02E020I

Project Numbers: IQP-DSA-43-0382
-5497
-1925

TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

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Date: August 21, 2002

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ABSTRACT

The purpose of this project was to explore the topic of transgenic animals and the effects this technology has on society. Using various web resources and journals, the project team describes the popular methods of creating transgenic animals and some of their beneficial uses. The team then discusses the ethical and legal implications of genetically modifying animals for human gain. Through the completion of this project the team has attained a pro-transgenic attitude except in cases of extreme animal suffering.

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EXECUTIVE SUMMARY

The purpose of this IQP was to explore the topic of transgenic animals and the effects this technology has on society. The first chapter in this project involves a description of what a transgenic animal is and the two most popular methods of creating one. A transgenic animal is any animal whose genetic composition has been altered to insert a foreign gene. The first transgenic animal was produced in 1982 by transferring the human growth hormone (HGH) gene from a human DNA library to the embryo of a mouse in such a way that HGH would be expressed in both the original mouse and its offspring. There are two main methods for creating a transgenic animal. The first method, and also the most popular, involves the microinjection of DNA into the male pronucleus of newly fertilized eggs. In this procedure, the transferred gene is randomly inserted anywhere in the DNA sequence. The second method involves the transfer of engineered embryonic stem cells into blastocysts. Using this technique, and homologous recombination, one may place the transferred gene in a particular location in the DNA sequence.

The second chapter involves a discussion of the classification and different examples of transgenic animals. Four main categories of transgenic animals were discussed: transfarmers, xenotransplanters, food sources, and disease models. Transpharming, or animal pharming, is the production of pharmaceutical human proteins in the milk of transgenic animals. The added genes make it possible for the animal to secrete valuable proteins that may be used to treat many human ailments such as arthritis, cystic fibrosis, malaria, and HIV. Xenotransplantation is the transfer of living organs,

cells, or tissues from one species to another. This process is very important because of its potential to supply an unlimited amount of organs like livers and kidneys for transplantation into humans. Current transplant research is focusing on minimizing potential immuno-rejection of the transplanted organ by engineering the donor animal, usually pigs, to not synthesize certain HLA proteins on their cellular surfaces. The food sources category involves improving the production characteristics of farm animals for a variety of human benefits, including cows that secrete more milk, pigs that are leaner, and chickens that lay cholesterol-free eggs. Disease models involve creating designer species for use in the study of human illness. In some cases, the animal models are absolutely required for producing subsequent vaccines. While there is wide protest to this practice if the animal is made to suffer, not all disease model animals suffer noticeably, and disease models can be an invaluable tool for discovering cures and treatments for human diseases. While the prospects of this transgenic technology sound beneficial, progress has been slow as a result of our limited knowledge of gene function and regulation.

The third chapter in this project is a discussion of the ethics of making transgenic animals. While most scientists feel that animal experiments are worthwhile and beneficial to humans, many people are against them due to the way some animals are treated. Some of the pro-transgenic arguments include looking at many of the past benefits. Many products in veterinary medicine are derived from human therapeutics that were developed using research on animals. Many anti-transgenic activists hold that there are too many physiological differences between humans and other animals for the scientific research to be valid, however the authors of this report feel that some valid

scientific comparisons are still possible, even given some differences. Others believe that higher-order animals deserve equal consideration to humans regarding humane treatment and should not be allowed to suffer at all for human gain. We feel that in those transgenic cases where the medical gain is potentially great, and the animal suffering is insignificant, the benefits out weigh the negatives, and the experiments should be continued.

The fourth chapter in this project deals with some of the legal aspects of making transgenic animals. In this discussion two cases are discussed in detail, including Diamond vs. Chakrabarty, and the oncomouse case. The diamond vs. Chakrabarty case is a landmark because it involves the first patent of a living organism, although it was not transgenic, and it was only a microbe. The oncomouse case is even more important because it is the first animal to be patented. This chapter concludes with a discussion of the pros and cons of trying to patent life.

Finally, the fifth chapter includes a conclusion of some of the major points made in this project, and a summary of the groups own opinions formed following our investigation. Overall, this group gained an appreciation for this fascinating new biological technology, and argue that transgenics holds great medical promise, but should be tightly regulated to minimize animal suffering.

PROJECT OBJECTIVE

The purpose of this project was to explore in depth the controversial biological topic of transgenic animals and the effects this technology has on society. Using a variety of web resources and scientific journals, the IQP project group gained valuable insight into several of the different biological techniques used to create transgenic animals, which we attempted to put into layman's terms. The next goal was to show some real world applications and societal benefits of this technology. In chapter three, our goal was to explore the different sides of the transgenic ethical debate. In chapter four, our goal was to investigate some of the legal issues involved in the patenting of transgenic animals. In completing this project, the IQP team has gained a broad sense of awareness about how transgenic technology affects society as a whole.

CHAPTER 1 - DESCRIPTION AND CONSTRUCTION OF TRANSGENIC ANIMALS

1.1 Description

1.1.1 What is a Transgenic Animal?

The **nucleus** found within most mammalian cells contains a combination of genes that comprises of the **DNA** molecule. Each one of these genes contains information that controls form and function. With the advent of modern technology, genes can be altered artificially, changing some characteristics of the animal. A transgenic animal is one whose genetic composition has been altered in some way.

While transgenic rats, rabbits, pigs, sheep, cows, and fish have all been produced, over 95% of all existing transgenic animals are mice (Frame Website, 2002). Figure 1 contains a picture of a genetically-altered 'fat' mouse that is used to study diabetes in humans. Mice are generally used in transgenic experiments because of the cost effectiveness of using a small species with a high fertility rate and rapid generation time (Mullins et al., 1997).

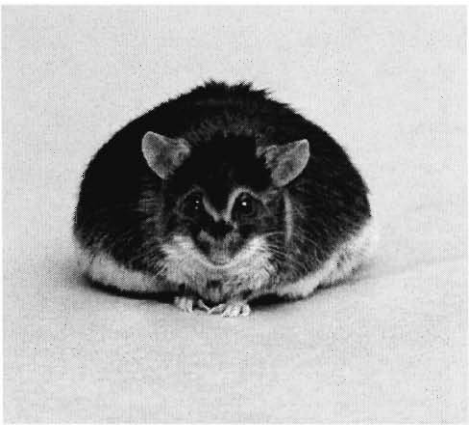

Figure 1: Genetically Altered 'Fat' Mouse (Gallepp, 2002)

1.1.2 Background

Before the current revolution in applied genetics, the only way to study the regulation and function of mammalian genes was to use spontaneous mutants. To show that the basis of the mutation was genetic, the animals had to transmit a specific trait to the offspring. The main problem with this method is that a large amount of DNA around the area being tested is transmitted from animal to animal during meiotic recombination, along with the genes in question (Transgenic Animal Science, 2002).

It has been possible, since the 1970's, to insert foreign DNA fragments into prokaryotic and eukaryotic cells, using various techniques, and have the cell express the modified DNA. Also, gene sequences may be integrated into cell cultures, and the protein of the transferred gene may be collected. Unfortunately, the action of a gene at a cellular level does not give scientists any information on the regulation of the gene among the **physiological** interactions of the whole animal (Transgenic Animal Science, 1991). Even the most state-of-the-art cell cultures can not replicate organ systems and predict responses to complex environmental stimuli.

The first transgenic animal was produced in 1982 by transferring a gene from one animal to the embryo of a mouse in such a way that the gene would be expressed in both the mouse and its offspring (Palmitter et al., 1982). Palmitter and Brinster were primarily concerned with understanding how cells read the genetic code and then translate that information into different biological structures.

While transgenic techniques have only been around for 20 years, the use of them in various biological experiments has steadily increased. In Great Britain, the number of transgenic animals used has expanded more than ten-fold from 1990 to 1999 (Frame Website, 2002). This steady increase has maintained even while the total number of animals used in all areas of research has declined by 17% (Frame Website, 2002). Transgenic animals now account for 19% of all experiments conducted on animals in Great Britain (Frame Website, 2002).

Transgenic animals have proven to be an invaluable tool used for research in a wide variety of areas. Some of these areas include the study of normal physiology and development, to test vaccines and chemicals, to provide organs that can be used for transplantation, and to produce useful biological products (Frame Website, 2002). A more detailed discussion of the different transgenic animals that have been created and their medical benefits can be found in chapter 2.

1.2 Construction

There are two methods for creating a transgenic animal. The first method, and also the most popular, involves the microinjection of DNA into fertilized eggs. In this procedure, the *trans*gene (i.e. transferred gene) is randomly inserted anywhere in the DNA sequence. The second method involves the transfer of **embryonic stem cells** into **blastocysts**. Using this technique, one may place the *trans*gene in a particular location of the DNA sequence.

