

There are several reasons why transgenic animals are created. They are used to help in the study of normal physiology and development, to study diseases and to help in the development of treatments, to produce useful biological products, to test the safety of vaccines and chemicals, to provide organs that could be used for transplantation, and to increase the quantity and the quality of products from farm animals. For example transgenic sheep have been developed to produce factor VIII proteins in milk, which can then be harvested and used to treat hemophiliacs, who lack this important blood-clotting factor. Transgenic pigs have been generated in order for them to have superior growth rates and meat quality. Some of the other proteins that have been able to be expressed in transgenic animals include: **anti-thrombin III**, which treats intravascular coagulation; **collagen**, which treats burns and bone fractures; **fibrinogen**, which is used for burns and after surgery; **human fertility hormones, human hemoglobin, human serum albumin**, used for surgery, trauma, and burns; **lactoferrin** an antimicrobial compound which is found in mother milk; **tissue plasminogen activator** used for treating heart attacks, and tumor specific **monoclonal antibodies** which are particularly effective against colon cancer.

Ever since the 1970's it has been possible to introduce foreign DNA fragments into prokaryotic and eukaryotic cells in vitro and to induce the expression of the foreign DNA in these cells. There are several ways this DNA can be introduced into a cell, including cell shock, precipitation, electrical poration of the membranes, viral carriers, and direct microinjection. Approximately one out of several thousand treated cells usually takes up and expresses the foreign DNA. One of the problems scientists face is that in order to produce transgenic offspring, large numbers of one-cell embryos are needed at the pronuclear stage, for gene injection. Such zygotes can be obtained in vivo following super ovulation and insemination. However this procedure is expensive and leads to variable yields of embryos depending on the technique used, the individual response of the donor, semen quality and other factors. Spreading of the ovulation and fertilization over several hours, which is common in these animal species, results in eggs at different stages and a low cost of recovery.

There is however a more efficient way to produce large numbers of embryos. This technique is called in vitro fertilization, it is the conception of a human embryo outside the mother's body. This is a more cost effective way to produce the large numbers of embryos needed, and therefore more useful for scientists. In order for this process to be achieved there are two main steps that must be accomplished. These steps are the maturation of ovarian oocytes (IVM), and the capacitating of spermatozoa and fertilization events (IVF). An in vitro oocyte with full developmental capacity must be able to support meiotic maturation and embryonic development. They must also be put in a culture where they can be ensured full oocyte maturation.

This page shows a diagram of how the DNA microinjection process takes place. It is a detailed representation of how scientists go through the step by step process.

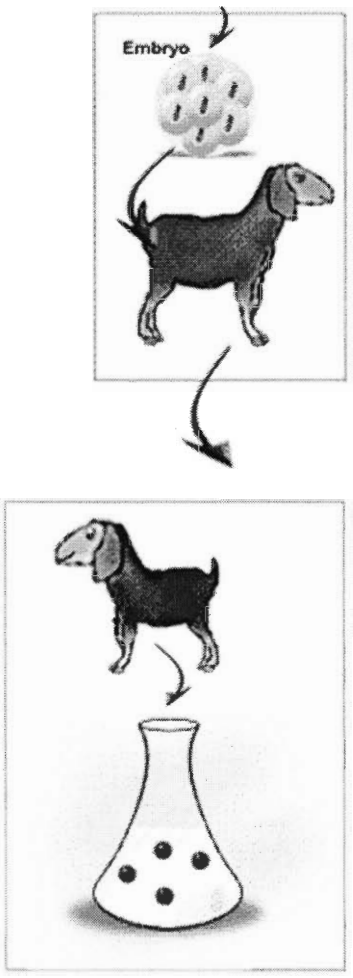


Gene for AT III Protein Isolated

Zygote with Injected AT III Gene

AT III Gene Incorporated into Zygote Genome

AT III Ready to be Administered to Patients Undergoing Open-Heart Surgery to Help Regulate Blood Clottin



The process shown above shows us how a transgenic animal is able to produce the human therapeutic protein AT-III in milk. In order to produce the therapeutic milk that humans will use for various diseases, a transgenic animal must carry the correct genetic information inserted by a scientist. Once the milk is produced, then these recombinant proteins can be purified to use in humans.

1.2 Embryonic Stem Cells

The second main method for preparing a transgenic animal uses embryonic stem cells. Embryonic stem (ES) cells are harvested from the inner cell mass of the animal's blastocysts see figure 6. These ES cells are pluripotent, meaning they can develop into any type of tissue. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, including its gametes.

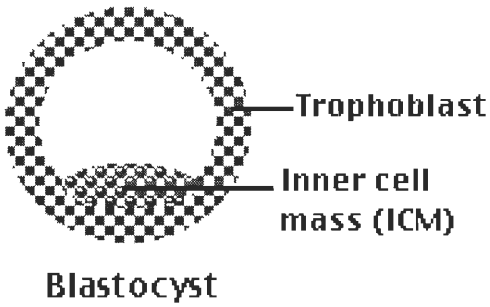


Figure 6 Diagram of a Mammalian Blastocyst as source of ES Cells.

Figure is from http://www.ultranet.com/~jkimball/BiologyPages/T/TransgenicAnimals.html

There are two main ways in which ES cells can be introduced into an animal:

1. Injection of ES cells into the mouse blastocyst or moraula by micromanipulations.
2. Aggregation of a clump of ES cells with an eight cell stage embryo.

ES cells properties make them a splendid cell vector for the modification of the mouse genome versus the complicated and inefficient technique of DNA injection into pronuclei. This technology takes advantage of four unique characteristics of the ES cell system:

1. ES cell lines are derived from a single mouse embryo.
2. ES cells can be maintained permanently in culture.
3. ES cells are pluripotent cells (capable of producing descendants representing all of the hundreds of differentiated cell types in the newborn born baby).
4. ES cells are germline compatible if they are introduced back into chimeras.

Figure 7 summarizes the production of a transgenic animal using ES technology.

