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TRANSGENIC ANIMALS

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ABSTRACT

This project examined the methods of creating transgenic animals, the reasons for doing so, and the effect of this controversial new technology on society via ethical and legal issues. A detailed description of what a transgenic animal is, how they are created, and their use in society today and in the future was followed by how transgenic animals have already provided much societal benefit, including information on human diseases, drugs to save human lives, and knowledge of the biological function of newly discovered proteins. Transgenic technology should have a positive impact on society as long as animal suffering is kept at a minimum and used solely for the purpose of helping humans.

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PROJECT OBJECTIVES

The objective of this IQP project was to examine the topic of transgenic animals and to discuss the effect of this controversial new technology on society. The report explains to readers what transgenic animals are, how they are created, and describes the types of transgenic animals created to date. The project then touches upon the ethical and legal issues encountered with such a breakthrough and complex technology. Because transgenesis has both positive and negative impacts on animals and on society, there is much controversy and unclear projections on where this technology will place us in the future. The goal of this paper is not to implant the idea that transgenic animals are good or bad, but to provide enough knowledge to allow readers to explore the topic and develop opinions of their own.

CHAPTER-1: TRANSGENIC TECHNOLOGY

Travis Abele

Transgenesis is a relatively new technology in which the DNA of an organism is altered to produce a new and unnatural desired trait. The overall genetic pool of the organism is kept relatively the same, but a foreign gene is inserted into an embryo prior to birth. The goal of this technology is to produce animals with desired benefits for mankind. When done successfully, all cells and tissues of the resulting animal contain the foreign gene, including their germ cells (sperm or ova), so these animals will be able to pass this desirable gene or genes to their offspring (Wheeler, 1991). By inserting certain genes into the genome of animals, thus recombining the DNA, many issues in the world today, most importantly health care, can be addressed. Such animals will be able to model life-threatening diseases, giving researchers a better chance to develop cures, produce human organs for patients who need them, and produce cost-effective pharmaceuticals.

Brief Transgenic History

The first genetically modified organism was a bacteria created in 1973 by Stanley N. Cohen and Herbert Boyer that contained genetic information from a variety of different species (Morrow et al., 1974). Soon after the technology was discovered, the Asilomar Conference was held in Pacific Grove, California to confirm that further research in this field should proceed under strict guidelines set by the National Institutes of Health in the United States, and by comparable organizations in other countries (Transgenic History, 2005).

The first transgenic animals were mice created in 1974 by Rudolf Jaenisch, a professor of biology at Massachusetts Institute of Technology (Jaenisch and Mintz, 1974). These mice contained viral SV40 leukemia genes, and the transgenes were not only present in the mouse but also in its offspring (Jaenisch, 1976; Transgenic History 2005). Then, in 1982, Ralph Brinster of the University of Pennsylvania inserted the structural gene for human growth hormone into mice embryos, and noticed the mice with the foreign gene grew much larger than those without the gene and also passed this trait to its offspring (Palmiter et al., 1982; Kwiram, 1996). The transgenic mouse is shown in **Figure-1** on the right next to a non-transgenic mouse on the left.

Figure-1: Picture of An Early Transgenic Mouse. The transgenic mouse on the right contains a foreign gene for human growth hormone thus it grows larger (Palmiter et al., 1982; Kwiram, 1996).

Recombinant DNA Technology

Because much of the creation of transgenic animals involves the manipulation of DNA, an overview of exactly what DNA is, and how it is used in the development of transgenic animals shall be discussed in detail. DNA, or deoxyribonucleic acid, is the molecule containing all the hereditary information for humans and almost every other

organism on earth. Most DNA is found in the cell nucleus, but small amounts can be found in the cell's mitochondria. Every cell in the organism has essentially the exact same DNA. DNA contains four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). (A) pairs with (T) and (G) pairs with (C) forming bases on a helix, which consists of a sugar (deoxyribose) and a phosphate, as shown in **Figure-2** (United States, 2008). The sequence of these base pairs is what makes each organism's DNA unique, and is what gives each organism its unique traits. Genes are sections of the DNA strand that encode a defined biochemical function, usually the production of a protein. The sequence of the DNA bases determines the structure of a protein, and the structure of a protein determines its function. Therefore, the genetic code determines what proteins an organism can make and their function (Seline and Friedman, 2007). These proteins then make the organism.

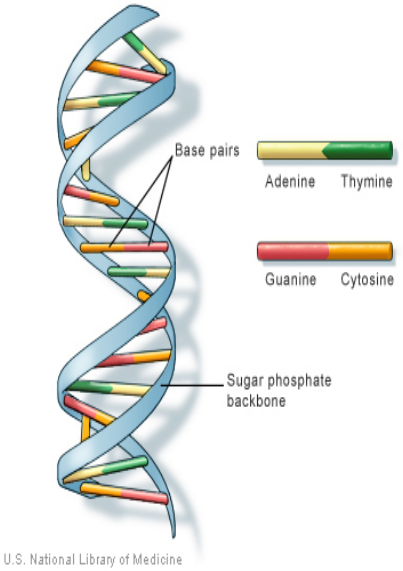

Figure-2: Diagram of the Structure of DNA. DNA is a double helix with base pairs (horizontal rungs in the diagram) in a distinct sequence (What is DNA, 2008).

To change the DNA to make it recombinant, scientists combine the original DNA with a different strand of DNA, creating a new strand of DNA. This recombinant DNA is

also referred to as a "chimera." There are three different ways recombinant DNA is created: Transformation, Non-Bacterial Transformation, and Phage Introduction.

Transformation involves inserting a piece of DNA into a vector (a plasmid DNA molecule acting as a carrier). Both the plasmid vector and insert are cut with a restriction enzyme to create compatible ends, then the two molecules are sealed together using DNA Ligase. The insert contains a marker, usually an antibiotic resistance gene, which allows identification of cells containing recombinant molecules. The recombinant plasmid is then inserted into a host cell, such as *E. coli* specially prepared (competent) to take up DNA.

Non-Bacterial Transformation is similar to Transformation except it does not use bacteria as the host cell. In this process, the DNA is usually directly microinjected into the nucleus of the cell being transformed. Finally, with Phage Introduction, a bacterio-phage (virus that infects a bacterium) is used to introduce the DNA into a cell. This process is similar to transformation except a virus is used to transfer the DNA to the cell.

Recombinant DNA is only effective when the host cell expresses the gene (makes RNA and protein). Thus the transgene is usually flanked by controlling DNAs that ensure correct expression of the transgene (Kuure-Kinsey et al., 2000).

Three Most Common Ways to Create Transgenic Animals

Currently, the three most widely used procedures for creating transgenic animals are microinjection of the cloned gene(s) into the pronucleus of a fertilized egg, injection of recombinant embryonic stem cells into embryos, and the use of retroviruses. Some other less common ways are also possible, and will be discussed in later sections.

MICROINJECTION

The main purpose of the microinjection method is to expose the fertilized egg to the transgene before cell differentiation begins, thus allowing the gene to be prevalent in the organism before the organism begins to develop. If the process works as planned, all cells in all tissues of the soon to be organism will contain this crucial gene. An egg and sperm are fertilized *in vitro*, and before the two pro-nuclei fuse inside the new zygote the male pronucleus is microinjected with the recombinant DNA (Figure-3). A fine point glass pipette (upper portion of the figure) immobilizes the embryo on one side, while on the other side the foreign DNA is inserted into the male pronucleus with an ultra-fine needle (lower portion of the figure). As one can see in the figure, the pronucleus visibly swells as it is microinjected.

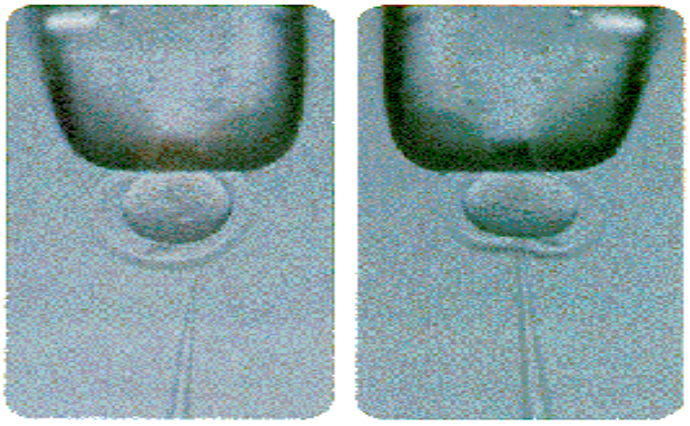

Figure-3: Photograph of the Microinjection of DNA into the Male Pronucleus of a Single Celled Embryo. An in vitro fertilized embryo is immobilized using a suction pipette (upper portion of the figure). The male pronucleus is microinjected with foreign DNA using a very fine pulled glass needle (Wheeler et al., 1991).