As discussed earlier in section 1.1.1, the mammalian DNA molecule is contained in the nucleus. This molecule contains genes that control how the organism is formed and functions. All living beings use the same genetic language built from the same four letters, or bases: adenine, guanine, cytosine, and thymine. These bases are often abbreviated A, G, C, and T for simplicity reasons.

Figure 2 contains a common analogy used to describe how the different terms are related. The DNA molecule itself can be thought of as the text, or sentences of a language. A gene may be thought of as a specific word in a sentence, while the specific bases may be thought of as the letters that make up the words. Because the genetic code is universal and common to all species, it is possible to express part of the genetic material of one species in another species.

Figure 2: Genetic Analogy (Genoway Website, 2002)

To complete the analogy one may think of the first method of creation of transgenic animals, i.e. pronuclear microinjection, as inserting a word or gene anywhere in the text. The second method of transgenesis, by transfer of ES cells into the blastocyst, may be viewed as inserting a word into a precise sentence in the text.

1.2.1 Method 1 - Microinjection of linear DNA into the pronucleus

While the first successful transgenic mouse produced through pronuclear microinjection was in 1980 (Gordon et al, 1980), the **phenotype** of the *trans*gene was not expressed. It wasn't until 1982 when a phenotypic change was seen and passed down to the offspring (Palmiter et al, 1982). Since its inception in the early eighties, the microinjection technique has become the most popular method of producing transgenic animals (Polites and Pinkert, 1993).

The series of pictures in figure 3 describe the process in which a transgenic animal is generated using the DNA microinjection technique:

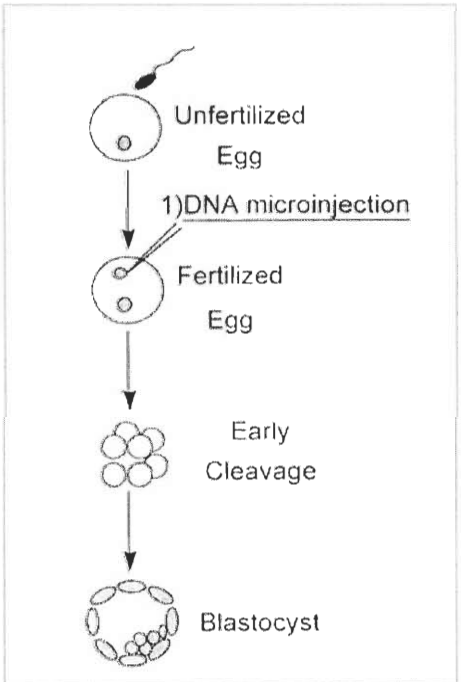

Figure 3: Transgenic animal creation methodology (Abbud and Nilson, 1999)

The pronuclear microinjection method of creating a transgenic animal results in the introduction of linear DNA sequences into the chromosomes of the fertilized animal egg. The foreign DNA must be introduced into the host genome before the doubling of the genetic material that precedes the first **cleavage**. Because of this reason, the transgene DNA is integrated into the **zygote** at the pronuclear period, which is the earliest possible stage following fertilization (See Figure 3). If this does not occur, a mosaic animal may be created in which only certain cells contain the new gene.

Several hours after the sperm enters the **oocyte**, the male and female pronuclei are visible under a microscope as individual structures and are not yet integrated into a zygote. While the transgene may be injected into either of these (male or female) pronuclei with equivalent results, the male pronucleus is usually chosen because of its large size compared to that of the female nucleus. Also, the male pronucleus may be chosen because it is closer to the oocyte surface, therefore making the microinjection process much easier. It is important to remember that X and Y (sex) chromosome integration sometimes does occur and is influenced by which pronucleus the genetic material is microinjected into. If the transgene is integrated with one of the embryonic chromosomes, the animal will be born with a copy of this new genetic information in every cell.

Figure 4 contains a microscopic image of a pronuclear microinjection that is about to take place. The embryo is in the center of the image and is being held in place by the holding capillary on the left. This device provides a light suction force that keeps the embryo from moving while the injection takes place. The actual microinjection is

accomplished by using the injection capillary which is shown on the right side of the image.

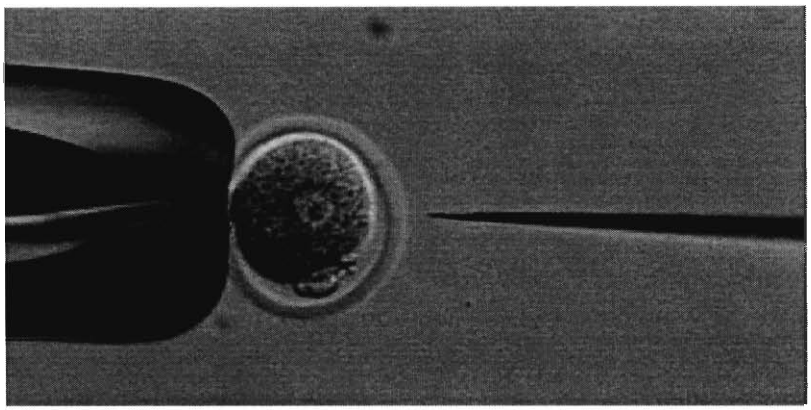

Figure 4: Pronuclear DNA Microinjection (Genoway Website, 2002)

After the DNA has been microinjected into the pronucleus, the embryo is then transferred into the **oviduct** of a recipient female, or a foster mother that has been induced to act as a recipient by mating with a sterile male. The resulting animal that develops after receiving the transgene DNA is referred to as the founder (Fo) of a transgenic lineage (Transgenic Animal Science, 2002). If the founder's **germ cells** end up transmitting the transgene stably, then all descendants of the animal are members of the unique transgenic lineage.

The insertion of the DNA using the microinjection technique is, however, a random process. Because of this, it is highly probable that the introduced gene will not insert itself into a site on the host DNA that will allow its full expression. The success rate of this microinjection technique depends largely upon the careful collection of a large group of accurately timed embryos from a reproductively synchronized group of female embryo donors (Transgenic Animal Science, 2002).

1.2.2 Method 2 - Transfer of ES Cell into Blastocyst

The second method for creating transgenic animals involves the transfer of embryonic stem (ES) cells into the blastocyst. This method is also commonly referred to as targeted insertion. Figure 5 contains a series of pictures that describe the process that is needed to go through to obtain a transgenic animal using this method.

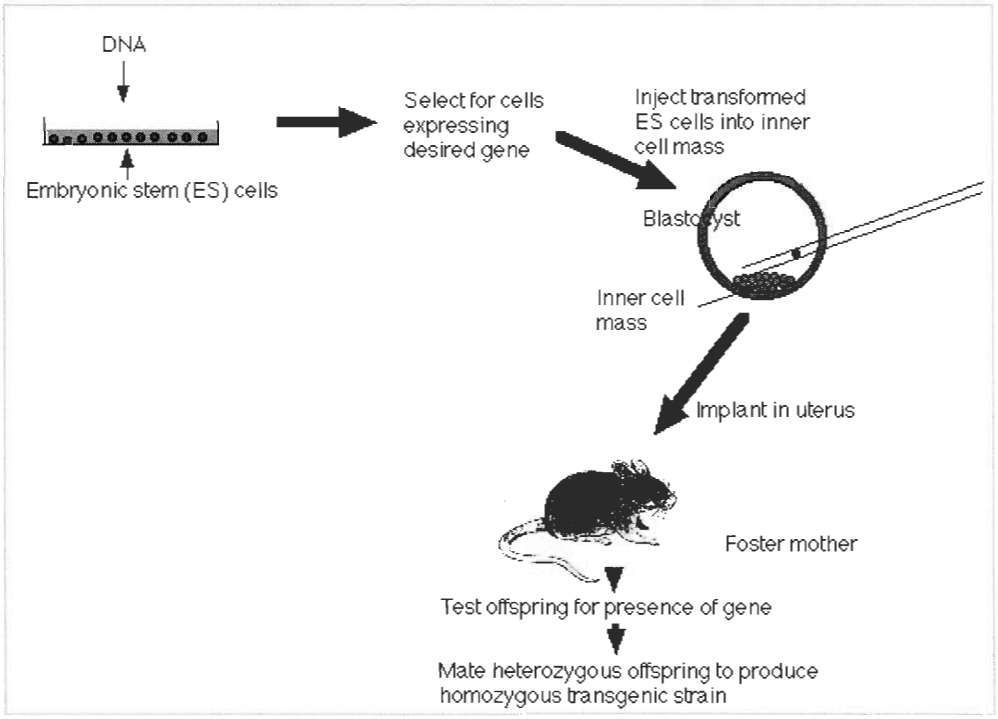

Figure 5: ES Cell Method Flow Diagram (Transgenic Animals, 2002)

An embryonic stem cell is an undifferentiated cell that has the potential to grow into any type of cell (i.e. somatic *or* germ cell) giving rise to a complete organism. These ES cells are harvested from the inner cell masses of normal blastocysts. The blastocyst is the ball of cells that exists during the early embryo stage. Figure 6 contains a pictorial view of what the blastocyst and inner cell mass look like.