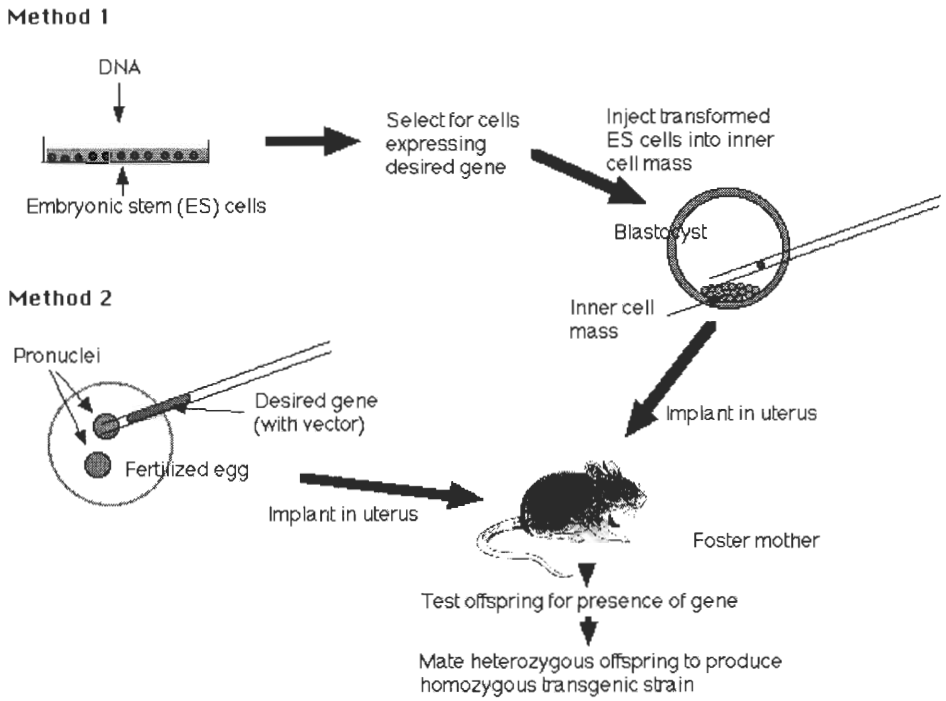


Figure 7 Creation of a Transgenic animal using the Embryonic Stem Cell Method.

Figure is from http://www.ultranet.com/~jkimball/BiologyPages/T/TransgenicAnimals.html

The real advantage of ES cell-mediated transgenesis is apparent when the identification of special, low frequency gene integration is required. Some of the uses of ES cells are: the analysis of the characteristics of totipotent cells, cell cycle studies, detection of unknown genes involved in developmental biology, function of an unknown gene, confirmation of the role of known gene, analysis of gene structure/ function by mutation, gene imprinting, study of the effect of chromosome rearrangements, gene therapy, acquisition of resistance to diseases, creation of models for human thologies, and the production of animals secreting hormones or other useful proteins.

CHAPTER 2: TRANSGENIC EXAMPLES

Again, a transgenic animal is one that carries a foreign gene inserted into its genome. This foreign gene was constructed through the means of recombinant DNA methodology. Recombinant DNA is DNA that has been created artificially. DNA from two or more sources is incorporated into a single recombinant molecule. This DNA also includes other sequences that would enable it to be incorporated into the DNA of the host or to ensure its true nature (expression) by the cells of the host.

Due to the progression of this technology, there have been many outcomes in the field of transgenic creations. New transgenic animals are now being created to serve specific purposes because of transgenics. Scientists are finding new uses for transgenic animals, some being a production source of medicine in their milk (transpharmers), and others being Disease models, and even food sources.

2.1 The First Transgenic Animal: Supermouse

In 1982, an article in Nature was published on the first transgenic animal created. Supermouse was created by the hands of Richard Palmiter and Ralph Brinster. Palmiter, an HHMI investigator at the University of Washington in Seattle, Ralph Brinster of the University of Pennsylvania, and their colleagues injected a modified rat growth-hormone gene into a fertilized mouse egg. Under normal circumstances, the gene would only produce small quantities of growth hormone in the pituitary, but in this case the scientist wanted to see the outcome of higher levels of the hormone. Prior to the injection of the

rat GH gene into the mouse egg, it was attached to a promoter. Promoters are regions of the DNA that control which tissue expresses a gene. These promoters ensure the redirection of the gene's expression to specific cell types, such as liver. Because of this act, the GH gene will now be freed from its normal controls, and would now produce large quantities of the hormone in all cells. Following preparations of the gene and the egg, it was now implanted into a surrogate mother. This mouse, with the implanted egg, gave birth to normal sized mice that eventually grew at an extremely fast rate. These mice became giant mice (fig. 1), nearly two times the size of normal mice.

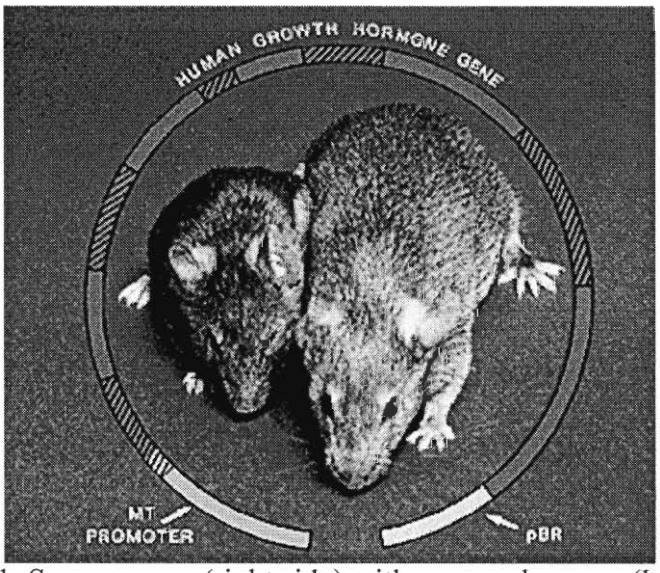


Figure 1. Super mouse (right side) with a normal mouse (left side) (http://www.washington.edu/alumni/columns/dec95/mice.html)

2.2 Transpharmers

There is a new kind of farming in today's world that has emerged. It began from the research and development labs of several universities and small biotechnology companies that have deemed their field as "pharming." Pharming is the production of human needed pharmaceuticals in farm animals. Since the creation of Supermouse in

1982, the new transgenic animals can be used for such purposes as above stated, an example of such is an animal to produce the human drug tPA that is used to treat blood clots.