Following microinjection, the embryo is cultured to the blastocyst stage *in vitro*, then placed back into a pseudo-pregnant female or foster mother. The embryo develops the same way as a typical embryo into a fetus, and normal pregnancy is observed. This microinjection procedure is the most efficient way today to create transgenic animal lines, even though only about 25% of these embryos actually produce transgenic offspring (Wheeler et al., 1991). The reason for this low efficiency is unknown, but likely includes destruction of the embryo during microinjection, and spontaneous abortion of the fetus (Eide, 1997). A visual description of the microinjection method is shown in **Figure-4**.

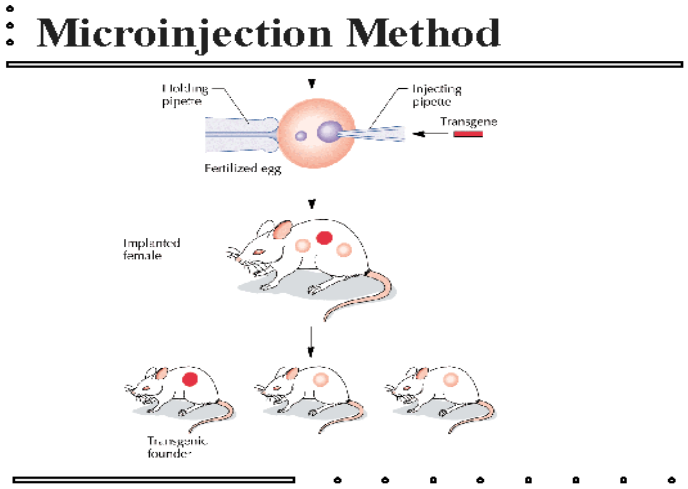

Figure-4: Diagram of Construction of a Transgenic Animal by Microinjection into Zygote. A transgenic mouse is created using the microinjection method. Implantation of the manipulated embryo is in the center of the diagram (Eide, 1997)

EMBRYONIC STEM CELL INJECTION

The second method for creating transgenic animals is somewhat similar to the first method discussed, but involves making embryonic stem (ES) cells transgenic instead of a male pronucleus. An embyo is created by *in vitro* fertilization (IVF) but instead of being injected with foreign DNA, the embryo is cultured to the blastocyst stage. The

blastocyst stage occurs about 5-7 days after fertilization. The blastocyst consists of an inner cell mass of embryonic stem (ES) cells and an outer trophoblast. The word blastocyst means "bud" or "sac" referring to the fetus which at the time is only a cellular sac with a central cavity. The ES cells are isolated then injected with foreign DNA. Once it has been determined that the transgene is present in the ES cells, they are injected into another blastocyst. That blastocyst is then implanted into a surrogate mother as before to create transgenic pups (Wheeler et al., 1991).

As with transgenic DNA microinjection, with this procedure every organ system of the animal usually contains transgenic DNA, including the reproductive system (Wheeler et al., 1991). And the ES cells can be pre-screened to ensure transgene insertion prior to injection into the blastocyst, which improves efficiency. A summary of the ES cell method is shown in **Figure-5**. This method is particularly important for studying the development of transgenic organisms while being able to control their genes, and works very well with mice. However, the DNA microinjection method works better on a wider variety of species.

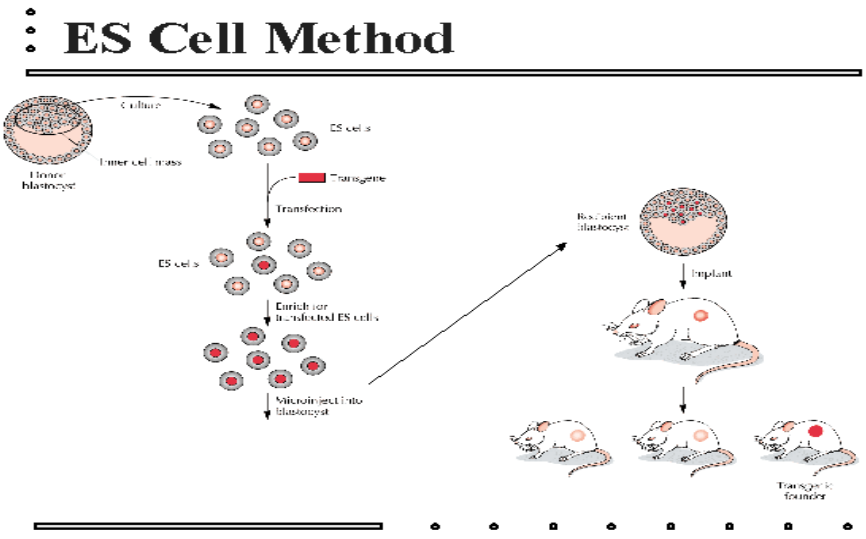

Figure-5: Construction of a Transgenic Animal Using ES Cells. ES cells are isolated from a blastocyst (upper left). The transgenic DNA is then inserted into them via viruses or microinjection, then the ES cells are injected back into another blastocyst (upper right) for further development (Eide, 1997).

VIRUSES FOR DELIVERING DNA

The third method for creating a transgenic animal is somewhat different from the first two discussed. When DNA is microinjected into the fertilized egg, DNA randomly inserts into the genome, and there is a possibility that the animals' normal gene function will be disrupted, leading to health problems such as birth defects, brain damage, cancer, etc. (Gillespie, 2008). However certain viruses can be used to target where the transgenes are inserted. Adeno-associated viruses (AAV) have been shown to insert at specific locations that do not damage a host cell's function. However, retroviruses are more efficient at integrating foreign DNA, although the integration site for retroviruses is often random. The biggest advantage of viral delivery is the fact that the cellular infection rate is very high.

A retrovirus is a virus that contains its genetic information in RNA rather than DNA, and this virus can be engineered to contain a transgene. As shown in **Figure-6**, the virus is then used to infect a cell, such as an ES cell, with its RNA. An enzyme from the virus (reverse transcriptase) copies the RNA to double stranded DNA. Once in the nucleus of the cell, the DNA sequence from the virus is inserted into the cell's genome at the target spot (Gillespie, 2008).

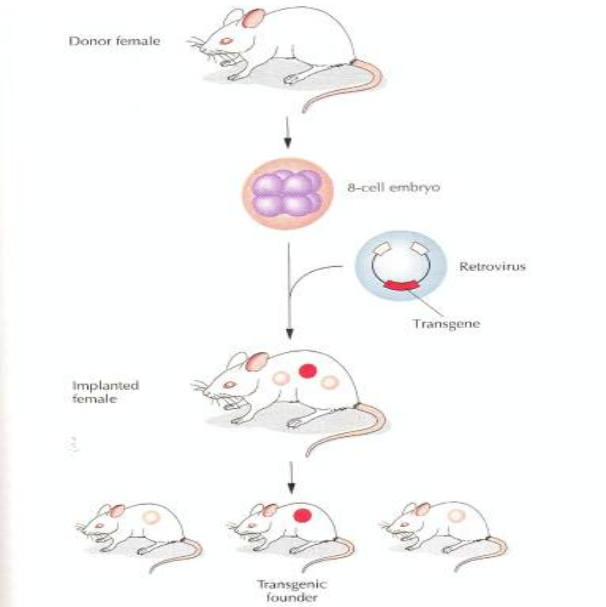

Figure 19.1 Establishing transgenic mice with retroviral vectors. Cleavage-stage embryos, usually at the eight-cell stage, are infected with a defective retrovirus carrying a transgene. Implanted females (foster mothers) give birth to transgenic pups. Matings are carried out to determine which pups have the transgene in their germ line cells. Transgenic lines can be established from these founder transgenic animals.

Figure-6: Creation of a Transgenic Animal Using a Retrovirus. In this case, an 8-cell embryo (purple) is infected with a virus containing a transgene (right), then the embryo is inserted into a foster mother as usual (center). The offspring of a mouse with a foreign gene is shown (lower). Of the three mice the foster mother gives birth to only one that is transgenic

Even though the retrovirus method usually leads to fewer problems with the organism's development, there are concerns about new viruses being created by recombination with naturally occurring viruses within the animal. Also, the success rate for this method is very low, with only 1% of new animals possessing the transgene.

