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TRANSGENIC ANIMALS AND SOCIETY

An Interactive Qualifying Project Report

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ABSTRACT

This project's purpose was to study the topic of transgenic animals and their effects on society. The first two chapters examine the technological aspects, namely how the animals are made and how they are categorized, while the third and fourth chapters explore the ethical and legal sides of the controversial topic. The authors then provide their own opinions based on their research.

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PROJECT OBJECTIVES

This project's objective was to study the science of transgenic animals and to discuss the effect this technology has on society. Chapter 1 explains the technical aspect of the science, what a transgenic animal is and how they are made. Chapter 2 classifies the different types of transgenic animals that have been engineered, and lists examples in each category that have already been made. Chapter 3 discusses the ethics of transgenic animals, weighing the benefits to society against the detriments to the animal. Chapter 4 investigates transgenic legalities and the major court cases involving transgenic animals. The conclusions sums up the information given in the previous four chapters and gives the authors' final opinions on the concept.

Chapter-1: Transgenic Animal Technology

Neil Crawford

The focus of this chapter is to define what a transgenic animal is, and give an overview of the technology and methods used to create them. Understanding the process by which such animals are created is crucial to understanding the possible applications and ethical issues of this technology, discussed later in the project.

What is a Transgenic Organism?

A transgenic organism is simply an organism that has DNA from another species inserted into its genome. But this definition only scratches the surface. There are many different *types* of transgenic organisms with a wide range of characteristics. As new technologies are developed the definition may have to change to incorporate all the varying types of genetically engineered organisms. For now though, perhaps the best definition is that of R.J. Wall: **"**The definition of transgenic animals is evolving, but a transgenic animal is one containing recombinant DNA molecules in its genome that were introduced by intentional human intervention" (Wall, 1996). The reason there must be a specific mention of intentional human intervention is because transfer of genetic material also happens regularly in nature. Two of the most common examples of this natural DNA transmission are the transformation of bacteria and the incorporation of viral DNA into its host cell. As we will see later, both of these natural processes can also be manipulated to produce transgenic animals.

DNA Cloning for Transgenesis

Before you can create a transgenic animal, you first have to decide what DNA to insert into the animal and then prepare it by cloning. To clone DNA it is inserted into a vector, such as a plasmid or virus, which is used to amplify the DNA and to express it in the host animal. The transgene of interest can be isolated from the rest of the genomic DNA of the donor organism using restriction enzymes that cut at specific points, or it can be amplified by polymerase chain reaction (PCR). The transgene is then packaged into a carefully selected vector. The vector is crucial to the successful production of transgenic animals for several reasons, the first of which is to insure the successful incorporation of the transgene into the DNA of the target animal. For example, viral vectors usually contain specific sequences at either end that allow the insert to be easily incorporated into the target animals genomic DNA. The vector can also contain certain promoter or regulatory genes. The promoters increase the level at which the gene is expressed in the target animal, and their effects can be drastic, sometimes up to one thousand times more expression than insets not containing a promoter. Regulatory sequences can also genes can also allow the transgene expression to be controlled, such as being turned on by external stimuli like the presence of heavy metals or specific proteins. By controlling these stimuli, the expression of the transgene can be controlled. Once the transgene insert is cloned into a vector, the vector is then grown to amplify the material.

After enough of the insert has been obtained it can be introduced into the target animal. There are several methods by which this can be done, but the two predominant methods are pronuclear manipulation and embryonic stem cell manipulation.

Pronuclear Manipulation

This is the most common method for producing transgenic animals (**Figure-1**). It begins by fertilizing an egg by *in vitro* fertilization (IVF). The fertilized egg is then harvested before the sperm and egg pro-nuclei are able to fuse (diagram upper). At this point, the foreign cloned DNA is introduced into the egg, usually by microinjection (diagram left). After the foreign cloned transgene DNA is injected, the fertilized egg is allowed to develop to the blastocyst stage (diagram right), then the egg is implanted into the uterus of a pseudopregnant foster mother (lower right) that has been induced to be receptive to the egg by mating her with a vasectomized male. The egg is allowed to develop normally within the foster mother, and after the gestation period the transgenic animal will be born.

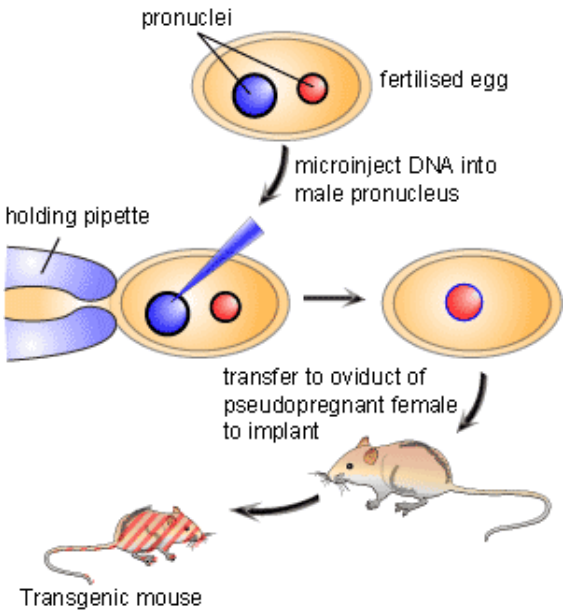

Figure 1: Schematic of the Pronuclear Injection Method. A suction pipette (diagram left) is used to hold a newly fertilized egg in place so the male pronucleus (large blue circle) can be injected with DNA. The embryo is then cultured (diagram right) and transferred into the uterus of a foster mother (lower right). (Walinski, 2004)