The creation of transgenic animals can remedy the call for large quantities of needed drugs/medicine. They are the most efficient means of meeting such a demand since there is flexibility in production capacity through the number of animals bred, and the maintenance of its own fuel supply. And of course, this protein drug can be delivered in a most suitable way, in its milk.

2.2.1 Transpharming Mice

Mice are well suited for making transgenic animals, and two companies have picked up the ball. Mice have a gestation time of 19 to 20 days, and mature in one to two months. They also produce a good number of offspring, making it a suitable organism for pharming. But the con for this animal would be its 1-month lactation period yielding low amounts of the recombinant protein. But Abgenix and Medarex chose the mouse to produce humanized antibodies. These two companies have patents in the production of fully human antibodies in transgenic mice. Under these two companies, the production of Fibrinogen Surfactant Protein B (procollagen recombinant antibodies) have been made. Another example of mice as transpharmers is the production of CFTR, cystic fibrosis transmembrane conductance regulator, which is used in the treatment of CF. CF is a disease that affects the pancreas, intestines, liver and reproductive system, as well as the lungs.

Abgenix (located in Fremont, CA) uses its Xenomouse transgenic mice to generate various antibodies for different therapeutic functions, while Medarex (Annandale, NJ) uses its HuMab-Mouse for the same purpose. Medarex has also created the TC Mouse. This was made possible because of a partnership with Kirin Brewery Co., Ltd (Tokyo, Japan) pharmaceutical division. The TC Mouse is a transgenic animal that contains 100% of the human genes required for the production of human antibodies using transchromosomic technology.

2.2.2 Transpharming Goats

Goats have been created for the production of many pharmaceutical drugs. Some of the most important protein therapeutics produced by goats include Antithrombin III, tPA (tissue plasminogen activator), monoclonal antibodies, α1-antitrypsin, and growth hormone. Goats can transpharm an annual amount of 4 kg per female (see Table 1). Second only to cattle, which produce an annual yield of 40-80 kg, goats produce the next largest amount of recombinant protein yearly. Goats are also high in the charts in terms of their dollar value as transpharmers, showing an estimated $75,000 per animal to produce tPA (table 2).

Key features of a selected list of transgenic animals used in therapeutic protein production

Animal	Time of gestation (month)	No.of off-spring	Time to sexual maturity (month)	Lactation period (month)	Annual milk production (L)	Annual yield of recombinant protein per female (kg)	Recombinant proteins expressed	Companies (numbers correspond to footnote)*
Cattle	9	1	16	33	8000–9000	40–80	Lactoferrin, α-lactalbumin	2, 7, 9, 11, 15
Chickens	20 days	250/yr	6			0.25 kg	Monoclonal antibodies, lysozyme, growth hormone, insulin, human serum albumin	4, 8, 18, 19, 20, 21
Goats	5	1–2	8;3–6 months in BELE** goats	18	800–1000; 365 in BELE goats	4	Antithrombin III, tissue plasminogen activator, monoclonal antibodies, α1-antitrypsin, growth hormone	10, 13, 16
Mice	19–21 days	10–12 L/ 3–4 weeks	1–2	1	0.015	750 μg–3 mg	Fibrinogen surfactant Protein B, procollagen recombinant antibodies	1, 12
Pigs	4	10	6	16	300	1.5	Factor VIII, Protein C, hemoglobin	3, 5, 14, 16, 17
Rabbits	1	8	5	7	4–5	0.02	Calcitonin, extracellular superoxide dismutase, erythropoietin, growth hormone, insulin-like growth factor 1, interleukin 2, α-glucosidase, glucagon-like peptide	15,16
Sheep	5	1–2	8	18	500	2.5	α1-antitrypsin, factor VIII, factor IX, fibrinogen, insulin-like growth factor 1	16

* 1. **Abgenix** (Fremont, CA); 2. **Advanced Cell Technology** (Worcester, MA); 3. **Alexion** (New Haven, CT); 4. **Avigenics** (Athens, GA); 5. **Biotransplant** (Charlestown, MA); 6. **Chromos MolecularSystems** (Burnaby, Canada); 7. **Gala Design** (Sauk City, WI); 8. **GeneWorks** (Ann Arbor, MI); 9. **Genetic Savings and Clone** (College Station, TX); 10. **Genzyme Transgenics** (Framingham, MA); 11. **Infigen** (De-Forest, WI); 12. **Medarex** (Annandale, NJ); 13. **Nexia Biotechnologies** (Montreal, Canada); 14. **Nextran** (Princeton, NJ); 15. **Pharming** (Leiden, The Netherlands); 16. **PPLTherapeutics** (Roslin, Scotland); 17. **ProLinia** (Athens, GA); 18. **Origen Therapeutics** (Burlingame, CA); 19. **Sima Biotechnology** (Minneapolis, MN); 20. **TranXenoGen** (Shrewsbury, MA); 21. **Vivalis** (Rouossay, France).

**Breed Early Lactate Early.

Source:Nature Biotechnology, October 2000.

Table 1: Key features of selected transgenic animals.

(http://www.iscpubs.com/pubs/abl/articles/b0102/b0102.das.pdf)

Drug	Animal	Value/Animal/Yr*
AAT	sheep	$15,000
tPA	goat	75,000
Factor VIII	sheep	37,000
Factor IX	sheep	20,000
Hemoglobin	pig	3,000
Lactoferrin	cow	20,000
CFTR	sheep, mouse	75,000
Human Protein C	pig	1,000,000

*Current market price of the drug and supply produced by one animal.