Other Ways to Create Transgenic Animals

NUCLEAR TRANSFER TECHNOLOGY

Somatic cell nuclear transfer (SCNT) technology allows the construction of transgenic cells with the same genetic background as the host. In nuclear transfer, a nucleus from a donor cell (usually a skin fibroblast cell nucleus) is removed and transplanted into an enucleated fertilized egg cell. This process was used to create Dolly,

the world's first cloned mammal. A modification of this process (**Figure-7)** uses unfertilized oocytes whose membranes are fused with membranes of adult transgenic cells. This process was used to create the sheep Molly and Polly. In each case, the embryo will gave rise to an organism containing the same background genetic information as the nuclear donor. Also, these clones only share nuclear DNA, not mitochondrial, unlike identical twins (Strachan and Read, 1999). Nuclear transfer technology is generally more efficient than microinjection.

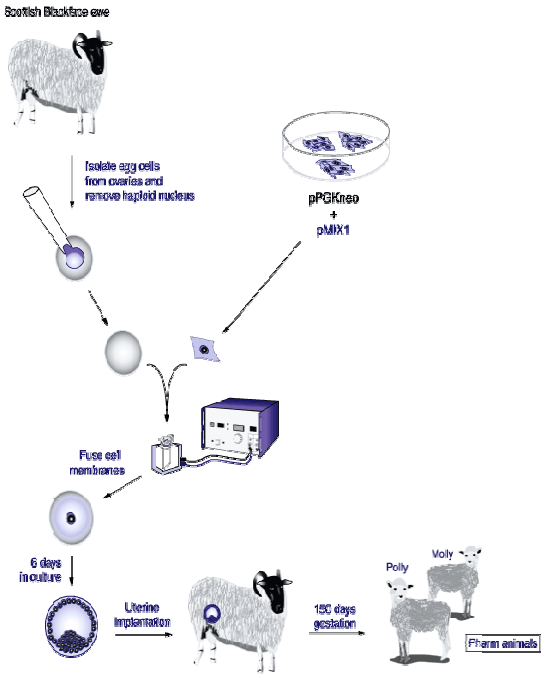

Figure 7: Diagram of the Somatic Cell Nuclear Transfer Technique. Shown here is the process in which the transgenic sheep Molly and Polly were created by means of nuclear transfer technology (Miesfeld, 2001).

An important aspect of nuclear transfer is that the cells, cultured *in vitro*, once genetically modified, all will contain the transgene. And depending on whether a tissue-specific promoter was used, the transgene will be expressed in the tissue of interest. Also, because the cells are cultured, site-specific genetic alterations can be made by homologous recombination (Akagi, 2008). Homologous recombination involves the

breaking and repair of DNA to produce a precise exchange of material between two DNA strands, one containing the transgene plus some host DNA, and the other being host DNA (Homologous Recombination, 2008).

A related technique, called the "Honolulu nuclear transfer technique", is a technique for generating diploid egg cells, cells having a normal two similar complement of chromosomes. Microinjected early stage diploid oocytes are placed into calcium-free media containing strontium, which activates the oocytes to divide *in vitro*. Cytochalasin-B is also included in the activation medium to prevent polar body formation, which would cause chromosome loss (Miesfeld, 2001). This procedure is shown in **Figure 8**.

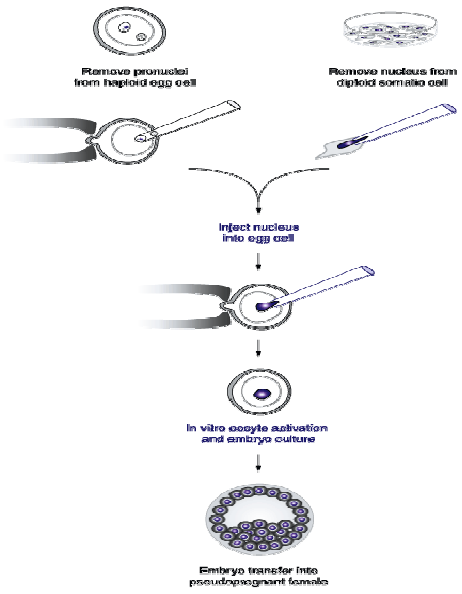

Figure 8: Diagram of the Honolulu SCNT Technique. This parthenogenic technique creates diploid transgenic eggs from early stage diploid oocytes (Miesfeld, 2001).

The SCNT technique, which has successfully been applied to animals but not humans yet, has been acclaimed as a method for treating some diseases. For example, ES cells genetically identical to a patient could be created from a patient's skin fibroblast cell nucleus, the ES cells could then be used to create new tissue in that same patient for healing a heart tissue following a heart attack. Or in another example, skin cells could be reprogrammed into insulin producing cells and then placed into the pancreas of a diabetes patient, allowing them to produce insulin (The Future of Cloning, 1998).

SCNT provides faster development than microinjected animals. For example, microinjection requires 44 months of development for sheep, but only 18 months for SCNT. And pre-screening of the nuclei to ensure transgenesis is possible which helps ensure ideal protein expression (Nuclear Transfer Technology, 2005). **Figure 9** shows a recent history of different animal breeds genetically modified by means of SCNT.

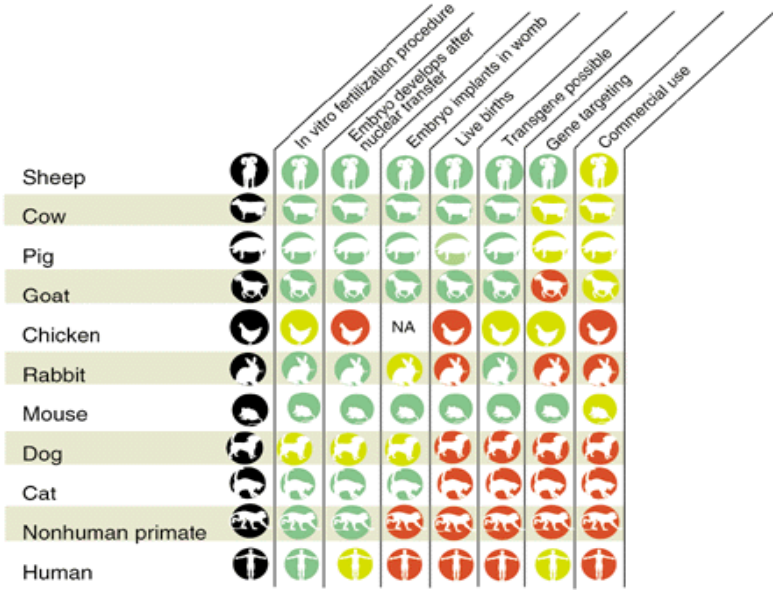

Figure 9: Recent History of Transgenic Animal Production by SCNT. Green denotes success, red means not done yet, and yellow means very close to being done successfully (Miesfeld,

GENETICALLY MODIFIED SPERM

The final method for producing transgenic animals discussed here is less common than the other methods. In the past, it has been difficult to genetically modify sperm cells in animals prior to fertilization because treated sperm have failed to mature under *in vitro* conditions. Noriyoshi Sakai, Ph.D, and Kayoko Kurita were able to allow immature sperm cells from zebrafish to survive long enough *in vitro* to receive foreign genes inserted by a retrovirus (Kurita et al., 2004). These genetically modified sperm were then allowed to fertilize eggs in culture, producing transgenic embryos and zebrafish. These transgenic zebrafish carried the foreign gene in every cell of their bodies (not mosaic), including the germ cells, allowing them to produce transgenic offspring. Scientists were easily able to tell whether the offspring were transgenic (Spencer, 2004). This process was discovered in 2004, so it is a very new technology, but there have also been reports of successful transgenic pigs and mice produced by sperm-mediated gene transfer.

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Chapter-2: Transgenic Applications

Michelle Miller

Transgenic animals have multiple uses in modern science and medicine. Transgenic animals have been used as disease models for human ailments such as AIDS and Alzheimer's disease. They have been modified to produce insulin in their milk through a process called Transpharming. Xenotransplantation can offer hope to people waiting for an organ transplant by growing new organs within a different species. They can even be altered to produce more food. The benefits to society that these transgenic animals provide as documented in this chapter will serve as a prelude to the ethical discussion in chapter 3.

Disease Models

One of the easiest ways to examine and experiment with human diseases is to use animal models, to allow experimentation on the animal instead of humans. Mice have always been a favorite model since they are small, easy to care for, have short life cycles, and produce large litters to provide large amounts of statistical data. The problem with testing diseases on mice is that the do not get most human diseases. Transgenics can solve this problem by inserting key genes for the disease into the genome of the entire animal. The animal then suffers from the same symptoms, or a part of them, that a human with the disease would, which in turn enables scientists to study treatments and medications for the disease. Scientists can also see the side effects and efficiency of the treatments. In terms of the FDA, if a medication satisfactorily passes the tests on animals,

it is then ready for human clinical trials which is the step before the drug can be released to the public (Transgenic Models, 2007).