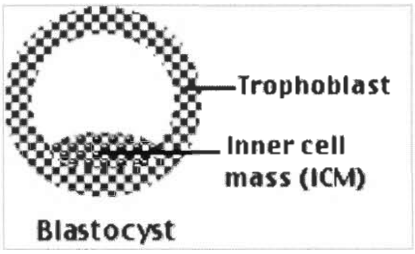

Figure 6: Pictorial view of blastocyst and inner cell mass (Transgenic Animals, 2002)

Once the ES cells have been collected and cultured in vitro they are then exposed to the modified DNA so that some will incorporate it. Cells that have undergone homologous recombination with matching genomic sequences are then selected. **Homologous recombination** is a process by which the two homologous pairs of sister chromatids (in the DNA molecule) align and crossover during **meiosis**. This is a very common phenomenon and could happen several times per meiosis.

The transformed cells are now collected and approximately 10-15 are inserted into the inner cell mass of a mouse blastocyst. Figure 7 contains an image of ES cells being inserted into the blastocyst. Notice that, similar to the microinjection technique of method one, the holding capillary on the left holds the blastocyst into place while the injection capillary inserts the ES cells directly into the inner cell mass.

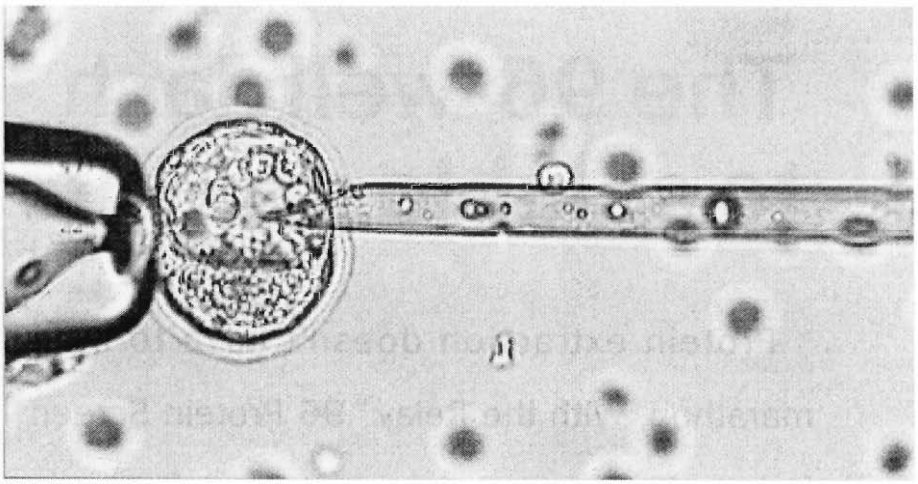

Figure 7: ES Cells inserted into blastocyte (Genetic Engineering News, 2002)

Once the ES cells have been injected into the blastocyst they may now be inserted into the female. Similar to the microinjection technique, a pseudopregnant mouse may be created by mating it with a vasectomized male. The stimulus of mating itself creates the hormonal changes needed to make the uterus receptive (Transgenic Animals, 2002). Approximately eight to ten blastocysts are implanted into the uterine horn of each recipient (What is a Transgenic Mouse, 2002).

Once the mouse is impregnated the process is not yet complete. Approximately one-third of the pups will grow into healthy individuals (Transgenic Animals, 2002). A small piece of tissue then can be removed and the DNA can be examined to see if it contains the desired gene. Approximately 10-20% of the pups will have the new transgene (Transgenic Animals, 2002), and they will only be heterozygous for that gene. To complete the process, two heterozygous mice are then mated together. One-fourth of their offspring will be homozygous for the transgene. These offspring are the founders (Fo) of the new transgenic lineage.

1.2.3 Advantages/Disadvantages of Each Method

Table 1 contains a concise breakdown of some of the advantages of disadvantages of each method of creating transgenic animals. The pronuclear DNA microinjection method allows for a much easier vector construction (i.e. modified DNA sequence) than the ES cell method. On the other hand, the ES cell method provides a precise model in which the insertion site into the genome is known, thereby increasing the probability of transgene expression. Also, using the ES cell method, the genome may be manipulated in several different ways, including deletion and mutation, which are out of the scope of this chapter. While the vector construction is much simpler with the pronuclear

microinjection method, it does come at a cost, because the transgene is inserted randomly into the DNA sequence and could cause some unknown effects.

Pronuclear DNA Microinjection		**ES Cell Method**	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Easier Vector Construction	Insertion site and number of copies inserted unknown	Insertion site known	Vector Construction Longer
Short Development Times	Effects of the insertion site on the expression of the transgene?	Several Possibilities to manipulate the genome (deletion, insertion, mutation)	
Applications		Precise Model	

Table 1: Advantages/Disadvantages of the two main methods for making Transgenic Animals (Genoway Website, 2002)

CHAPTER 2 - CLASSIFICATION AND EXAMPLES OF TRANSGENIC ANIMALS

2.1 Transfarmers

2.1.1 What is animal pharming?

Animal pharming is the production of pharmaceutical human proteins in farm animals that have been genetically altered by the introduction of foreign genes into their chromosomes. The added genes make it possible for the animal to create valuable proteins in its milk, blood, urine, sperm, or eggs. These proteins have numerous uses, such as the treatment of cystic fibrosis, anemia, hemophilia, osteoporosis, arthritis, malaria, and HIV. They are also used to produce monoclonal antibodies, which are a key element in vaccine manufacturing.

The first successful products of genetic engineering were protein drugs like insulin, made in 1982, and growth hormone, made in 1987. *E. coli* can be used to inexpensively manufacture these drugs in bioreactors, but bacteria and other microorganisms cannot produce more complex human proteins. Higher organisms, such as mammals, are needed because bacteria lack the enzymes needed to recognize the human protein sequences that need modification. Maintaining mammalian cell cultures is far too expensive in comparison to the small amount of product that can be obtained from them. Certain protein drugs that require post-translational modifications such as phosphorylation or glycosylation, or that are needed in a large supply, therefore, are most efficiently produced in transgenic animals (Pharmaceutical Production in Transgenic Animals, 2002).

2.1.2 Examples

As of right now, milk of transgenic goats and cows used for the production of human therapeutic proteins is in the process of clinical trials. Many have reached Phase I and some have even reached Phase III and early stages of product development. The Genzyme Transgenics Corporation is an example of cutting edge transgenic research with mice, goats, and cows. The corporation, located in Framingham Massachusetts, is a 383-acre farm working in collaboration with Louisiana State University and Tufts School of Veterinary Medicine to make the word's first transgenic goats. These goats produced human antithrombin III in their milk, which is a human plasma protein that regulates blood clotting. It would be used as a treatment for patients that have had strokes and heart attacks. It is the first transgenically produced protein to begin human clinical trials and make it all the way to Phase III tests (Milking For Medicine, 2002).

Nexia Biotechnologies Inc., a partner of Genzyme Transgenic Corp., is also working on the development of proteins from transgenic goats in Ste Anne de Bellevue, Quebec. They are looking for a growth hormone that will boost immune systems of transplant recipients and reduce the horrible side effects of chemotherapy. The farm where they operate is only one of three in the entire world. The other two are Genzyme's and PPL Therapeutics in Scotland, which became the first company to clone a mammal when they introduced Dolly the sheep. To speed up their research, Nexia has developed a goat that matures earlier than normal and breeds all year long. This goat is called BELE, which stands for *breed early, lactate early* (McGovern, 2002).

Transgenic goats may even be a key element in the fight against HIV/AIDS. PRO 542 is a fusion protein made by Progenics Pharmaceuticals in partnership with Genzyme

that may neutralize the HIV virus. Genzyme is producing the transgenic goats for Progenics to use in their clinical trials. The goats secrete PRO 542 in their milk, which has already been shown to reduce HIV levels in adults and children who did not benefit from antiretroviral therapies. The drug has been tested in Phase I and Phase II clinical trials so far. Some of the most exciting news is the fact that in high doses, PRO 542 lowered the HIV virus count to undetectable levels for prolonged periods. Ronald J. Prentki, president of Progenics, said, "given the increasing worldwide prevalence of HIV and the potential demand which would be expected for PRO 542, we consider transgenics to be the most appropriate manufacturing approach to meet the bulk volume and cost of goods requirements for a commercial product (Transgenic Goats May Help Battle HIV/Aids, 2002)."