Drug descriptions:

AAT	alpha-1-antitrypsin, inherited deficiency leads to emphysema
tPA	tissue plasminogen activator, treatment for blood clots
Factors VIII, IX	blood clotting factors, treatment for hemophilia
Hemoglobin	blood substitute for human transfusion
Lactoferrin	infant formula additive
CFTR	cystic fibrosis transmembrane conductance regulator, treatment of CF
Human Protein C	anticoagulant, treatment for blood clots

Table 1

Table 2: Current market price and drug produced by selected transgenic animals.

(http://www.biotech.iastate.edu/biotech_info_series/bio10.html#anchor319736)

In the field of transpharmer goats there are three leaders: Genzyme Transgenics, Nexia Biotechnologies, and PPL Therapeutics. Among these three, Genzyme Transgenics, located in Framingham, MA, stakes claim to the highest number of produced therapeutic proteins, which includes some of the most hard to express proteins.

Currently goat's milk is the source of production of more than 60 biopharmaceutical proteins. Some of the transpharmed proteins, such as antithrombin III, are already in Phase III clinical trials. Antithrombin III (produced by Genzyme Transgenics) is a protein used to help prevent and regulate blood clotting for example during coronary artery bypass grafting. Another protein that is only in Phase II of testing

is α1-antitrypsin (AAT). The AAT protein produced by PPL Therapeutics is used in the treatment of cystic fibrosis.

2.2.3 Cows

The worlds first transgenic bull/cow was baby Herman produced in 1990. Herman was created to carry the human gene Lactoferrin, which is used as an antimicrobial agent. Herman has become a father to eight transgenic female calves, increasing the production line for Lactoferrin production in milk.

Lactoferrin is a protein found in mother's breast milk. So, Herman's human gene was a substance that had anti-bacterial, nutritious properties. These properties are useful in medicine for treating those with a weak(ened) immune system.

According to table 2, in the prior section, the transgenic cow producing Lactoferrin has a value of $20,000 for the protein produced in its milk. Bovine Transpharmers are currently used mainly to produce Lactoferrin. Lactoferrin's future use is as an ingredient in human baby milk powder.

2.3 Disease Models

Humans, like any other organism, have health problems. But with health research and development comes the need for a means of testing: Gathering data on a possible marketable drug takes a lot of time, money, and consent on the patients' part. Scientists, not to long ago, had only cells in tissue culture to work on. These studies gave researchers valuable information on the function of specific genes and their roles in

diseases. But as the research went on, many questions were raised compared to those that were answered. Often this was the case because a disease is a function of a whole organism, and not just that of a single cell. So animal models were needed that mimicked aspects of human disease.

Transgenics give a means of understanding, which gene is mutated in a particular human disease, and this can be the source of development of a model with the same mutation. In today's world, transgenic disease models mimicking certain diseases such as cystic fibrosis, cancer, and Alzheimer's give new insights into the genetic basis of these diseases. In the creation of Disease Models a great medical benefit can become apparent, but ethical problems will arise, for sometimes the animal is born to be in pain. Ethical issues are covered in a later chapter.

An example of a Disease Model can be seen in the creation of a mouse (by Palmiter and Brinster) with a faulty liver, then successfully implanting it with healthy mouse liver cells, which in turn cause functioning livers. According to professor Palmiter of University of Washington:

> "The most important thing that comes from these mouse models is that you can see what you could expect if everything worked perfectly, because the genetics are very carefully controlled (in transgenic mice). Knowing it's feasible is worth a lot," he continues. "If you don't know whether something is going to work in the first place, it's hard to press ahead on a new therapy."

Another scientist notes that mice are an ideal disease model, since they are easy to handle, they can reproduce rapidly, and they can be manipulated at the molecular level. With such advantages the mouse has become the industry standard for disease models.

2.3.1 Alzheimer's Mouse

Scientists describe Alzheimer's disease as a neurodegenerative disorder in which neurological changes are associated with the accumulation of senile plaques and deposition of amyloid β-protein. In other words it is a disease that causes memory loss and disorientation. Plaques present in the brain cause the memory loss and disorientation in an Alzheimer's patient. Latter it was found that these plaques were made of amyloid. To aid our understanding of the cause of this disease a Disease Model was created (Games et al., 1995).

Professor David Adams (Dept. of Biotechnology at Worcester Polytechnic Institute) and some of his colleagues at the former TSI Corp (Worcester, MA) set forth to create of this disease model. The first step was to clone the human gene for amyloid (one of the causes of Alzheimer's). This amyloid gene was made in large quantities. The pure amyloid DNA was inserted into the mouse. Using a maze test, within 6 to 8 months there were signs of the disease present in the mouse. Microscopy performed on brain sections showed the presence of senile plaques. This transgenic mouse became the first functioning Disease Model for Alzheimer's (Games et al., 1995).

Elan Pharmaceuticals purchased the Alzheimer mouse from TSI Corp, and used it to test the world's first Alzheimer's vaccine. This vaccine was able to remove these amyloid plaques from the brain of the mouse, restoring brain function (Shenk et al., 1999).

2.3.2 Oncomouse

The patent to this transgenic mouse was awarded to Philip Leder and Timothy A. Stewart of Harvard University on April 12, 1988. Oncomouse is a transgenic animal line created to develop cancer tumors all over its body. It was created by the implantation of a cancer causing gene (the oncogene sequence) making these animals susceptible to cancer in a mouse fetus.

With these mice, the reasoning behind how genes and other exterior and environmental factors interact to cause cancer, make it possible for a cure.

The research on Oncomouse was supported and then licensed to DuPont. This transgenic animal became famous for reason's not dealing with its original purpose as a Disease Model for cancer, but for the idea of patenting a living being.

2.3.3 Aids Mouse

Over time, new diseases have become man's burden. AIDS or Aquired Immune Deficiency Syndrome has become modern man's burden. With the use of Transgenic Technology, American scientists developed of a disease model for AIDS. Dr. John Leonard and his colleagues of Bethesda, Maryland have succeeded in the creation of the first strain of transgenic mouse that suffers from AIDS.