HIV-1 Rat

Acquired Immune Deficiency Syndrome (AIDS) is actually the later stages of infection from a retro-virus called Human Immunodeficiency Virus (HIV). In 2007, it was estimated that 33.2 million people lived with HIV on the planet. The problem with normal animal testing of HIV is that the virus is specific to humans; instead SIV (Simian Immunodeficiency Virus) was often used to test a highly related virus on primates such as chimpanzees. However primates are expensive and rare, whereas rats or mice are much more abundant and easier to study for genetic tests because of their relatively short life cycle and large litters (Baylor Scientists, 2001).

HIV infected transgenic rat and mice have both been created, however the rat model seems to work better because of its larger size. Rats provide more blood to collect and analyze and easier organ inspection. A team of scientists from University of Maryland's Biotechnology Institute first created a rat with a transgenically modified genome that contained the mutated genome of HIV (Bunce and Hunt, 2004). The implanted HIV genome had the genes *pol* and *gag* spliced out to prevent transmission of the virus (Kohn, 2001). The original transgenic female rat which carried the spliced HIV genome was bred with a wild type rat; the offspring were a mix of uninfected and infected rats. The rats, who displayed a phenotype of cataracts, were separated based on the severity of their cataracts (Reid et al, 2001). A southern blot test was then performed

to compare wild type rats with the transgenic rat's offspring to confirm that the offspring of the female transgenic rat contained the same HIV gene. The southern blot test indicated that the rats were indeed transgenic. By the time the rats reached the age of 5-9 months they displayed symptoms similar to that of an AIDS patient. In addition to the cataracts, the rats developed kidney disease, heart problems, weight loss, and skin lesions.

These rats provide a solution to the small animal experimentation problem for HIV/AIDS research. The University of Maryland recently licensed the HIV-1 transgenic rat to Harlan Sprague Dawley, Inc., one of the world's largest providers of rats to medical research facilities, which will hopefully allow for a vaccination for HIV be developed faster by allowing widespread availability of small animals to use for HIV research (University of Maryland, 2002).

Alzheimer's Mouse

Alzheimer's disease (AD) is a progressive and fatal brain disease with an unknown cause. It is thought however that the cause of AD could be the accumulation and development of senile plaques, which are abnormal clusters of β-amyloid protein fragments deposited between nerve cells. Also important are neurofibrillary tangles, which are twisted strands of a tau protein found within dead and dying nerve cells (Information, 2008). Plaques and tangles show up mostly in the cerebral cortex and hippocampus, the areas of the brain that control memory and cognitive thought. The buildups of these neurotoxic proteins associated with plaques and tangles cause a

dysfunction in the sending and processing of information in the brain, which in turn cause the symptoms of AD. The common age for onset of symptoms for AD is 70; however there is an early onset AD which develops in patients 30-40's (Alzheimer's Disease, 2008).

In 1995 the first transgenic mouse to show the pathology of AD was announced in *Nature* (Games, Adams et al, 1995). Professor Dave Adams from Worcester Polytechnic Institute and his fellow researchers used a known mutation from an early onset Indiana family to reproduce the symptoms of AD. The mice developed senile plaques at about 6-8 months, and their brains showed similar damage to the brain that you would see in an AD patient. However Alzheimer's mouse did not form neurofibrillary tangles like an AD patient (Games, Adams et al, 1995). This same mouse model was tested by Elan Pharmaceuticals (California) with an antibody vaccine that removes β-amyloid, and the mouse showed improved cognitive function (Schenk et al., 1999). Subsequently Elan has begun human clinical trials with this vaccine.

In the past few years Frank LaFerla, a professor from the University of California, created transgenic mice who exhibit both plaques and tangles (Kingman, 2004). Previously researchers could only test medications that would get rid of the plaques on mice; however this new transgenic model allows them experiment with ways to get rid of both the plaques and tangles. Laferla and his team of scientists tested the effectiveness of antibodies to the beta-amyloid protein injected into the brain in order to remove the plaques and tangles. Within a week of the tests the plaques and tangles had disappeared. The same test done with antibodies to the tau protein did not work as well (Kingman,

2004). The creation of the first mouse displaying AD symptoms was a huge breakthrough and was a catalyst for an invigorated search into the cause and possible cures for AD.

Transpharmer Animals

Transgenics has also been used to make an animal produce a drug that can later be purified and given to the public. A transpharmer is created, like all other transgenic applications, by knocking out part of the animals genome and replacing it with a gene to produce a certain protein. Originally this was done by secreting the protein into the animal's blood, however the problems that arose with that were that the proteins would adversely affect the animal's physiology which restricted its uses. But by localizing the production to breast tissue, there was no longer an issue of the proteins entering the blood stream. The proteins would secrete into the milk that the animal produced (Ziomek, 1998). The ease of using milk for making mass-produced proteins and other chemicals is that there is much more freedom with the range and variety of compounds that can be produced since very little enters the bloodstream of the animal. In addition the drug can be delivered in milk form and little subsequent purification, if any, is required. The animals most commonly used for transphamering are sheep, cows, and goats (Houdebine et al., 1997).

Goats

Human antithrombin III (hAT) is a serum glycoprotein that controls blood clots by inactivating the clotting factor thrombin, as well as inhibiting other clotting factors. This protein is very useful anticoagulant for when a person is undergoing coronary bypass surgery (What are the HD…2007). Genzyme Transgenics Corporation first created cloned transgenic goats containing the gene to produce recombinant hAT. It was found that the transgenic goats did in fact produce recombinant hAT in their milk (Genzyme, 1999). Subsequently, this hAT became the first FDA approved transpharmed medicine.

Recently GTC Biotherapeutics has been advancing research in recombinant hAT produced from goats. In August of 2006, ATryn® was granted market authorization by the European Commission. ATryn® is GTC's treatment for people who have Hereditary Antithrombin Deficiancy (HD) and are undergoing surgery where they have a high chance of deep vein thrombosis (ATryn®, 2008). In the United States ATryn® has been granted fast track status by the FDA which is a great sign for getting it on the market sooner (GTC Biotherapeutics, 2008). GTC is also developing research into many other recombinant proteins such as albumin and alpha-1 antitrypsin (AAT) all produced from well-cared for transgenic herds of animals (Recombinant, 2008).

Nexia Biotechnologies is also developing a product from transgenic goats that is significantly different from that of GTC. Nexia has developed a product they call Biosteel, which is spider silk proteins isolated from the milk of transgenic goats, which are then spun together (Products, Nexia). Nexia is still determining how and to what

specifications to use the Biosteel for medical purposes. Biosteel also has other possible uses, for example spider's silk is three times as strong as Kevlar, the material used for bullet-proof vests. Nexia is also researching a chemical called butyrylcholinesterase (BChE) which is found in small quantities in human blood. BChE has been shown to protect the body against nerve agents which are toxic chemicals absorbed via skin or from inhalation. Nerve agents then travel through the blood stream to the nervous system where they proceed to wreak havoc by interrupting communications between nerves. Currently there are treatments for nerve agents but they must be administered quickly after exposure, and in most cases permanent damage still occurs. The U.S. military conducted studies and showed that elevated levels of BChE in the blood stream protected lab animals from nerve agents. The hope is that recombinant BChE, derived from transgenic goats milk, can be used as a protective measure for troops in a war setting where a nerve agent would be used, thereby reducing damage done by the nerve agent significantly.

Sheep

Alpha-1-antitrypsin Deficiency in humans generally results in problems with the lungs and respiratory functions. Alpha-1-antitrypsin (AAT) is a protein normally produced in the liver, which protects the lungs by stimulating an enzyme that fights bacteria and cleans the lungs of dead tissue to keep them functioning properly. AAT-deficient patients can display a number of symptoms since AAT deficiency opens them up to develop many other diseases including cirrhosis, asthma, aancreatitis, gallstones,

emphysema, and cancer (Researchers, 2007). The current treatment for this deficiency is to replace the missing AAT with Prolastin, which is a medication produced by Bayer pharmaceuticals. Prolastin is a solution whose main active ingredient is AAT derived from human plasma. The problem with this medication is that human plasma is expensive and of short supply. In addition Prolastin has to be administered intravenously which a lot of patients find uncomfortable (rAAT, 2008).