The American Red Cross has collaborated with the Virginia Polytechnic Institute in Blacksburg, Va. to invent a way of making proteins in the milk of transgenic pigs. Their goal was to find a treatment for hemophilia, which affects more than 15,000 people in the United States. These people cannot produce the necessary proteins called Factor VIII or Factor IX which allow blood to clot. Previously, Factor VIII and Factor IX had to be derived from human plasma, which was expensive and time consuming. Genie, the first transgenic pig, could make about 200 times the concentration of protein found in human blood (Producing Abundant Plasma Proteins, 2002).

On April 2, 2001, researchers at Novazyme Pharmaceuticals Inc. and the University of Florida demonstrated how injections of the enzyme alpha-glucosidase restored normal muscle function in lab animals suffering from Pompe disease, which is a rare form of muscular dystrophy. The enzyme breaks down glycogen, which can interfere

with efficient muscle development and function. Alpha-glucosidase was shown to clear up glycogen that had accumulated in both skeletal and cardiac muscles of affected animals (Unprecedented Response to Enzyme Therapy, 2002). In Geel, Belgium, rabbits are grown to produce alpha-glucosidase in their milk. Pharming Group N.V. has gone through many clinical trials involving the enzyme. In one trial, four infants lived to be 12 to 17 months old, which is remarkable for a patient born with Pompe Disease. The transgenic enzyme was present in normal levels following treatment and muscle regeneration was also observed in the children.

GD3 is an antibody that is used to treat melanoma and some other forms of cancer. It works by targeting a tumor antigen on the surface of the cancer cells. Viragen in Florida along with the Roslin Institute in Scotland produced it for the very first time from transgenic chickens in October 2001 (Medicinal Eggs Closer to Reality, 2002). Obtaining a transgenic product from chicken eggs is sometimes favorable to obtaining them from milk because egg whites are less complex and the proteins are easier to extract (Gene-tinkered Chicken Lays 'Designer' Eggs, 2002).

Alpha-1-antitrypsin, has been produced in sheep by PPL Therapeutics. It is used to treat lung disorders such as cystic fibrosis. Some people are born with genes that either don't work or are insufficient for making Alpha-1-antitrypsin. This results in damage to the lungs and sometimes liver. The transgenic sheep used to produce this protein have been extensively studied. It has been shown that expression levels and transgene copy numbers have been steady over at least five generations of sheep (Cooper, 2002).

Fibrinogen is a protein in blood plasma that forms a fibrous network in the process of blood coagulation. It is used to help stop severe blood loss, reconstruct bone,

or deliver chemotherapy drugs. Because fibrinogen is such a large, complex protein, it cannot be produced commercially in any culture system existing today. The use of transgenic animals has been the only successful way to date of obtaining fibrinogen (Plasma Derivatives, 2002).

Researchers have designed goats that secrete a substance called Tissue Plasminogen Activator (TPA) in their milk. It is used to help dissolve blood clots in heart attack patients. Extremely high yields have been observed and scientists even believe that one goat can produce as much TPA as a 1,000-liter bioreactor. Goats have also been designed to produce Human C1 inhibitor, which is used to treat patients with hereditary angioedema. This is a disease that causes swelling of body tissue and can be disfiguring and potentially lethal. Clinical trials have been done and the results are successful so far (Pharming Group N.V, 2002).

2.2 Xenotransplantation

2.2.1 What is Xenotransplantation?

Xenotransplantation is the transfer of living organs, cells, or tissues from one species to another. It is important because of its potential to supply an unlimited amount of biological products for transplantation from animals into humans. For example, People could receive a new heart, lungs, liver, kidney, pancreas, cornea, bone, or skin graft from a pig (Biologic and Genetic Therapies, 2002). According to the United Network for Organ Sharing, only 23,000 of the 77,000 people currently waiting for a transplant each year actually are able to get one. Demand for human organs is growing almost five times as much as the supply and many people are left to suffer (Zwillich, 2002).

Xenotransplantation could possibly reduce the need for human donors and provide patients with a transplant when and where they need one.

2.2.2 Risk of Viruses and Disease

There are many disadvantages and risks associated with xenotransplantation. The most serious risk is the chance of an unidentified animal infectious agent being transferred to humans and endangering the entire population. A whole new epidemic could arise that we don't know to treat. Since the immune systems of transplant patients are suppressed to avoid rejection, this is an especially important concern (Biologic and Genetic Therapies, 2002). Animal parts placed inside the human body are potentially very dangerous. Any viruses present in the transplanted organ have already bypassed the mucosal and skin barriers that would have normally prevented them from infecting the host. Because the virus has circumvented all natural barriers, new diseases may emerge resulting form a virus that used to be unable to enter the human body. To date, no record of a disease has been traced to a transplant, but it is definitely a possibility (Xenotransplants, 2002). Even if a particular patient is willing to take a chance and receive a transplant from an animal source, their choice could affect others if a pathogen is contracted and exposed to the public.

Many researchers believe that HIV evolved from the simian immunodeficiency virus (SIV) found in primates. Almost all monkeys in Africa have their own kind of AIDS virus that is completely harmless to them. If they are introduced to humans or Asian monkeys however, they cause AIDS. Another example is a form of herpes that causes fever blisters in monkeys, but if a human contracts the virus it is fatal (Xenotransplants, 2002). In 1918 there was a Spanish flu epidemic that killed 20 million

people and recently there was a Malaysian epidemic of a virus that killed a hundred people (Scott, 2002). In the 1950's the Asian flu killed a million people and in the 1600's the Hong Kong flu killed 700,000. All of these viruses are believed to have come from animals (Better Science, 2002). These examples show how it is difficult to tell which pathogens are going to be harmless in one species, but cause illness in another. Researchers are particularly concerned about the *pig endogenous retrovirus* (PERV), which could jump to humans in a similar way as HIV may have done. In 1997, researchers at the London Institute of Cancer Research ran some tests and observed the pig virus enter human cells and initiate infection. In 1998 however, BioTransplant Inc. announced that they have bred transgenic, miniature pigs that cannot transmit PERV to human cells, which is a major advancement.

2.2.3 Risk of Rejection

Another disadvantage to using animals such as pigs for human transplants is the problem of rejection. Humans reject pig organs much more strongly than other human organs because of a certain sugar that lines the pig's organs. It is called alpha- 1-galactose and it is extremely similar to a bacterial sugar the human immune system recognizes as a pathogen As a result, the human body destroys many transplanted pig organs right away. In January of 2002, two companies announced that they are close to solving the alpha-1-galactose problem in pig organs. Immerge BioTherapeutics and PPL Therapeutics both have created a pig lacking one of the two copies of a gene that makes the sugar. If scientists can knock out the other gene and also if pigs can be shown to live

without any production of alpha-1-glucosidase, the success rate of pig to human transplants would be much greater (Zwillich, 2002).

2.2.4 History of Xenotransplants

Xenotransplantation began in the early 1900's and there have been many different attempts through history. However, there has never been a successful whole-organ animal to human transplant. Most of the human recipients die in just hours or weeks after receiving an animal organ. The organ usually causes a severe, life-threatening rejection. Many scientists have attempted to transplant animal organs into humans however. In France in 1905, pieces of a rabbit kidney were placed into a child who, unfortunately, only lived for two more weeks. A lamb kidney was transplanted into an American woman in 1923, but she died only nine days later. Several people received chimpanzee kidneys in 1963, but the organs were rejected quickly. In 1964, the heart of a chimp was put in a man who only lived for two additional hours because of an immediate rejection. "Baby Fae," a child born with a malformed heart, received a baboon heart in 1984, but only survived for 20 days. Remarkably, a man lived for 71 days after receiving a liver from a baboon in 1992. Also in 1992 a pig liver was transplanted into a woman, but she only lived for a day following surgery (Better Science, 2002).

Xenotransplantation is a technology that scientists will continue to work on. In the future it may become common to use histocompatible pig organs for human transplants, but for right now it seems like there are just too many barriers to overcome before that is possible.

2.3 Food Sources

2.3.1 Background

Approximately the past two decades have been spent trying to improve the production characteristics of farm animals for a variety of human benefits. The technology of transgenic farming is becoming more and more useful as we continue to solve the mystery of how genes operate. The future looks promising for breakthrough discoveries that will change the way farmers operate agricultural business. The goals are to increase the size of the animal or to increase the quality of the products obtained from the animal. The prospect sounds beneficial; however, progress has been slow as a result of our limited knowledge of gene function and regulation. Also, many factors have a limiting effect on scientific advancement such as the health of the animals and the safety of human consumers.

Safety, in fact, is a huge issue when it comes to using genetically modified animals as food sources. Scientists are still learning about this technology, which has the potential to some day be used as a more efficient way of farming. For right now though there are many problems regarding the ways that genetically engineered foods are regulated and tested for safety. Even minor chemical changes in food could significantly alter the way the human body reacts to it. It is extremely difficult to identify the numerous changes that could take place and what their long-term effects will be after consumption. New toxins could emerge as a result of people eating inserted genes that have never before been in the food chain.