AIDS is a disease caused by HIV that infects human cells by copying its own genetic "blueprint" into human DNA. In a way, the AIDS infected human cell has become a transgenic being itself containing information on the reproduction of the AIDS

virus. The copied genetic code from the original AIDS virus, that is now located in the human DNA, is referred to as the "provirus". The provirus, can be copied over and over again.

Understanding the nature of the disease, Dr. Leonard and his associates have taken newly fertilized mouse eggs and injected them with the AIDS provirus. Theses eggs were then transplanted into the uterus of a female mouse, acting as a surrogate mother. This process yielded three kinds of offspring. The first offspring had no evidence of the AIDS provirus, meaning the crossover process failed. The second liter born brought with it the provirus in its DNA, but did not produce any viruses. The final offspring (one female mouse), which had the viral information, produced antibodies to the virus. This one female demonstrated that the provirus was being copied and expressed into viral proteins.

This female was bred and produced four litters, which totaled 40 offspring. Of the 40 offspring, 15 had the full form of the AIDS disease. They all died within one month, all showing the symptoms exhibited by typical victims of AIDS. The creation of the AIDS mouse allow experimentation into the real workings of this disease, but more research has to be performed to understand if this strain of mice really model AIDS exactly as humans live with it.

2.4 Food Sources

If transgenic animals can be created for the purposes of Drug producers and Disease models, why can't they be used as a means of a food source? Transgenic Food Sources are in the works, but the ethics behind making such artificial organisms do not

weight as heavy in development as those spoken prior to this section (Transpharmers and Disease Models). This form, out of the many blooming forms under transgenic science, is as accepted as the others described here. But still, transgenic animals as food sources are becoming reality in the market, today. Reasons for their creation vary, but some may be concerned with a leaner and more abundant food source.

2.4.1 Super Fish

As the name implies, super fish is a specially made fish that will be superior to its natural kin. Super fish is a transgenic animal given extra copies of growth genes, thus it is larger than non-transgenic fish. One particular breed of salmon was genetically made and programmed to grow eight times faster, and has a maximum size that can reach 37 times larger than normal. In addition to their tremendous size they need much more food, since their metabolism is set in overdrive. These fish can be an ample yet inexpensive food supply.

Presently a Waltham, MA based company awaits approval from the Food and Drug Administration for their engineered salmon. These salmon, according to their maker, Aqua Bounty Farms Inc., grow to market size twice as fast as normal salmon. Aqua Bounty's aim would be to sell these "super fish" at a reduced price, while easing the pressure on wild or hatchery-raised fish.

Beyond the marketing value of such fish, some issues on their viability have come up. The issues brought to mind include a lack of any would be the medical benefits, and the suffering connected to this super fish. Super fish was produced to make a new kind of food source, which was cheap and affordable, but in contrast this food

problem can also be remedied by increasing the breeding of normal fish. In terms of the suffering related to the super fish, they may suffer malnutrition since they have a higher metabolism and may not swim as well as a normal fish. In the end/beginning, the super fish marketing venture will be decided by the FDA, and in their decision many other "new" transgenic food sources may or may not arise.

2.4.2 Super Pig

This was another venture in the same direction as the "Super fish". Through the means of "molecular tinkering" with embryos' growth genes resulted in the super pig. This new transgenic animal, first created in the 1980's was made to grow faster, bigger, and would yield leaner meat. This source of food was highly publicized but drastically took a turn for the worst. When these super pigs came into the world artificially, it became apparent that they were physically and mentally in pain. Super pig soon developed arthritis, ulcers, and became blind and impotent. Suffering was definitely present in the life of super pig. As was the case with super fish, super pig did not really have any medical benefits to mankind, and in addition did show great signs of suffering in the animal, so scientists voluntarily stopped creating such animals.

Transgenic animals are slowly but surely coming into the view of the public. Such animals will surely become an answer to medical problems that inflict many people, but issues dealing with the life lived by these creatures will come up. Ethics as well as morality will be tested by the creation and government acceptance of these transgenic animals.

CHAPTER 3: TRANSGENIC ETHICS

When discussing transgenics it is also necessary to look at the ethics of this technology. In order to look at the ethical issues presented by transgenic animals, this term needs to be defined first. One definition of ethics "…suggests a set of standards by which a particular group or community decides to regulate its behavior – to distinguish what is legitimate or acceptable in pursuit of their aims from what is not. Hence we talk of 'business ethics' or 'medical ethics'"(Straughan, 2000). Two strong reasons exist for reviewing the ethicality of this subject, as stated in Roger Straughan's booklet *Ethics, Morality and Animal Biotechnology*:

"i. No new scientific or technological development can claim immunity from ethical scrutiny. The fact that new technologies exist does not mean that they necessarily ought to be employed. Science cannot be pursued in a complete moral and ethical vacuum in any society that claims to be healthy and civilized, and in practice the legal and regulatory systems of such societies can be seen to rest upon an ethical basis." (Straughan, 2000)

"ii. More specifically, surveys have shown that moral and ethical concerns are of considerable practical importance in influencing public attitudes towards modern biotechnology. Worries are being increasingly expressed that the potential benefits of modern biotechnology may be lost if the new processes and products fail to gain 'consumer acceptance' because of moral concerns, which surveys in many countries show to be widespread. There must, then, be a strong practical argument in favor of examining the ethical basis of such concerns, not in order to try paternalistically to

persuade people to accept the technology, but to raise the level of debate and to encourage judgments to be made on a rational and considered basis."(Straughan, 2000)

Due to the complexity of transgenics, it is difficult to come up with exact answers to the ethical questions that are raised. It is only possible to provide information to clarify those questions and allow you, the reader, to have an educated opportunity to determine your response to the issues. After examining transgenic ethics it is understandable why transgenics is such a controversial subject.