In response to the need for a better method of AAT production, PPL Therapeutics successfully created a transgenic sheep that produced recombinant AAT in its milk (Hughes, 2000). Obtaining ATT from transgenic sheep's milk offers a much easier method for AAT production; in addition the ability to mass produce AAT will help lower the cost of the medication for AAT deficient patients. Currently Bayer and Arriva Pharmaceuticals are working on getting a recombinant AAT derived from yeast, delivered in aerosol form, as a treatment for hereditary emphysema through FDA clinical trials (rAAT, 2008).

Cows

Human lactoferrin (hLF) is a protein that helps to protect the body from infections and strengthens the immune system. hLF has been found in human tears and lung secretions, and has been shown to fight bacteria that cause infections of the eye and lungs (Lactoferrin, 2008). It has also been found in large quantities in a human substance called colostrum, which is the milk produced by the mammary glands in the few days right before and after birth. Colostrum, which is also known as immune milk, helps build up

the newborn's immunities, deliver essential nutrients, and helps clear the baby's digestive system. In 1989 Dutch company Gene Pharming was allowed to try to genetically engineer a cow which produced hLF in its milk (Krimpenfort et al, 1991). Eventually they produced one very famous male, Herman, who was the world's first transgenic cow, and who carried the gene to produce hLF, however as it was a male it could not produce milk for tests. A few years later that male cow fathered a female cow that had recombinant hLF in her milk (Van Berkel et al, 2002). Gene Pharming then proved that the recombinant hLF was so similar to natural hLF that they expected it to work the same. In 2001 the FDA put out a "generally recognized as safe" notice for the use of purified hLF from bovine milk for the use in sports foods and functional foods (Tarantino, 2003). The ability to produce hLF in cow's milk is very useful since milk is already a well established food worldwide.

Xenotransplanters

One of the current major problems with getting transplants is that a patient waiting for an organ will oftentimes die before one becomes available. The supply of organs needed for transplants is so small compared to the need that a lot of people started to look into alternatives (Corporate, 2007). Pigs have been experimented with for quite some time because they have a physiology similar to humans, and are relatively cheap and easy to get compared to a chimpanzee (Catez, 2005). The current problem with xenotransplantation, the transplanting of tissue from one species to another, is that pigs naturally create sugars on the surface of their cells which humans recognize as foreign

causing immunorejection (Mooney, 1999). When the human body notices that those sugar shouldn't be there, it kick starts an immune response to attack the new organ which is the most common reason for organ rejection. The basis behind xenotransplantation is to generate genetically altered animals who are missing the genes that encode the production of the sugar that initiates the immune response.

Two major things hold back the transplantation of porcine organs into humans, immuno-rejection, and the fear of spreading diseases across the species border. Currently a company called Xeno Transplants Corporation is testing whether baboons have an immune reaction to organs transplanted from a transgenic herd of pigs they created (Corporate, 2007). Their pigs are missing the gene which encodes the animal protein markers that cause rejection. So far their tests on rats and mice are promising (Kaiser, 2002). In addition, their herd of pigs are also missing the Porcine Endogenous Retrovirus, which is one of the biggest concerns for spreading diseases between humans and pigs. It is hoped that these advances in transgenics can have a huge benefit to the thousands of people waiting for organ transplants (Xenotransplantation, 1996).

Transgenic Animals as Food Sources

Transgenic animals can also be genetically modified to provide additional nutrients or even to grow larger. Transgenic animals are not commercially available on the market because of ethical and safety issues, but they are still made and used for research (Harper, 2006).

Superpig

The hope behind research into pigs was to create a pig who grew faster, larger, and ate less food. In an attempt to do so, scientists spliced the gene for Human Growth Hormone (HGH) into the pigs genome. The pig, who was has been termed Superpig, grew larger and quicker than normal pigs, consumed less food, and had less body fat (Pursel 1997). However, Superpig suffered from several painful side effects of the gene transplant. He suffered from arthritis, gastric ulcers, stomach lesions, lack of coordination, and severe muscle weakness. It was then determined that the pain that was caused to the pig was too great to warrant further research, so scientists placed a voluntary moratorium on farm animal transgenesis with human growth hormone. While it was a good original idea, the negative side effects of human growth hormone on pigs makes it not viable for human consumption or the commercial market (Rexroad and Caird, 1994).

Super Salmon

While there were setbacks in the research of human growth hormone transgenesis on farm animals, fish, salmon in particular, were found to react well to this inserted gene. The reason why fish react better to human growth hormone research is that they swim in water so if they were to grow larger their muscles are not needed to support themselves against the force of gravity (Devlin et al, 2001). Transgenic salmon have been shown to grow 3-6 times faster than normal salmon, and can reach marketable size a year earlier

than other commercially produced salmon. They are also 10-30% more efficient at converting their food consumed into muscle weight (Fletcher and Shears, 2002).

There are fears for the environment and the current wild salmon population if some of the transgenic salmon get loose (Stokstad, 2002). However transgenic salmon producers are taking steps to avoid those dangers (5 Myths, 2008). At the moment there are no transgenic animal food products on the market, but many are in development. For example, AquaAdvantage© Salmon from AquaBounty Farms are salmon who can grow from egg to market size in 1-1 ½ years, compared to traditional commercially farmed salmon who take 2-3 years to be marketable. AquaAdvantage© salmon and similar products could solve a much needed supply demand on salmon in the general market which would also hopefully decrease the over-fishing of wild salmon (Transgenic Animals, 2008).

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Chapter-3: Transgenic Ethics

Michelle Miller

This chapter will spotlight the arguments for and against making different types of transgenic animals. General arguments in favor of transgenic animals focus on the medical, scientific, nutritional, and financial benefits that the animals provide. On the other hand arguments against transgenic animals include animal welfare, environmental issues, and religious conflicts.

Animals have long been genetically modified through selective breeding to be better at their job or to look better. However it has not been until recent technological advancements that gene modification was even possible so it's emergence as a new technology obviously raises questions on the basis of its experiments. The scientific community has taken steps to create ethical boundaries based on failed experiments or mistakes. Some general worries about transgenic animals include that containment can never truly be enforced, which could lead to a transgenic animal out competing its own species, or mating and spreading the modified genes. Also connected to the control issue are worries of what genetically modified food would do to a human who ingested it. There is also a religious conflict in that genetically altered animals go against the natural order of things. In addition there are activists who argue that genetically altering animals affects their general welfare.

However transgenic animals have proven to provide many benefits to humans including the production of drugs or simply more food, reduced need to use pesticides or herbicides, and a reduction in the overall number of animals used for testing. Some examples of things that transgenic animals have produced are outlined in the previous

chapter. There is also the argument that discoveries about humans should not come at the cost of the suffering of the animals used for the tests. The question is where does that balance lie, but the main trouble is that it is different for different people and for different types of experiments. Another trouble lies in trying to outline an ethical code for transgenic experiments when new discoveries in the field are happening so rapidly, which creates worry that scientists are doing things they will regret later. The attempt to create legal boundaries on transgenic research is discussed in the next chapter.

Transgenic Positives

The positive benefits that transgenic animals offer fall into four broad categories: medicinal, scientific, food enhancement and productivity, and financial.

Medicinal uses for transgenic animals include disease models, transpharmers and xenotransplanters. Disease models such as the Alzheimer's mouse and the AIDS rat have given scientists a way of understanding viruses and diseases in a way they couldn't before. It is because of the disease models that scientists were able to use that knowledge to create cures and better treatments for diseases (Baylor, 2001). The use of humans for such experimental tests is highly unethical, therefore disease models are greatly needed. Transpharmers are medically beneficial because of their ability to produce pharmaceuticals in their mammary cells which gets secreted into their milk. Those pharmaceuticals can then be extracted from the milk in much larger quantities than could be produced using traditional methods. The production of drugs in the milk of farm animals has been shown to create very little side effects to the animal itself. Animals that

are xenotransplanters are created to have organs that are transplantable in humans. This is done by removing certain markers on the surface of the animal cells which usually cause rejection in humans. The need for transplant organs currently greatly exceeds the supply; the number one preventable death in the United States is waiting for an organ transplant (Edwards, 2006). The use of animals for organ transplants would relieve great suffering that many people live through while waiting for an organ.

Scientific models are used to identify the functions of specific newly discovered proteins. For example by removing a certain gene that encodes for a protein that has an unknown function scientists can then observe the phenotype of the genetically altered animal to discover the function of that protein. Doing such experiments allows researchers to explore the gene modification as a treatment for certain disabilities.