2.3.2 *Examples*

Lately, more and more fish are grown in fish farms rather than being caught at sea. Aquaculture production has been steadily increasing every year and is projected to keep growing in the future. More than 20 kinds of fish have been genetically engineered for either commercial production or scientific study. Genes are inserted to improve the quality of the fish such as to speed up growth, increase cold tolerance, increase disease resistance, or alter the flesh quality. Growth hormones that have been used include those from pigs, other fish, and humans.

AquaBounty Farms from Waltham, Massachusetts, engineered an Atlantic Salmon that contains a growth hormone gene from a fish called the chinook salmon. They claim that this fish can grow 400 to 600 percent faster than the nontransgenic ones. Another Waltham based company called A/F Protein has engineered salmon with genes from the cold water eel pout, also making them grow significantly faster than normal fish. These discoveries could make food production more economical, especially for poor areas of the world (Goldburg, 1998). Table 2 contains a list of fish that have been genetically modified so far.

Fish Species That Have Been Genetically Engineered	
Abalone	Medaka
Atlantic salmon	Mud carp
Bluntnose bream	Northern pike
Channel catfish	Penaeid shrimp
Coho salmon	Rainbow trout
Common carp	Sea bream
Gilthead bream	Striped bass
Goldfish	Tilapia
Killifish	Walleye
Largemouth bass	Zebrafish
Loach	

Table 2: Fish Species that have been Genetically Engineered (Goldburg, 1998)

Pigs have been given a cow growth hormone to try to make them produce more lean meat. Many problems existed with the use of this hormone including the pigs having arthritis, ulcers, kidney disease, and fertility problems. A new growth hormone gene has been discovered that produces enhanced meat production without all of the nasty side effects. The new transgenic pigs carry the gene IGF-1, which is an insulin-like gene already present in the pig's cells in small amounts. IGF-1 is produced in muscle and can safely be increased in the muscles without interfering with any other tissues. This fact makes the new transgenic pigs unique. They have less carcass fat and more lean body mass without the severe health problems associated with other transgenic pigs bred for the same purpose (Pigs Genetically Engineered with a Growth Factor Gene, 1998). The pictures below show the difference between modified and unmodified pork. The image on the left shows meat with a normal amount of fat. The image on the right shows leaner meat from a transgenic pig.

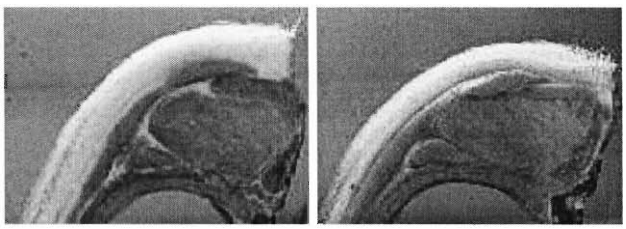

Figure 8: Unmodified vs. Modified Pork (How are Researchers in Animal Science Using Biotechnology, 2002)

Another way to improve pork was done at the Kinki University in Japan. Scientists inserted genetic material from spinach into pigs in an attempt to produce healthier meat. The FAD2 gene converts saturated fatty acids to linoleic acids, which are believed to be much better for human consumption. These FAD2 pigs are currently being tested for safety (Spinach Improves Pork, 2002).

AviGenics plans to produce a line of transgenic chickens that have bigger breast muscles and faster growing rates. The chickens would yield significantly more meat and would be marked with a DNA tag to prevent anyone from breeding them without permission. AviGenics is also working on transgenic birds that have greater disease resistance, making them have a better day to day life even though they will eventually be slaughtered (New Super Chickens fuel GM Food Row, 2002).

People that suffer from problems with lactose in milk may be in luck. Researchers are developing ways to reduce or eliminate the amount of lactose without changing the content of protein and fat in the milk. The transgenic mice used in experiments were manufactured to make the lactose-digesting enzyme lactase in their mammary glands. The lactase broke down lactose in high amounts especially when the milk was allowed to collect in the mammary glands. In the future, this same technology could be used in cows to enable lactose intolerant people to drink milk (Lawrence, 2002) Another modification of milk for health benefits is the increased transgenic production of lysozyme in milk. Lysozyme has antimicrobial properties that improve animal health, give milk a longer shelf life, and help reduce diarrhea among children in poor countries (How are Researchers in Animal Science Using Biotechnology, 2002).

2.4 Disease Models

Developing transgenic animals for disease research is an extremely controversial subject. Individuals who are concerned for animal welfare label this technology cruel and unnecessary. It is important for the public to understand, however, that animal models of disease can be invaluable tools for discovering cures and treatments for human illnesses.

The models give scientists a way to study symptoms in a living system so they may better understand the disease in humans. They can test drugs and vaccines on the animals that otherwise would not be able to be tested. It is reasonable to say that if animal experiments are carried out in a responsible way, then the huge benefit to society balances out the relatively small number of animals used in testing.

An effective way to study the role of how genes work in human health and disease is through mouse models because most human genes have a mouse equivalent. Chella David, Ph.D. from the Mayo Clinic in Rochester, Minn. says "transgenic mice are a bridge between laboratory analysis and human trials of new treatments" (Mighty Mice, 2000). More and more genetically modified animals, especially mice, are being used in experiments and the numbers are predicted to keep rising in the future. There are thousands of genetically altered mice made to study all different diseases. For example there are mice with immune deficiencies, mice prone to cancer, diabetes, blindness, Lou Gehrig's disease, Huntington's disease, obesity, anxiousness, aggressiveness, mice with nervous disorders, and even mice made with alcoholism and drug addiction. They are made by either inserting a transgene or by mutating a specific gene to produce a knockout. In some cases, the seriousness of a human disease may be a sufficient reason to make a mouse model, but some genetically modified animals are just made simply because they can be.

It is true that many researchers sometimes abuse their scientific power with unnecessary experiments and pointless animal suffering. It is also true that animals are used in ways designed to truly benefit the human race. The Alzheimer's mouse model (Games et al., 1995) is one example of an invaluable invention crucial for studying the

causes of the complex disease. Alzheimer's is our nations fourth leading killer and effects millions of people around the world (King, 1995). It is a serious brain disorder that effects a patient's ability to carry out even the simplest every day activities. It is characterized by extreme confusion, loss of memory, and skewed judgement. This loss of mental ability goes along with an abundance of protein clumps in the brain called amyloid plaques. Also present are networks of tangled fibers called neurofibrillary tangles. These two trademarks of Alzheimer's accompany the loss of nerve cells in vital parts of the brain (Alzheimer's Disease Fact Sheet, 2002). With the large amount of people suffering from Alzheimer's and the devastation it causes to its victims, it is no surprise how important a disease model is to current research.

Prof. Adams and a team from the former Transgenic Sciences, Inc. (Worcester, MA) engineered the world's first Alzheimer's mouse (Games et al., 1995) by cloning the gene for human amyloid, changing it to mimic the mutations seen in early-onset human cases, then inserting the gene into a mouse. After about 3-6 months, the mouse develops plaques in the brain, and also performs less efficiently on a maze test. This mouse line proved that deposition of amyloid in the brain was sufficient for initiating Alzheimer's disease, and appeared on the cover of Nature magazine. Layman's coverage also appeared in the New York Times (Feb 9, 1995), the Wall Street Journal, and U.S. News and World Report (Feb 9, 1995).

What's good about the mouse disease model is the fact that no suffering is observed in mice bred to have Alzheimer's. They are of normal size and weight, and do not display any abnormal eating, mating or behavior patterns other than some memory loss. Because of these facts it is safe to say that the transgenic mice with Alzheimer

symptoms are humanely kept and do not suffer. Even more exciting news is the discovery of an Alzheimer's vaccine that directly resulted from the use of mouse models of the disease. The vaccine, which was produced by Elan Pharmaceuticals worked in mice to prevent amyloid plaques from forming at all or stopped the formation of them once it had started (Schenk et al., 1999). It was made from beta amyloid because researchers believed it would trigger the immune system to attack the amyloid deposits. This is exactly what happened in the mice. The Alzheimer's mice ended up making antibodies that tagged brain amyloid deposits so they would be attacked. In humans, it has been shown that the vaccine is safe in phase 1 trials, but as of right now scientists aren't sure exactly how effective it is (Possible Alzheimer's vaccine shows promise, 2000).