3.1 Reasons For Transgenic Animals

There are many reasons for producing transgenic animals. Some of those reasons are in species propagation, food production, and life saving pharmaceuticals. One of the largest arguments in favor of transgenic animals is their use in medical research. Mostly to provide models for disease, and with those models help develop treatments and cures for those diseases. All of these reasons are intended to help not only mankind but the animal kingdom as well, when disease-resistance genes are inserted.

Assisting in the propagation of species is one of the least used forms of transgenics. However it is still a valuable asset in the fight against extinction. If a species was threatened with extinction due to some disease, it is possible to give that species disease resistance through transgenic applications. If a disease plagues a particular species then it could be stopped. Also because of mankind's expansion throughout the world, he has changed the climates and terrains of many areas where different animals have lived. Due to this changing and destruction of these habitats they

have had to either adapt to their new environment, migrate somewhere else, or in many cases if they cannot adapt fast enough, die. Transgenics could make it possible to help these animals adapt faster to the new environment, or even make it possible for them to live somewhere where they are not usually found. This would not only help those animals, which would be saved, but also it would help keep the diversity of life around on the planet that we, as humans the dominant species, should try to maintain.

Transgenics are starting to play a major role in food production. There are many reasons for transgenic animals in the food industry. One such reason is for the production of animals with leaner meat. They also are making transgenic animals that grow larger and faster. The benefits of this are that while humans are getting leaner meat and eating healthier, it also cuts down on the number of animals that are killed for food each year. Since there are fewer animals slaughtered, it also means there are fewer animals raised, by which there is less land cleared, particularly the rain forest, for grazing for these animals. As the rainforest is a vital part of the world ecosystem it is important to retain this resource of life.

Pharmaceutical companies are employing the use of transgenic animals more rapidly as this technology gets developed. The pharmaceutical industry is using transpharmers to produce different proteins and drugs in animal milk. Having these things being produced in the milk of animals is very beneficial. Since it is being made in the animal milk it keeps from having to slaughter the animals to harvest it. Not only does it provide an easy way to harvest the drugs, it also provides a constant supply of them. Also it allows for different varieties of these drugs to be produced in case that some people are allergic to one kind from a specific type of animal. For example some

diabetics are allergic to bovine or pig insulin and they use the more expensive human insulin instead.

Many medical researchers are extensively using transgenic animals. They use them to model many diseases that affect the human race. Three of the major diseases modeled by animals are Alzheimer's, Cancer, and AIDS. Researchers are able to make a very accurate model of these diseases and with these models determine how each disease is contracted and how it affects the body. With this knowledge researchers can find ways to detect these diseases early and then treat them before they adversely harm someone. They also can find treatments and cures for people inflicted with these diseases. As these diseases afflict a large percentage of the world's population it would stop a vast amount of people who suffer because of diseases, not only those with the disease, but their family and friends who suffer along with them.

3.2 Reasons Against Transgenic Animals

After looking at the positive reasons for making transgenic animals it is time to look at their negative aspects. It is because of these negative aspects that transgenic animal research is being hindered. There are many animal rights groups and religious leaders lobbying against transgenic animal use and research. They feel that the costs of this technology greatly outweigh the benefits that are achieved. They feel that transgenic animal research is "playing god" and that mankind does not have that right.

Many people have large concerns about introducing a new species to an environment, especially when that species is specially adapted to survive in that

environment. They are concerned that when a new species is introduced, it will overrun the area in which it is at and become a pest, similar to what has happened in the past with the starling, Dutch elm disease, Chestnut blight, fire ants, and the gypsy moth. Also if this species is resistant to disease, it is possible that these diseases could mutate and affect other species that have a natural resistance to the disease. Another aspect that the animal rights groups are worried about is the concept of introducing foreign DNA into an animal, that it is changing the "nature" of that animal. They feel that by doing this researchers are going against the natural order of life.

One of the biggest reasons why animal rights groups are against transgenic animals is their use in food production. Besides the fact that animals are raised to be slaughtered for human meals, the animal rights groups are concerned about how these animals are treated. Many of the transgenic animals that have growth hormones to grow larger, end up growing too fast and develop arthritis and other debilitating diseases that cause severe suffering. There are some groups who feel that the application of transgenic animals in food production is going to go too far, that scientists are going to make "animal factories" that are not real animals. These "animal factories" would essentially be animals but have no reaction with anything around them. Also some religions are against this due to their beliefs, like the Hindu's belief that all cows are sacred and should be left alone. Most farms that would use transgenic animals would have to be the large users with lots of money. They would then take over the market because they would be able to produce faster. This would bankrupt small farms that could not keep up and there would be many jobs lost throughout the world.

Transgenic animal use in pharmaceuticals is under scrutiny as well. The techniques used to make the animals are not very efficient. The number of animals that are born with the necessary proteins is a very small percentage of the population used. Those that are not of use are discarded like waste products. Also in the transgenic process there are many of the animals that do not survive at all do to the foreign genes. It is also unclear whether using proteins and drugs that are from transgenic animals could end up being harmful to humans. Since the proteins and drugs are harvested from the animals' milk, they could be forced to constantly produce milk. "The production of pharmaceutical proteins in animal milk might increase pressure to extend the length of lactation or the frequency of milking"(Straughan, 2000).

When researchers model a disease in a transgenic animal there are a lot of precautions that need to be taken, many of which cause many people to think that this research should not be undertaken. One such precaution is making sure that the animal does not escape. There are numerous possibilities that have been come up with if this were to happen, and many more still to be thought of. The biggest concern if an animal were to escape into the wild would be the outcome if it were to breed. Would whatever type of disease it had transform into some type of "super" disease that could be transmitted much more easily between hosts? Another concern is how well these animal models will represent a human. What may cause cancer or something else in a mouse is not guaranteed to cause cancer in a human. Groups are concerned that because of this it may be a waste of money and animal life to continue with the research. With the possible inaccuracy in the animal models it is also unsure on the validity of using treatments created from these models on human subjects. Even though the possible human lives that

could be saved is great it is still necessary to look at the sacrifices these animals are making, not necessarily by their own choice.