Food enhancement through transgenics has been used to create animals such as super fish, who as explained in the previous chapter is a fish who is has been altered to produce extra growth hormones and therefore grow larger and faster on less food than other normal fish. Other animals have also been altered similarly to mature faster, grow more muscles, and consume less food. Animals can also be altered to be resistant to diseases which help with losses at farms.

All of the categories above also provide a financial benefit since they all relieve a demand on a product. While this financial benefit is not generally ethically relevant, it is relevant in when it comes to sponsorship of research. All of these benefits that transgenic animals provide do not come without the costs of the mistakes and failures that occurred in their development.

Transgenic Negatives

The negatives of transgenics usually arise from mistakes or unexpected results from experiments, and most of the time the animal involved ends up being deformed or suffers which create an ethical issue. Disease models such as the AIDS rat or Alzheimer's mouse have disease symptoms induced upon them that can lead to suffering, deformities, and a reduced life span (although the suffering varies widely with the disease being modeled, Alz mouse only gets initial symptoms of the disease while oncomouse can die of cancer). Animals that have increased growth hormone expression do provide more food and faster growth than normal animals. But as a side effect of that abnormal growth, the animals usually develop symptoms such as arthritis, stress, irregular heart and lung function, and early death. These animals have been shown to suffer greatly while alive.

The main ethical issue that lies within creating transgenic animals is that by using them and their lifespan to solve human problems, the animals are then worth less than humans. This usually does not become a public ethical issue with mice, but changes if the animal involved is more human like such as a primate. A lot of the ethical issues surrounding transgenic animal creation stem from religious sections who feel that science is going too far, and that altering the very way an animal works is like trying to play god. Many people are afraid that altering an animal in such a way will have consequences that affect not only that animal but also the human race in general. The possibility of transgenic animals escaping into the wild and forever altering the ecosystem of our planet forever is also a huge concern. The transgenic animals could possibly wipe out their wild

type counterparts (Donnelley, 1993). The possibility of creating a new, dangerous organism that we can't control is also a huge fear. The Beltsville Pig or Super Pig is one the clearest examples of negatives of transgenic research since the animal suffered so horribly.

Alzheimer's Mouse Ethics

Alzheimer's disease affects more than 5 million Americans, and if the sixth leading cause of death in the United States ("Alzheimer's Disease", 2008). The Alzheimer's mouse that was created in part at WPI is an example of a transgenic animal model that provides a great benefit to the scientific and medical community that has little to no suffering for the animal. The mouse, which was genetically altered to form senile plaques, performs slower on a maze test which appears to be the extent of its suffering. With respect to a medical benefit, that mouse was subsequently used by Elan Pharmaceuticals Inc. to develop a vaccine to remove the plaques. The vaccine was successful in mice (Schenk et al., 1999), but the first human clinical trial was aborted due to inflammation in 1% of the patients (Young, 2002), however Elan has since re-entered new clinical trials with a second generation vaccine that appears to cause no inflammation in the AD patients. In this case of transgenesis, the medical benefits seem to outweigh the harm being done to the animal, if any harm is done at all.

Transpharmer Ethics

Transpharmers are animals genetically altered to produce a desired chemical. Originally transpharmers were made by altering the genome of the animal to produce the chemical in its blood; however this had multiple negative side effects on the animal. Most commonly the animal just didn't know how to react to such a large concentration of foreign chemicals in their blood. In response to that, scientists started making the gene alterations to only produce chemicals in the mammary epithelium which then get secreted into the animals milk, with minimal amounts actually affecting the animal's physiology. So far this new method of transpharming shows very little damage done to the animal. At the same time these animals can produce large quantities of pharmaceuticals that require little if any purification. Current transpharmers are cows, goats, and sheep, and they have been used to create a host of useful proteins including those to help phenylketonuria (PKU) and cystic fibrosis, as well as insulin, growth hormone, and blood anti-clotting factors. There are people who feel that transpharming from animals is more ethical than growing them to kill and eat their meat. Even religious activists find a hard time arguing against transpharmers.

Xenotransplanter Ethics

For quite a while there has been a very serious need for organs in developed countries. Animal organs are immunorejected because protein and sugar markers on the surface of animal cells tend to aggravate the human immune system and incite an

immune-rejection response. Transgenics alters the genome of the animal so that they do not produce the cell markers that cause rejection (Kaiser, 2002). Currently pigs are the best option for xenotransplantation because of their similarity to human physiology and their low cost compared to simians. The main concern with using xenotransplanted organisms is the infection and spreading of pig retro-viruses in humans. That kind of jumping from species to species is dangerous since it is not a virus normally residing in the human population. In 2000 guidelines for xenotransplantation were issued by the Public Health Service that requires xenotransplanters to "procure source animals from herds or colonies that are screened and qualified as free of specific pathogenic infectious agents, and that are maintained in an environment that reduces exposure to vectors of infectious agents (U.S. Public Health Service, 2001)." However there is still the question of animal welfare, and some groups argue that just keeping the animals in a non-native environment is enough to make the experiments unethical. The use of animals for transplantation requires the animal to live without an organ which can result in death or shortened lifespan. The ethical challenge behind xenotransplanters is whether a human life is worth that of an animal and vice versa.

Food Sources

People are also concerned about genetically modified food reaching the general public when there has been little testing on the effects of genetically modified food on the human body. It is worried that these foods will cause some kind of harm to humans. The majority of the transgenic foods currently on the market are plants; however genetic

engineering has been used to make cows resistant to diseases such as mad cow disease. The thing holding back genetically altered foods from booming on the marketplace is the lack of consumer acceptance. A possible use for transgenic food sources includes making animals that are resistant to diseases to send to impoverished villages to fight global hunger. Transgenic food sources can also be used to bolster the nutritional value of a food by having it produce extra vitamins and minerals. Using transgenic plants could help produce more crops in less space which could save the destruction of natural environments of animal species. The use of transgenics on plants have significantly less ethical issues since it is generally accepted that plants do not have consciousnesses.

Super Pig Ethics

The hope behind super pig was that it was a means of producing more meat for less money in less time. However Super pig ended up starting a large discussion over the ethical boundaries of transgenic experimentation. Although Super pig's creators surely had better results in minds, they did achieve their goal, the pig they had designed to grow faster and bigger did in fact grow bigger faster, but what they had not expected was the potential side effects of such an experiment. Because of his larger size, super pig developed arthritis, ulcers and stomach lesions, and overall muscle weakness. After the effects of the experiment were seen, scientists unanimously agreed on a moratorium on growth hormone experiments on farm animals. They did so because the discovery they had made was overshadowed by the intense suffering felt by the animal. Super pig is one of the clearest examples of a negative effect of trangenics.

Super Fish Ethics

The fish supply of the world are currently in danger, demand for fish as a food source is rising so most species are being over fished to meet the need. Nowadays extra fish are bred in captivity at hatcheries, but even they are not enough so scientists decided to create a transgenic fish that could grow faster than normal fish and consume less food. The way Aqua Bounty Farms did so was by making a fish that produced growth hormone year round as opposed to wild type fish that only use growth hormone in spring and summer. Aqua Bounty Farms salmon grow to be the same size as other salmon they get there about six times faster. Currently they are in the process of getting their salmon FDA approved for testing on human consumption (Stokstad, 2002). The biggest concern about transgenic fish is the possibility of them escaping into the environment. Since they grow faster than the native species there is fear that a large scale release of transgenic fish could completely wipe out a species. Companies like Aqua Bounty Farms are combating that issue by first sterilizing the transgenic fish so that if they were to escape they would not mate with a native species and would not hunt in the areas where mating occurs and baby salmon grow. In addition they are feeding the fish food pellets and when the fish escape they tend to look for items that look similar to the pellets for food such as tree bark. The difference between growth hormone experiments on fish versus other animals is that fish do not develop the same kind of negative side effects as other animals since they are floating in water. In fact the genetically altered salmon experience no suffering that we can measure. Their benefits are numerous not only to human fisheries and fish

markets but also to the native salmon race. These super fish could be a plausible solution to the demand for fish.

Trangenic Ethics in Religion

Ethics are the basic principles of conduct governing an individual or group. Many individuals learn their moral values through religion, which in turn creates societies with the same or similar ethical values. Almost all of the world's religions preach a kindness and respect for animals since they are God's creatures too. Hinduism reveres the cow above all other animals since it is the staple of life in many countries; it is a sin to harm a cow. Buddhism promotes vegetarianism since animals are divine creatures who are greatly involved in the staple of the religion, reincarnation. When it comes to Christianity there are mixed messages since animals are said to be divine creations of God but that they have no souls and humans have dominion over them. It has been suggested that since humans evolved to the point to create this technology, that it might have been God's will to have transgenic experimentation happen. Either way, religious values often are a source of friction to the advancement of transgenic sciences.