The OncoMouse, which contains a recombinant activated oncogene sequence, was engineered and made in 1981 to have a genetic predisposition to develop tumors. These certain mice will probably suffer a lot in their lifetime and die a painful death because of the seriousness of the disease. Since cancer is such a big problem in the world today it is acceptable to make animal models that will some day help save millions of human lives. Scientists use the mice to study a range of possible treatments so that they don't have to be tested on humans. Research using the genetically engineered mice has already given great results. In July 2000, scientists at the University of California found five proteins that trigger the immune system to attack cancer. This information makes it easier to find targets for drugs and vaccines to boost natural cancer-fighting ability. The human counterparts for these mouse proteins have been identified as a result of the initial mouse testing. Mouse models have also been helpful in the search for ways to genetically alter tumor cells to fight cancer in humans. Researchers at Columbia University are

working on a way to block the interaction of two molecules in tumor cells to block growth and spreading in mice. Hopefully the data collected from these types of experiments in mice will lead to similar treatments that will be effective in stopping cancer in humans (Mighty Mice, 2000).

CHAPTER 3 - ETHICS OF MAKING TRANSGENIC ANIMALS

3.1 Introduction

3.1.1 The ethical debate: for and against

The ethical debate on whether or not transgenic animals should be created and used for scientific research or increased food production has been going on for years. There are many reasons for both sides of the argument. Scientists mostly believe that transgenic animals should be made, as they can greatly improve and speed up research on deadly diseases, or help produce more food for our ever growing world population. Animal rights groups are against transgenic animal production because they believe these animals are treated unfairly and sometimes suffer during experiments, and they don't believe experiments using transgenic animals work. Some religions, like Judaism and Islam, have strict dietary rules, and question the acceptability of genetically engineered animals for food. When taking into account people's acceptance of transgenic animals, we have to ask ourselves some key questions. Some of these questions include whether or not the animals suffer or are harmed during the experimentation period, and what happens to the animals after we are no longer studying them? Do the benefits of transgenic animal creation take into account the animal's interests, when compared with the risks? Is it ethically acceptable to change animal natures through genetic engineering (Kslab Website, 2002)?

3.1.2 Public acceptance vs. Ethical test

We should not, however, be so quick to assume that public acceptance of transgenic animals is a decisive test for the ethics of creating them. "Practices such as

slavery or racial and ethnic discrimination have been 'publicly acceptable' at one time or another, and... many ethically controversial products, from pornography to so-called nutritional dietary supplements, meet the market test of economic viability" (Kslab Website, 2002). In the case of transgenic animals, we have to be sure that what we are doing is ethically fair to the animals, not just that people approve of and accept it.

3.2 Pro-Transgenic Arguments

3.2.1 Reasons for making transgenic animals

There are many potential benefits of transgenic animals. Scientists genetically engineer animals to help in the study of normal human physiology and development, provide organs that may be used for transplantation, increase food supplies from farm animals, find out how genes contribute to the development of a disease (Frame Website, 2002), and give them characteristics that mimic human diseases. "These research resources, for example, rapidly advanced the understanding of oncogenes – genes that have gone awry and are responsible for causing cancers. Moreover, researchers now seek ways to genetically modify the organs of animals, such as pigs, for possible transplantation into humans" (About.com Website, 2002). Also, through a type of transgenic process known as transpharming, scientists can make animals produce protein-based drugs in their milk. These drugs would be used to treat diseases, with the patients only having to drink the milk of the animal, minimizing injections and other forms of uncomfortable therapy.

3.2.2 *Why transgenic animals are necessary*

The Biotechnology and Biological Sciences Research Council of the United Kingdom does not agree with opponents of the use of animals in research that computer modeling or other non-animal techniques can successfully replace all animal experiments.

They believe that despite the increasing use of cell culture technology, mathematical modeling, and other alternatives, there are many areas in which the use of whole animals is essential. "Many physiological, behavioral, welfare, nutritional and biomedical studies by definition require the use of whole animals. Research into the use of animals as producers of large quantities of therapeutic proteins in their milk is designed to overcome the limitations of cell culture technology in producing large quantities of product economically." The BBSRC does not accept the argument that differences between species invalidate the use of animals as model species in biomedical and pharmaceutical applications. "The conservation of many genetic features during the common evolutionary history of animals, including humans, means that laboratory animals can be extremely good models for diseases of livestock and humans, and for increasing knowledge of these species." Many products in veterinary medicine are derived from human therapeutics that were developed using research on animals.

However, the BBSRC recognizes that the value of animals as models, particularly in the study of disease and pain, brings with it a responsibility for their welfare. They also believe that the welfare of laboratory animals is important not only for ethical reasons but also for obtaining the best possible data. Excellent scientific reasons exist for optimizing the welfare of these animals (BBSRC Website, 2002).

3.3 Anti-Transgenic Arguments

3.3.1 Reasons for not making transgenic animals

In the mid 1970's, Peter Singer, an Australian philosopher, wrote a book called *Animal Liberation*. In it he outlined the ethical principle of equal consideration of interests. This principle is designed to help us work out if the ways we use animals are acceptable or not. "It is applied to those higher order animals (which include all mammals [but not human beings], birds, amphibians, reptiles, and in some cases, fish [bony or cartilaginous], octopus, squid, crab, lobster or crayfish [fresh and salt water]. In the context of research, teaching and testing, higher order animals are also considered to include mammalian fetuses during the last half of pregnancy [i.e. an animal's babies before they are born], the unhatched young of birds or reptiles during the last half of their development in the egg, and marsupial pouch young) which can suffer or can be harmed by our actions. It is not applied to any lower order animals (which include some micro-organisms and non-vertebrate animals) which are unable to suffer or be otherwise harmed." According to this principle, when thinking about whether particular animal uses are right or wrong, we should give the same weight to the interests of the animals involved as we would give to our own interests if we were used in the same ways. It means that the interests of animals, whether or not they are the same as ours, should be regarded as being just as significant for them as our human interests are for us. Singer argued that if we say it is alright to use animals in ways that cause suffering or other harm simply because they belong to another species, because they are 'only animals', this is a sort of prejudice like racism or sexism, and is just as morally unacceptable (ANZCCART Website, 2002). I don't believe that Singer's argument is valid. We need to try and use

the technology we have available to us to try and make our lives better. As long as the experiments don't hurt the animals all that much, I believe they are alright to do.

For every advantage of transgenic animals that scientists can come up with, people have found some disadvantage to talk about. Although transgenic animals help scientists study diseases that naturally occur in humans, the animals might not be adequate or relevant models of the human disease. PETA's website (2002) points out that "enormous physiological variations exist among rats, rabbits, dogs, pigs, and human beings. A 1989 study to determine the carcinogenicity of fluoride illustrated this fact. Approximately 520 rats and 520 mice were given daily doses of the mineral for two years. Not one mouse was adversely affected by the fluoride, but the rats experienced health problems including cancer of the mouth and bone. As test data cannot accurately be extrapolated from a mouse to a rat, it can't be argued that data can accurately be extrapolated from either species to a human." Also, since these animals are used to closely mimic the human disease being studied, sometimes that the animals will suffer the pain and/or distress associated with the disease. PETA's point is a good one, but some diseases are transmittable from humans to rats and mice, and therefore using transgenic animals would be a good thing, as long as the animals aren't hurt much.

Creating organs in genetically engineered animals for transplant into humans is a risky task. The organs, more than likely, will be rejected by the human body, even when human genes are inserted into the animal's organ. Although scientists are working on alleviating this problem, there is also concern that such transplants could increase the likelihood of animal to human disease spreading. I don't believe that organ transfers from

animals to people is a good idea right now, because of all the risks, but I do think scientists should keep working on it, because it could be a really beneficial experiment.

Transgenic farm animals that are created to produce more milk, wool, or meat are considered unnecessary by some people. Why create a giant pig when you can just have two pigs of normal size? Transgenic farm animals sometimes have been found to suffer from arthritis, ulcers, kidney disease and fertility problems. Also, people are skeptical of scientific reassurances, especially dealing with food production. Mostly, in the wake of Upton Sinclair's The Jungle, an expose of the meat-packing industry of the early 1900's, some people might not be likely to trust the assurance of the government that transgenic farm animals are safe to create and eat (Kslab Website, 2002). I do think some transgenic foods should be made. For example, meat with less fat in it. If scientists can make this without actually raising the live animals first, it would be better. I don't know how ethical it would be creating animals with less fat.

Transpharming, which can cheaply create great amounts of biological products in animals' milk, can also cause a leak into the blood supply of the animal producing the product, although this is quite rare. The leaking of certain products can interfere with the animal's normal bodily functions (Frame Website, 2002). I think transpharming is a good idea. It is an easy and painless way to give people medications that could save their lives. I think that scientists need to work on the process a little more though, to make sure that the animals don't have the chemicals leak into their blood and make them sick."

There are also many welfare problems associated with transgenic animals. Only a very small percentage of embryos used in transgenic animal creation will survive and be born expressing the foreign gene. Those that do not express the gene are sometimes used

for other purposes, but most are killed. Also, transgenic experiments are unpredictable and the animals created can suffer "from deformities, disease and organ failure due to the insertion of the "foreign" gene into the wrong place in the DNA of their cells" (Frame Website, 2002). Uncontrolled expression of inserted genes may result in an increase in the mortality rate of the transgenic animal (ANZCCART Website, 2002).