3.3 Ethical Examples

An example of a transgenic animal that the authors feel should be done is the Alzheimer's mouse. This mouse is a very good model of Alzheimer's disease. Not only is it a good way for scientists to research the disease in all it's aspects, but this mouse has helped develop the world's first Alzheimer's vaccine. This vaccine helped the mouse restore its brain functions, demonstrating that it may be possible to help humans who are inflicted with the disease.

An example of a transgenic animal that we feel should not be done is super pig. Although the concept of super pig was a good idea, its application was not. When super pig was created it was evident that it was suffering. Super pig developed many ailments including arthritis, ulcers, blindness and impotentence. The amount of pain that super pig was enduring was unethical and thankfully the scientists voluntarily ceased creating such animals.

When looking at these examples it is shown that there is no right or wrong answer to the transgenic animal questions. It is hard to tell if there is some sort of middle ground that can be taken given the examples above.

3.4 Is there a balance?

After this discussion about transgenic animals it may be asked why there has not been as much public debate on the ethics of this subject. "At one time in our society, it was accepted, almost without question, that scientific breakthroughs and technological developments were progress that would improve the quality of our lives."(Albrecht, 2001) However nowadays that is not the case.

One of the reasons is the lack of information that is presented to the public. "Developments in biotechnology are occurring so quickly that work is often outdated even before it is published"(Albrecht, 2001). This paper is trying to help solve this problem. Not many of the biotechnology companies have been researching public opinion of transgenic animals. Which is not necessarily the actual researching scientists fault. "This research has not been neglected because social scientists have not attempted to obtain research funding. Numerous efforts to obtain funding have met in failure"(Albrecht, 2001). It is this lack of knowledge on both sides that is causing much of the controversy of this topic.

Many people consider the morality of something like transgenics but that does not mean they are taking ethics into the equation. It has been said "moral concerns are felt about what it is right or wrong to do, while ethical concerns are about the reasons and justifications for judging those things to be right or wrong" (Straughan, 2000). The issue that people have is the difference between who is benefited and who makes the sacrifices. In most cases it is "humans who reap the benefits and animals who incur the costs" (Straughan, 2000).

The question that comes from all of this is if there is a balance that can be achieved? Hopefully the answer to this question is yes, that way all of the earth's creatures can benefit. Unfortunately this paper cannot easily answer the ethical issues raised, it can only provide the different sides and ask you, the reader, to make an informed decision for yourselves. Just remember that, "In morals, as in politics, most people tend to shun extremes. However, a middle view is at once the most defensible and the most difficult to defend. Pitted against extreme or esoteric positions, the numbers on its side create a presumption in its favor. Yet a presumption given only by the weight of opinion will not amount to a moral justification. A belief is not shown to be true simply by counting the votes of those who accept it. Some basis for an opinion, independent of it being accepted, must be found"(OTA, 1986).

CHAPTER 4: TRANSGENIC LEGAL ISSUES

4.1 Introduction: Patents

To discuss the controversial topic of patenting life, a definition of a patent is first necessary. A patent protects the exclusive right to an invention and the rights to make, use, and sell that invention for a set period of time. In addition a patent is a grant issued by the government used by the inventor of said property or concept in order to recoup expenses generated in the research and development phases. The inventor also controls the invention and its exchange with society. A patent however is not the right to use an invention rather it is the right to prohibit the use of the invention by others for unauthorized gain (Houdebine, 1997).

There are certain criteria required to attain a patent. In order receive a patent it must be new, not be obvious and must be useful. Also in some countries, a patent "must not contradict basic ethical principles" and you cannot patent a discovery, only an invention (Haerlin, 2000).

Regarding biotechnology and patent rights, there is a gray area in which the American court system has made its judgments. The United States Supreme Court ruled, in a case concerning genetically engineered microorganisms, that one can patent "anything under the sun made by man" (Woessner, 1999).

Benefits and Disadvantages of Patents	
Benefits	**Disadvantages**
The patent holder retains an absolute monopoly on the product or process for the period of the patent (up to 20 years in some cases).	Knowledge is in the public expiry and could be valuable to competitors.
Administration of patent maintenance once it has been obtained is relatively easy.	Litigation can be expensive. Problems of lack of harmonization of patent laws and other trading blocks not covered by patent could allow misuse.

Table 4.1 (Smith, 1996)
Shows the pros and cons for obtaining patents.

A Brief History of Bio-Patents in the USA

- 1979 First Genetic Engineering patent issued
- 1980 Chakrabarty decision: Micro-organisms are patentable.
- 1985 Higher plants are included (*ex parte* Hibbert)
- 1987 Higher animals are included (a polyploid oyster first)
- 1988 First mammal patented (Harvard/DuPont's oncomouse, by now over 100,000 filed)

  Moore vs. UCA lost: No rights to his own cells
- 1996 Surgical methods limited subject to patenting

www.mikro.org/Events/OS/ref-texte/haerlin.html

4.2 Diamond v. Chakrabarty

In the case of Diamond VS. Chakrabarty, a specimen by the name of *Burkholderia cepacia* or *B. cepacia*, caused quite an uproar in the scientific community as well as Supreme Courts. This bacteria had the ability to degrade crude oil into byproducts that would no longer be harmful to the environment, but would also serve as food for marine life.

B. cepacia was the subject of a landmark U.S. Supreme Court decision specifying that forms of life created in a laboratory can be patented. The Supreme Court came to a five to four decision concluding that since the bacteria was created in a laboratory it was no longer made or found in nature but was manmade. Even if an organism remains 99% similar to its original composition, that 1% is what makes it new and thus patentable (web.mit.edu/invent).

The Supreme Court's decision affected more than Chakrabarty and the patent office. Biotechnology companies were elated by the decision, and as a result many pharmaceutical and chemical companies could not invest in research and development fast enough to utilize this new opportunity for future gains.