Ethics and Legality

A major issue in transgenics is patenting and whether discoveries made in the field should be open to all. The argument for patenting is supported by the private business sector since investors support research into transgenics in order to make their

money back when they sell the patent or a product derived from it. Without the return on their investments, funding for research would depress if patents were no longer issued. On the other hand, discoveries that could be used to help find cures and treatments for diseases should be available to the larger scientific community to facilitate a faster discovery process. In addition there is the sense of ownership that a scientists feels over their creations, how if those creations are living organisms can a person really own that or should they own intellectual property over the organism. This discussion leads to the age old question of what is property. Should an animal be the owner of themselves as a human is? The trouble with identifying ownership when it comes to transgenic animals is where does the animal begin and the transplanted gene end. It is argued that the animal owns all that is naturally occurring, and that once they have part of them that is not naturally occurring is when ownership changes, however even this system has problems. The next chapter will go into more detail on legal arguments surrounding transgenic animals.

Chapter Conclusions

This chapter discussed the negative and positive consequences of creating transgenic animals, and the ethical arguments that accompany each of the uses for transgenic animals. Although there are many potential negative side effects to transgenic research, the possible benefits to many aspects of society are great. With strong regulation and support, transgenic animals can be used to save many human lives. The ethical discussions outlined in this chapter serve as a prelude to the next chapter which

discusses the legal issues involved in trying to solve some of the ethical disagreements about transgenic animals.

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CHAPTER-4: TRANSGENIC LEGALITIES

Travis Abele

As mentioned in the previous chapters, the use and manipulation of transgenic animals has obvious advantages for humans, not just for healthcare, but also for the economy. But as is typical for any complex technology, laws are in effect to regulate its usage. And considerable debates have taken place regarding whether animals should be patented. Clearly, there can be many dilemmas for a new technology involving live creatures.

The biggest question revolving around transgenic animals is whether or not such animals should be patented. In 1793 , according to Thomas Jefferson, one of the nation's founding fathers, defined anything patentable as, "Any new and useful art, machine, manufacture, or composition of matter, and any new and useful improvement on any art, machine, manufacture, or composition of matter" (A Brief History… 2003). Not only can animals be considered an art, machine, manufacture, or composition of matter, but in theory humans could be classified as a composition of matter as well, possibly making them patentable according to Thomas Jefferson. The idea of patenting humans is out of the realm of reality, but the question of whether to patent animals is not.

Patents and Transgenic Animals

Patents for transgenic animals are somewhat complicated. Patents in the United States have history dating back to the colonial days. The earliest patents were supposedly passed in the 1640s by the Massachusetts Bay Colony, largely influenced by the English Parliament. It wasn't until the Constitution was drafted in 1776 that patents were fully

established. Article I, section 8, clause 8 of the United States Constitution states, "Congress shall have power… to promote the progress of science and useful arts by securing for limited times to authors and inventors exclusive right to their respective writings and discoveries." (A Brief History… 2003)

After a period of about one hundred years of revising rules for patents, such as whether non-U.S. citizens should be granted patents, and how long it should take, in 1870 the legislation regarding patents was brought together in a single act. A major clarification of the law was that the sale or use of the invention before the two year grace period of receiving the patent was illegal. In 1952, the present structure of U.S. patent law was adopted. Here, the law stated that an invention had to be novel, and for the first time it gave a description of patent infringement. Also, the word "art" mentioned earlier was changed to "process" in which if an invention required multiple elements, these elements were defined in functional terms.

Today, the three requirements to receive a patent are: novelty, utility, and non-obviousness. The novelty requirement simply states that the invention must be new and original. The utility requirement states that the invention must be useful in some way, and that the invention must do what the author says it will do. If it does not operate as it says it should, then the patent will be rejected. The non-obvious requirement states that the invention must result in new or non-obvious characteristics in comparison to similar previous inventions. Factors such as color, size, and texture are obvious features, and changes in these will not be granted patents (Patent Requirements, 2007).

First Patent for Microbes, Diamond v. Chakrabarty, 1980-1983

The first patent regarding life was granted in 1930, called the Plant Patent Act, which covered newly developed asexually reproducing plants. However, until 1980, no patents had been allowed on animals or microbes. During this year, the Supreme Court held the famous case *Diamond vs. Chakrabarty*, in which the Court ruled that a genetically modified microorganism was patentable. Ananda M. Chakrabarty was trying to obtain a patent for a genetically engineered bacterium that broke down crude oil into smaller chains, such as gasoline, diesel, etc. Chakrabarty wanted to obtain patents on the following:

1. "The method of producing the bacterium"
2. "An inoculum comprised of a carrier material floating on water, such as straw, and the new bacteria"
3. "The bacteria themselves" (Edwards, 2001).

Initially, a patent was issued for the first two, but not for the third because according to The Patent Office Board of Appeals, the bacteria themselves were products of nature, and life itself cannot be patented. However, The United States Court of Customs and Patent Appeals eventually reversed this rejection, and the patent covered not only the process but the organisms themselves. Apparently, the Court considered the bacterium as a "manufacture" and a "composition of matter" as outlined in the Patent Law of 1793. Shortly thereafter, the Patent and Trademark Office stated in the Official Gazette:

> "The Patent and Trademark Office now considers nonnaturally occurring non-human multicellular organisms, including animals, to be patentable subject matter. The Board's decision does not affect the principle and practice that products found in nature will not be considered to be patentable subject matter. An article of manufacture or composition of matter occurring in nature will not be considered patentable unless given a new form, quality, properties, or combination not present in the original article…"
> (Edwards, 2001).

This final Chakrabarty ruling in 1983 stirred lots of controversy, especially in the business world. McDonalds and Frito-Lay asked their suppliers not to supply them food from genetically modified seed, and many believed that modifying animals would eventually lead to the spread of disease and would be of great harm to the human race, causing humans to lack genetic diversity.

The Harvard Oncomouse Case, 1984-1988, 1992, 1999

Just one year later in 1984, Harvard University scientists Dr. Philip Leger and Dr. Timothy Stewart inserted human oncogenes into a mouse to study the impact these cancer causing genes would have on the animal. The oncogenes increased the probability of developing neoplasms, typically malignant tumors in the mouse. The purpose of this experiment was to produce laboratory test animals with an increased probability of developing cancer (Leder and Stewart, 1984). In 1984, these two scientists filed for a patent, and in 1988, the United States Patent and Trademark Office granted them U.S. Patent 4,736,866.

Within the patent, it claimed the mouse as, "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" (Leder, and Stewart, 1984). This of course caused a tremendous uproar, not only because animals had now been patented, but many believe at the discretion of their health and well being. The patent also stated how these mice would be tested for carcinogenicity, saying "If the animals are to be used to test materials thought to be only weakly carcinogenic, the transgenic mice most susceptible of developing tumors are

selected, by exposing the mice to a low dosage of a known carcinogen and selecting those which first develop tumors" (Leder, 1984). The mice are clearly harmed, causing animal rights activists to be upset, not caring whether these cancer studies had any implications on humans.

The two men also received two more patents, one in 1992, and the other in 1999. U.S. patent 5, 087, 571, issued in 1992, covered the method for preparing a cell culture from a non-human mammal, and U.S. patent 5, 925, 80 issued in 1999, covered the testing method using transgenic mice expressing an oncogene.

In Europe, Oncomouse was a different story. The European Patent Office (EPO) is the organization that grants patents, and it reviewed the Harvard Oncomouse case. As part of the European Patent Convention, Article 53 (a) excludes patents for "the publication or exploitation of which would be contrary to *ordre public* or morality, and Article 53 (b) excludes patents for "animal varieties or essentially biological processes for the production of … animals." (Bioethics… 2006). In 1989, the European Patent Office rejected the patent, believing that the mouse was excluded as per Article 53 (b). However, this was appealed shortly thereafter, while the Board of Appeal claimed that the article only excludes "animal varieties" and considered the Harvard mouse to not be an animal variety, but just an animal. In 1992, the European oncomouse patent was granted.

Many people did not agree with the ruling on the patent, stating that the other article 53 (a) about morality described the mouse. The European Patent Office had to weigh the benefits of the mouse, such as future impact on the lives of human being suffering from cancer, to the harmful effects the cancerous tumors had on the mice, and

whether or not these effects were important. In 2004, the European Patent Office officially ruled that the potential benefits of the mice experiments far outweighed the dangers the mice would endure, but amended the patent to only cover mice.