3.3.2 Alternatives to transgenic animals

Many people believe that there are alternatives to transgenic animal creation. Carla Bennett, advise columnist for PETA, believes that simply educating people about the importance of "avoiding fat and cholesterol, the dangers of smoking, reducing alcohol and other drug consumption, exercising regularly, and cleaning up the environment" can save more lives than all animal testing being done. She believes that human and animal cells, tissues, and organs are so different from each other that the way animal's bodies react to a certain drug or chemical is not necessarily how our bodies would react. "For example, a dose of aspirin that is therapeutic in humans is poisonous to cats and has no effect on fever in horses; benzene causes leukemia in humans but not in mice; insulin produces birth defects in animals but not in humans..." Carla points out that there are sophisticated computers out there today that can predict the effect of a drug or substance on any organ in the human body, and suggests we look into using these computers instead of unwilling animals (Ask Carla Website).

3.3.3 Survey: Public's opinions on transgenic animals

The European Federation of Biotechnology conducted a series of surveys to find out the public's perception on biotechnology. The first survey (EFB Website, 2002) asks about the American public's opinion of new developments in biotechnology. "The

majority feels that the benefits outweigh the risks related to scientific innovations. Two thirds expect that genetic engineering will make life better and a small majority would accept risks to the environment (but not 'unknown' risks) in order to gain potential benefits from genetically modified organisms. The issue of human cell manipulation is sensitive; a significant majority says it is morally wrong. For specific purposes (e.g. to cure illnesses), a majority approves of gene therapy. The majority feels that biotechnology research should be pursued. And, while Americans find the end products of biotechnology attractive, they are sufficiently concerned about potential risk that a majority believes strict regulation is necessary."

The second survey (EFB Website, 2002) asks American consumers about their attitudes towards the creation of transgenic farm animals for increased food production. "Lower pricing, as a result of biotechnology, was more appreciated than better quality... Food labeling information was 'very important' for the majority. More support for biotechnology applications was found in groups with higher education, more interest in science, technology and biotechnology, more awareness of biotechnology, and with higher incomes. Women are less favorable towards biotechnology than men... Negative attitudes towards biotechnology are mostly influenced by beliefs that the technology is morally wrong... Acceptance increases significantly when consumers learn that agencies like National Institutes of Health (NIH) have determined that foods from biotechnology are safe."

The third survey (EFB Website, 2002) asks Americans what some of their oppositions to genetic engineering are. "Moral objections are the strongest arguments against genetic engineering in agricultural production and are expressed mostly by

women. Moral objections are mainly correlated with a lower income and lower formal education rather than with a religious orientation."

In the third survey, the German journal 'Beobachter' wanted to know from German citizens how they felt about the debate on gene technology. "One third of the people would agree with the position expressed against the technology, one third would disagree, and one third did not know yet. Genetic engineering of animals for medical research was supported by 20% and was opposed by 65%. Gene patenting was supported by 9% of the respondents and was opposed by 79%."

The fourth survey (EFB Website, 2002) studied the ethical concerns of Americans associated with genetic engineering of food production vs. medicine. "The applications of genetic engineering to food production were seen as riskier and less beneficial when compared to those in medicine... Ethical objections were also greater to food applications than to those in medicine, and were dependent on the type of organism manipulated...

The fifth survey (EFB Website, 2002) studied public emotions towards gene technology in Germany. "Elder people and women are amongst the most skeptical. Attitudes towards medical applications are more positive than towards applications in the agricultural area... The majority of the population (64%) feels uncomfortable with gene technology... However, the wish to totally ban gene technology has decreased, from 40% in 1988 to 29% in 1996" (EFB Website, 2002).

CHAPTER 4 - LEGAL ASPECTS OF TRANSGENIC ANIMALS

4.1 The Diamond vs. Chakrabarty Case

Ananda Chakrabarty, Ph.D. is a professor at the University Of Illinois College Of Medicine. His most notable creation is the Burkholderia cepacia bacterium, which was supposed to have the ability to "eat" oil. In the event of an oil spill, B. cepacia could be spread over the area to neutralize the oil and turn it into simpler substances that serve as food for aquatic life. This process had the potential to greatly reduce harm done to animals and the surrounding environment (The Invention Dimension, 2002).

Chakrabarty, who was working for General Electric at the time, applied for a patent in 1974 to protect his hard work and ingenuity that resulted in the production of B. cepacia. The patent was rejected by the U.S. Patent and Trademark Office because of the fact that the bacterium was a living entity. They were skeptical of patenting a life form. General Electric appealed the rejection to the courts because they felt the bacteria was a novel invention created from intense research. The bacterium was manmade and had never occurred naturally in the environment (Catron, 2000). In 1980, the Supreme Court voted 5 to 4 that life could be patented and Chakrabarty finally received his patent for B. cepacia. Prior to this landmark case, life forms were considered products of nature and not human inventions.

The Diamond vs. Chakrabarty case marked the first occasion that a patent was issued by the U.S. Patent and Trademark Office for the production of a genetically modified organism (Catron, 2000). It was ruled that anything in the world created by man should be allowed to be patented, therefore extending the patent rights to biological inventions. Chief Justice Warren Burger stated the relevant distinction is not between

living and inanimate things, but rather between human-made and naturally existing creations (THE Invention Dimension, 2002). Microorganism patents can now be obtained in most countries as a result of this extremely important case. The decision was a precedent for future patents including animals that have had human genes introduced into them.

A patent gives the patent-holder the absolute right to keep others from making, using, or selling the invention for about 17 years. To be patentable, a product must be new, useful, and non-obvious as well as have a full written description that would enable others to use or make the invention. The U.S. Patent and trademark Office has already granted more than 1,000 patents on human genes and fragments since the Chakrabarty case. Over 20,000 more patents are still pending (Catron, 2000).

4.2 The Oncomouse Case

While the Diamond vs. Chakrabarty case stands as the first patent of a multi-cellular organism, the "oncomouse" case draws equal if not greater attention for being the first animal to be patented. The oncomouse, developed at Harvard Medical School, is a transgenic mouse that has the tendency to develop cancer. It was created by modifying the regulatory mechanism of the myc gene and injecting the gene into a mouse embryo (Lasker Foundation, 2002). Chapter one contains a more detailed discussion of the techniques of creating such a transgenic animal. This myc gene, also referred to as an oncogene, is a gene that is essential to normal functioning of cells and organisms. When the oncogene is genetically modified, or when the cellular mechanism controlling its

expression is genetically altered, affected cells may undergo a transformation thereby becoming neoplastic, or cancerous cells (Lasker Foundation, 2002).

The Harvard mouse case involves a myriad of appeals and rulings, only the important ones will be discussed here. Figure 9 contains a time line of significant events involving the oncomouse case. There are several important cases prior to the 1988 Patent and Trademarks Office (PTO) approval of the oncomouse patent which planted the seeds of approval for the case.

As discussed earlier in section 4.1, the Diamond vs. Chakrabarty case is an important one because it was the first patent received for a living organism. Patents on other living organisms were denied until 1987, when the PTO in *Ex Parte Allen* found that a radiation-induced variety of oysters was suitable for patent (Deftos, 2000). However, this patent was later denied by the Board of Appeals in *Ex Parte Allen* (Woessner, 2002). The important detail to note in this case was that the board said the fact that a multi-cellular animal was involved was not a bar to patentability (Woessner, 2002). The issue, they exclaimed, was simply whether the subject matter is made by man (Woessner, 2002).

1972 - Patent for oil-digesting bacteria developed for use in treating oil spills denied by patent examiner who reasoned that microorganisms are "products of nature" and not patentable under U. S. patent law.

1980 – Diamond vs. Chakrabarty Case. U.S. Supreme Court approves patent of oil-eating bacteria.

1984 - Harvard applies for US patent of "transgenic non-human mammal"

1987 - U.S. Patent Commissioner rules that "The PTO now considers non-naturally occurring, nonhuman, multi-cellular living organisms, including animals, to be patentable subject matter"

1988 - PTO issues the patent for the Harvard transgenic oncomouse.

1989 – Animal Legal Defense Fund challenges the patent in federal court. Court held they had no standing to sue.

1992 - Harvard Mouse patent in Europe

1993 – Canadian patent office rejects oncomouse patent arguing that the animal was made primarily by nature.

1994 – Oncomouse patent in Japan

1995 - Canadian commissioner of patents upholds rejection of Oncomouse patent.

1998 – Canadian federal trial court rejects Oncomouse patent.

2000 - Canadian federal appeals court reversed both rejections and approves the patent.