In 1987 the Board of Appeals denied a patent on a method of producing more oysters by processing them under pressure. The Board of Appeals reasoned that the process which was under questioning was "obvious" and it also refused to grant a patent on the oyster because it was a *multicellular* animal.

Three weeks after this decision, the PTO reversed its decision and decided to accept applications for "nonnaturally occurring nonhuman *multicellular* living organisms,

including animals." In order for the patent to be granted, the claim must be that the animal is "given a new form, quality, properties or combination not present in the original article existing in nature in accordance with existing law" (Woessner, 1999).

4.3 The Harvard & Dupont Oncomouse

Onco-mouse is a mouse that has a human oncogene, a gene that can cause certain cancers, implanted into its cells by genetic engineering. These mice are used to test anticancer treatments and were created within the US by Harvard University in 1988.

In 1989 the patent office declared the Harvard mouse "a composition of matter" and granted a patent on the strain that was developed by the scientists, Philip Leder and Timothy Steward. A patent was granted thereby protecting its exclusive rights to produce the animal and profit from its research.

Oncomice are created by splicing a DNA sequence of a human oncogene into the genetic material of a mouse and this combination was used to make a mouse that passed this material onto its offspring.

This is the claim of the 1989 patent:

> "A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage." [U.S. Patent No. 4,736,866]

A few things worth noting about this claim are that it not only covers mice but rather "all mammals." In addition to patenting the mice that were created and all mammals that may be created by this method, it covers any and all offspring of any mammals created by the oncogene. Dupont, the patent holder, now profits from the sales of the oncomice to research institutions (Woessner, 1999).

Many people were not pleased with the court's decision to allow patenting of transgenic animals. Animal rights groups adamantly contested that the PTO (Patent and Trademark Office) could grant patents on transgenic animals. However the Court of Appeals for the Federal Circuit decided to stand by the original ruling and found that the Animal Legal Defense Fund did not have a case against the PTO (Woessner, 1999).

4.4 Moore vs. UCA

Another landmark case concerning the patenting of life was Moore vs. UCA in 1988. Mr. Moore was a man suffering from leukemia. While undergoing treatment doctors had taken his blood and patented parts of it. Moore took UCA to court and lost. Moore's reaction was, "you cannot do this, you cannot patent my blood." But they could, and they did. The doctors had already made a vaccine out of his blood and the courts found that the intellectual property rights of doctors were more important to mankind. Moore had no right over his own cells once they left his body.

In 1996, for the first time in the US, patenting surgical methods was allowed. Up until then certain procedures were patentable but knowledge of the procedure was not. But since 1996 there has not been a formal decision on it (Haerlin, 2000).

4.5 European Patent Law

The oncomouse patent has been the topic of discussion in Europe as well. After the EPO (European Patent Office) issued patents on the Harvard mouse, the European Parliament "instructed" the EU to revoke it in 1993 and refused to grant any other patents on transgenic animals until further investigation could be done to make a more educated ruling on the legalities of patenting life. This instruction was more of a recommendation and could not easily be enforced (Woessner, 1999).

Activist groups such as Greenpeace and the European Green Party knew they did not have grounds to block the EU's plan of "legal protection of biotechnological inventions" in March of 1995. However in 1997, the EU did approve the plan with a new article 1(b) restricting patenting plants and animals to their usefulness. Meaning that the patent must be practical and can not limited to any one particular plant or animal (Woessner, 1999).

Article 52(1) of the EPC states that a "monopoly right can be granted only: for any inventions which are susceptible of industrial applications, which are new and which involve an inventive step." What the EPC means by this is that the invention must be of some use industrially, must not be a new invention and must not be clearly obvious to someone experienced in that field of industry. Most importantly there must exist an

inventive step, an inventive step can be classified as the action necessary to cause the final outcome, and this outcome is the claim of the patent (Houdebine, 1997).

However, the EPC does have exceptional cases where a patent may not be granted. These exemptions are the foundation for the arguments posed by the opposition of the patentability of transgenics. Examples of grounds for denying a patent are, however valid the claim may be, is if the claim is immoral or not in the best interest of society or for biological organisms or the production of biological organisms.

> "European patents shall not be granted in respect of:
>
> > Inventions the publication or exploitation of which would be contrary to order public or morality,…;
> >
> > Plant or animal varieties or essentially biological processes for production of plants or animals; this provision does not apply to microbiological processes or the products thereof" (Houdebine, 1997).

CHAPTER 5: CONCLUSIONS

Transgenic animals are created by inserting a foreign gene into its genome, and are able to pass that gene to future generations through gametes. There are two main ways to do this, one is through a process called microinjection into an enucleated egg, and the other is through a process that uses embryonic stem cells.

The techniques for making transgenic animals have been used to make numerous types such as tranpharmers, disease models, and food sources, from many different kinds of animals like mice, goats, cows, fish, and pigs. These animals have contributed a lot to biotechnology and mankind by producing proteins, and by modeling diseases such as Alzheimer's, cancer, and AIDS.

Although transgenics are helpful, it is necessary to look at the ethical issues that are raised. There are many reasons in favor of transgenic animals. They help cure diseases and manufacture pharmaceuticals. However, transgenic animals also have their drawbacks. Some times the animals suffer, and there can be health and safety risks if some of the animals were to escape into the wild.

Another controversial topic under transgenic animals is the concept of patenting life. A patent protects the exclusive right to an invention and the rights to make, use, and sell that invention for a set period of time. Regarding biotechnology and patent rights, there is a gray area in which the American court system has made its initial judgments. Many current transgenic legal cases are still under appeal.

After researching the transgenic animal process, the types of transgenic animals made to date, and the ethical and patenting issues accompanying transgenic animals, we feel that overall they are a benefit to society. The medical applications of transgenic animals can save many lives and ease much suffering throughout the world. We do not condone any inhumane treatment of the animals, especially when transgenic animals are used as food sources, we see no benefit in this. Also, as one last statement, we would like to add that the public awareness on transgenics needs to be increased. No matter what the benefits may be, if the public is not fully informed about what is going on transgenics will never be accepted in today's society.

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