Oncomouse in Canada

To this day, Canada remains the only industrialized country in the world to prohibit the patenting of higher life forms. The Canadian Patent Act is identical to that of the United States, claiming an invention to be "any new and useful art, process, machine, manufacture or composition of matter, or any new and useful improvement in any art, process, machine, manufacture or composition of matter" (Ching, 2003). Canada allows single-celled organisms, such as yeast and bacteria, as well as genetically modified crops to be patented, and animal or human cell lines. However, the Supreme Court believes that more complicated forms of life, such as humans and animals, fall into a completely different category and should not be patented. In 1993, Harvard obtained a patent for the oncogene and the *process* from the Canadian Intellectual Property Office, but not a patent for the mouse itself. Harvard tried to appeal this decision, but in 1998 the Trial Division of the Federal Court dismissed the appeal.

Just four years later, the Canadian Federal Court of Appeal overturned the trial judge and allowed the mouse to be patented. But the case was sent to Parliament, and with a 5-4 ruling, it was decided that a living animal cannot be patented. The Supreme Court concluded that the mouse is not a "manufacture" since it is a product of nature and not something artificially put together, nor can it be considered a "composition of matter". Justice Michael Bastarache said "Just as 'machine' and 'manufacture' do not

imply a living creature, the words 'composition of matter' are best read as not including higher forms" (Ching, 2003). He also wrote, "Higher life forms are generally regarded as possessing qualities and characteristics that transcend the particular genetic material of which they are composed" (Ching, 2003). He added, "A complex life form such as a mouse or a chimpanzee cannot easily be characterized as 'something made by the hands of man" (Kondro, 2002).

One of the major reasons why Canada was against the patenting of higher forms is because they believe that if an animal is allowed to be patentable, a living organism that lives and breathes similar to that of a human being, patenting of humans should be allowed as well. Clearly, patenting human life crosses a severe line, and they believe animal patents cross a similar line. Justice Bastarche further warned in 2002 after the famous Oncomouse patent denial that "there is no defensible basis within the definition of invention itself to conclude that a chimpanzee is a 'composition of matter' while a human being is not" (Kondro, 2002). Even though this may seem to put Canada behind other countries in the world as far as their biotechnology industry is concerned, some Canadian researchers believe that this benefits them. Arnold Naimark, Director of the University of Manitoba's Centre for the Advancement of Medicine in Winnipeg, and Chair of the federal government's Canadian Biotechnology Advisory Committee, stated in 2002 that "the patent only gives the patent holder the right to exclude others… If there is no [Oncomouse] patent in Canada, there is no restriction on people being able to do research on the Harvard Oncomouse if they get a hold of it" (Kondro, 2002).

Other Patented Transgenic Animals

Since the Harvard Oncomouse case, there have been over 600 patents awarded to various transgenic animals, leading to a variety of controversies. According to the law, an animal patent covers animals with a particular gene sequence unnatural to other animals within its species, and the patent allows the company to prohibit others from selling or using these animals without its permission for 17 years (Andrews, 1993). The patent also covers whatever the animal may be producing, such as organs, pharmaceuticals, or antibodies. The offspring of the transgenic animals are also covered by the patent, even if the chromosomes of the offspring are different than the chromosomes of the parents. However, the company must prove that these offspring carry the foreign gene and are capable of performing the special duties as outlined in the patent.

TRANSGENIC FISH

The first transgenic fish were produced in 1997 (Devlin et al., 1997), and strict regulations have been imposed on transgenic fish. Transgenic fish have been developed for the purposes of human nutrition, biological research, environmental monitoring, and aquacultural modeling. For aquacultural purposes, fish have been genetically altered to improve productivity, increase their resistance to disease, and contain greater nutritional value (Eenennaam, 2006). Fish tend to lay a large number of eggs, and embryonic development takes place mostly outside of the mother, giving them an advantage over other transgenic animals. However, if these fish are accidentally released into the environment, they are most likely to cause environmental harm than any other organism.

They are very difficult to contain because they are so mobile and pose the danger of invading native ecosystems.

In the United States, the Food, Drug, and Cosmetics Act, as well as the FDA's Center for Veterinary Medicine regulate the use of transgenic fish. To this date, no transgenic animals have been approved for use as food, however the Aqua Bounty transgenic Atlantic Salmon have been under review for five years (Eenennaam, 2006). These salmon are shown in **Figure 1**. The Food and Drug Administration (FDA) evaluates the environmental risks of transgenic animals as directed by the National Environmental Policy Act. As far as outside the United States, there are currently no international standards, related to the confinement of transgenic fish and their possible dangerous escape into the environment.

Figure 1: Picture of Transgenic Salmon Containing a Growth-Enhancer Gene. Such transgenic salmon are much larger than normal salmon (Eenennaam, 2006).

DOLLY THE SHEEP

The Roslin Institute, which originally invented cloned transgenic sheep, has been awarded many patents on the ground-breaking invention of nuclear transfer technology for transgenic animals. In December of 2007, the Roslin Institute was awarded U.S. Patents 7,304,204 and 7,307,198, which covered the methods of using differentiated cells to clone ungulate animals, fetuses, and embryos (Dolly the Sheep…2007). These new patents joined a number of other patents regarding somatic cell nuclear transfer technology as described in chapter 1 of this report. Roslin also possesses U.S. Patent 7,232,938 for the cloning process that uses "fusion or microinjection of a quiescent ungulent donor cells" (Dolly the Sheep…2007). This process has been used over the years to clone numerous animals, including farm animals, rodents, cats, and dogs. Although the process of cloning an animal using SCNT is by itself not necessarily transgenic if a foreign gene is not inserted in the host genome during the process, patents on the sheep SCNT procedure will pertain to transgenesis in the future.

Positives and Negatives for Patenting Animals

Clearly, patenting animals gives biological scientists the motivation to pursue research projects related to altering the genes of animals for potential benefits to humans, knowing that if they are successful in developing an animal for a specific cause, there is a reward for them down the road. And this reward could fund additional scientific advances in the future. Any kind of new technology or invention will create numerous jobs within that industry, thus helping the economy. Transgenic animal technology appears to have very little harm on human life, as long as animals are not patented for

food purposes. Even if animals are created to produce more food or provide better nutrition to humans, they provide no dangers as long as the technology is strongly regulated by the FDA and the resulting food is screened numerous times for defects or potential hazards to the digestion by humans.

With respect to transgenic patenting negatives, some scientists argue that awarding such patents actually will hinder scientific research by limiting access to the animals to researchers that can afford it, not to all scientists. This argument was made immediately following the award of the U.S. Oncomouse patent, but as the years went on, and Harvard and Dupont softened their licenses, this argument has more recently taken a backseat. Although the possibility of one day finding a cure for cancer using transgenic animals such as Oncomouse makes a strong case for allowing such animals to be created, especially if animal suffering is minimized using pain killers and early euthanasia, some scientists and politicians argue that some disease models do not portray disease in the same way humans would, thus the model is invalid. Although this may be the case, the thousands of peer review articles that have appeared highlighting new scientific facts learned from transgenic animals attest to their validity for mimicking at least a portion of the disease. Some scientists also believe no organs produced in an animal will work properly in a human, but this will only be known after we try it.

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CONCLUSIONS

This project examined several important topics with transgenic animals, including describing what a transgenic animal is, how one is created, different examples of transgenic animals, and discussing the ethical and legal issues surrounding their use. In the earliest days of transgenesis, the process was extremely inefficient resulting in dead embryos and negative screens for the presence of the trans-gene. But eventually the technology improved, and new techniques, including somatic nuclear transfer, have greatly improved the method with higher success rates and allowing the process to work on larger animals, such as cows and sheep.

The most controversial topics associated with this technology deal with ethical and legal issues. Clearly, animal rights supporters are against the use of transgenic animals for any purpose, but the fact that these animals will have a major positive impact saving lives far outweighs the pain associated with some of these animals. We strongly support the areas of transgenesis that produce no pain in the animal, including transpharming and models like Alzheimer's mouse. For those models that can produce some pain to the animal, we believe such research should be continued so long as animal suffering is kept to a minimum, either by using pain killers when advanced disease stages must be studied, or by sacrificing the animal prior to advanced disease formation. We also support transgenic areas such as fish food sources and xenotransplanters that involve animal sacrifice, because the animals do not suffer while alive, yet they clearly save human lives.

With respect to transgenic legal issues, we both agree with the U.S. Oncomouse court case in which animals were found to be legally patentable. However we also support laws designed to minimize animal suffering, and preventing transgenic experiments with no clear benefit to society. We believe that if transgenic animal technology is allowed to be pursued more heavily, society will benefit not only medically but economically as well.