Figure 9: Time line of significant effects in the oncomouse case (Deftos, 2000).

On April 21, 1987, only days after the Allen decision, the PTO announced that it would accept applications for "non-naturally occurring non-human multi-cellular living organisms, including animals" (Woessner, 2002). The PTO said that, in order to be patentable, the animals must be "given a new form, quality, properties or combination not present in the original article existing in nature in accordance with existing law" (Woessner, 2002).

With this decision, the U.S. Patent Office went ahead and awarded U.S. Patent No. 4,736,866 to Philip Leder and Timothy A. Steward of Harvard University on April 12, 1988. Below is an excerpt from the first claim of the patent:

> 1. A transgenic non-human mammal all of whose germ cells and somatic cells contains a recombinant activated oncogene sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage.

It is important to note that this patent covers all mammals, not only mice (the original tested species). Also, this patent covers all offspring of the original animal that received the oncogene. DuPont, who substantially funded the research, now holds the license to the oncomouse and sells the transgenic mice to research institutions.

With news of the patent out, numerous environmentalists and animal rights groups sought immediate protest. In 1989, the Animal Legal Defense Fund challenged the PTO rule in federal court. The Court of Appeals for the Federal Court determined that the ALDF had no standing to sue. So far, no court has ruled on whether or not animals are patentable subject matter (Woessner, 2002).

Other concern about animal patents has even arisen in Congress. In 1987 and 1989, U.S. senator at the time Mark Hatfield unsuccessfully attempted to introduce legislation to place a moratorium on animal patents (Deftos, 2000).

Since the 1988 United States PTO approval of the oncomouse patent, scores of other transgenic animal patents have been made across the globe. The oncomouse patent itself has been approved in Europe, Japan, and most recently in Canada. However, animal patent laws are far from settled. Large scale opposition to them still exists in Europe and even more so in undeveloped countries that characterize patent law as biopiracy (Deftos, 2000).

4.3 The Pros and Cons of Trying to Patent Life

While animal patents have been granted for many years now in America, there is still a much heated debate concerning whether or not patents should be granted to living organisms. Does the patenting of life represent just another example of corporate greed, or are scientists getting what is rightfully theirs?

Table 3 contains a listing of some of the pros and cons of patenting living organisms. By looking at this table we can see that the main benefit for allowing patents on genetically modified animals is that it offers a return on investment. When a firm places a patent on a specific living creature it ensures that no one else can create a copy. This allows the firm to license out the use of their patented organism, reaping a substantial profit. This return on investment benefit is a tremendous plus because investors feel more confident in giving their money to companies to do research. While this may seem like another case of monetary greed, society does, however, see some benefit from this in the increase in the rate and number of scientific advances.

While it may seem reasonable to give researchers credit and protection for their work, many people believe that patenting animal life is a dangerous practice. Many environmentalists believe there may be dangerous side effects to introducing genetically-modified species into the biosphere. They argue that patenting such animals only perpetuates their existence and therefore should be stopped. Likewise, many animal rights advocates and religious zealots believe that the tampering with nature can only lead to disastrous results. Others still hold that patenting mammals and other creates may set a precedent that would allow for future patents on genetically-modified higher mammals, even humans.

Pro	Con
Return on investment	Possible precedent for patenting other higher mammals, including humans
Profits offer incentive to invest in more research	Welfare groups fear introduction of mutant species into biosphere
	Some Farmers think the royalties from purchasing patented animals will be excessive
	Religious critics feel life should be exempt from exploitation for economic benefit

Table 3: The Pros and Cons of Trying to Patent Life

While there is no right answer to this debate it remains clear that genetically modified creatures do offer much benefit to human society (see chapter 2). The only way for research to continue is to ensure that scientists and firms get reimbursed for their efforts. While opposing groups continue with their protest, animal patents are still allowed in the United States and most other industrialized nations with new patents being applied for daily.

CHAPTER 5 - CONCLUSION

Through the completion of this Interactive Qualifying Project we have learned about what a transgenic animal is, how one is created, and the ethical and legal issues surrounding them. From this thorough research we were able to develop well-educated opinions on whether or not transgenic animal research should continue and if there is a large-scale societal benefit to such practices.

In chapter one we learned that a transgenic animal is one whose genetic composition has been manipulated in such a fashion that a foreign gene is expressed in the organism and its offspring.

While the technology used to create these animals is fascinating, it is important to realize the real world applications and societal benefit of such advances. As we have seen in chapter two, transgenic animals can be divided into four categories, including transfarmers, xenotransplanters, food sources, and disease models. Transpharming offers a great benefit to society in that proteins produced in the milk can help in the treatment of many human ailments such as anemia, cystic fibrosis, hemophilia, osteoporosis, and others. Yet at the same time, the transpharming animals do not appear to suffer (the new protein is merely secreted into the milk), and the animal is not sacrificed to obtain the beneficial drug. This IQP team agreed that animal pharming is an ethical practice because in most cases the animal is not even harmed.

Xenotransplantation is a technology that has been around since the early 1900's that allows the transfer of living organs, cells, or tissues from one species to another. While there are many serious issues concerning this practice, including possible infection

with an unknown virus, immuno-rejection, and others, research is still ongoing to minimize these risks. As of now, because of the amount of risks involved the IQP team feels that xenotransplants should be avoided unless a patient has no other option and is aware of the risks.

Food sources involve improving the production capacities of farm animals. The IQP group agreed that this practice should only be pursued in areas where the animal feels no effects.

Lastly, disease models use transgenic animals in the study of human illness. While models such as the Alzheimer's mouse which feel no effects are obviously fine, the IQP team feels that these experiments should only be performed when there is no viable alternative.

As we have seen there is widespread debate over whether transgenic animal experiments should even be performed at all. Many religious groups and animal rights advocates such as Peter Singer (Singer, 1990) feel that animal experiments should not be performed at all. After extensive research, this IQP team feels that transgenic experiments have enormous potential, and should continue. In cases where the medical gain is potentially great, and the animal suffering is insignificant, the benefits outweigh the negatives, and the experiments should be continued. The IQP team does feel, however, that some animal experiments should not be performed, such as on cosmetics. Also, the group feels that in cases where there is no medical benefit, such as increasing cow udder sizes with Recombinant Bovine Growth Hormone (RBGH) and reducing fat amounts in meat, the practice should only continue if there is no noticeable suffering or harm done to the animal.

While there is also opposition to the patenting of life forms, the IQP team could not find any good reason to stop the practice. Patents are necessary these days to protect intellectual property and allow research institutions to make a profit from their work. Most of the opposition to animal patents stems from an already existing resistance to animal experiments in general. Because transgenic and other animal experiments *are* allowed today, they should be patentable.

Overall, this group gained an appreciation for this fascinating new biological technology. While the technology itself is relatively new, many breakthroughs have been discovered already and there is great promise for the future. The IQP team does feel, however, that animal experiments should be closely regulated to minimize the amount of animal suffering that sometimes occurs.

GLOSSARY

Blastocyst	- Embryo development stage characterized by the formation of a cavity inside the morula (set of embryonic cells): the blastocœlium. ES cells can be injected into blastocysts in order to create chimeras or transgenic animals (Genoway, 2002).
Cleavage	- The series of mitotic cell divisions that produces a blastula from a fertilized ovum. It is the basis of the multicellularity of complex organisms. Also called segmentation.
DNA	- A large, self-replicating molecule that acts as the hereditary material by carrying information coding for all the characteristics and functions of an organism (Frame Website, 2002).
Embryonic Stem Cells	- An unspecialized cell that gives rise to a specific specialized cell, such as a blood cell.
Gamete	- A reproductive cell having the haploid number of chromosomes, especially a mature sperm or egg capable of fusing with a gamete of the opposite sex to produce the fertilized egg.
Genomics	- The study of all of the nucleotide sequences, including structural genes, regulatory sequences, and non-coding DNA segments, in the chromosomes of an organism.
Germ Cell	- An ovum or a sperm cell or one of its developmental precursors.
Homologous Recombination	- a process by which the two homologous pairs of sister chromatids (in the DNA molecule) align and crossover during meiosis
Meiosis	- The process of cell division in sexually reproducing organisms that reduces the number of chromosomes in reproductive cells from diploid to haploid, leading to the production of gametes in animals and spores in plants.
Nucleus	- A part of most living cells (except for those of bacteria), that contains the chromosomes, and functions as a store of genetic information and as the director of the cell's activities (Frame Website, 2002).
Oocyte	- A cell from which an egg or ovum develops by meiosis; a female gametocyte.
Oviduct	- A tube through which the ova pass from the ovary to the uterus or to the outside.

Ovum - The female reproductive cell or gamete of animals; egg.

Physiology - The biological study of the functions of living organisms and their parts.

Zygote - The cell formed by the union of two gametes before cleavage. A fertilized egg.

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