There are several different ways transgene DNA can be introduced to the fertilized egg before the nuclei fuse. The most common way is through microinjection where a tiny glass needle is stuck into the male pronucleus and the DNA solution is injected (**Figure-2**).

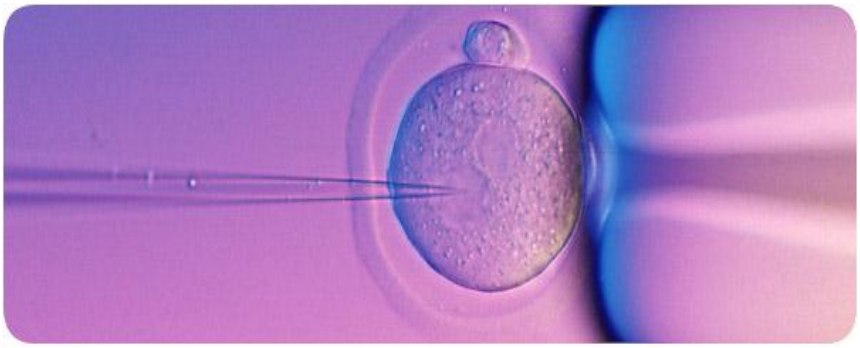

Figure 2: Picture of Pronuclear Injection. This picture shows a newly fertilized egg (diagram center) being held in place with a suction pipette (diagram right). A glass micropipette (diagram left) is being used to inject a DNA solution into one of the two pro-nuclei which have not yet fused. (Medical Research Council, 2010)

Other DNA introduction techniques include electroporation, retroviral infection, sperm mediated DNA transfer, and somatic cell nuclear transfer (Niemann, 2000). Each of these is aimed at getting the foreign cloned DNA into the fertilized egg before it begins to divide. In electroporation, the eggs are incubated in the presence of the DNA and are subjected to electrical pulses that cause the egg to take up the DNA. In retroviral infection, the foreign cloned material is actually RNA packaged into a gutted out retrovirus, such as HIV, which is then used to efficiently infect the egg. The RNA inside the virus is inserted into the host DNA by a viral reverse transcriptase protein, and proliferation continues. In sperm-mediated DNA transfer, the foreign cloned DNA is incorporated into the sperm before fertilization takes place, either by incubating the sperm cells with the foreign cloned DNA *in vitro*, or by injecting the foreign cloned DNA into the testis of the male before fertilization (Chang, 1999). The male is then

allowed to mate normally and transgenic offspring result. In somatic cell nuclear transfer, the nucleus from an adult animal has the foreign cloned DNA inserted into it, often using some of the procedures mentioned above, and is inserted into an egg that has had its nucleus removed (McCreath, 2000). Each of these procedures has their own advantages and disadvantages, and research is being done to increase the precision of the gene insertion and the efficiency by which the transgenic eggs produce animals.

Embryonic Stem Cell Manipulation

The next common method for creating a transgenic animal is embryonic stem (ES) cell manipulation (**Figure-3**). In this method, an IVF egg is allowed to grow until it enters the blastocyst stage, when ES cells are removed from the inner cell mass. These ES cells are then exposed to the foreign transgene DNA using any of a number of techniques similar to the ones used in pronuclear manipulation, such as electroporation, retroviral infection, and microinjection. In addition to these techniques, the use of ES cells allows other special techniques not used with pro-nuclear manipulation. For example, the ES cells may be chemically treated to take up DNA, and then grown in medium containing a selection antibiotic to allow only ES cells receiving the transgene to replicate, for example by fluorescence screening or drug resistance screening. In both cases, the inserted vector contains an extra gene allowing the selection. In fluorescence screening, the vector contains an extra gene encoding a protein that will fluoresce under certain conditions. The cells are then exposed to these conditions, and the cells that fluoresce are likely to be the ones that correctly incorporated the transgene. In drug resistance screening, the vector contains and extra gene that imparts resistance to a certain drug. The cells are then grown in a medium that contains that drug, and only the cells that incorporated the vector will grow (Keiser,

2001). The use of ES cells also allows assays to be performed to determine whether they have taken up the transgene by Southern blots or PCR. This helps improve the efficiency of the procedure.

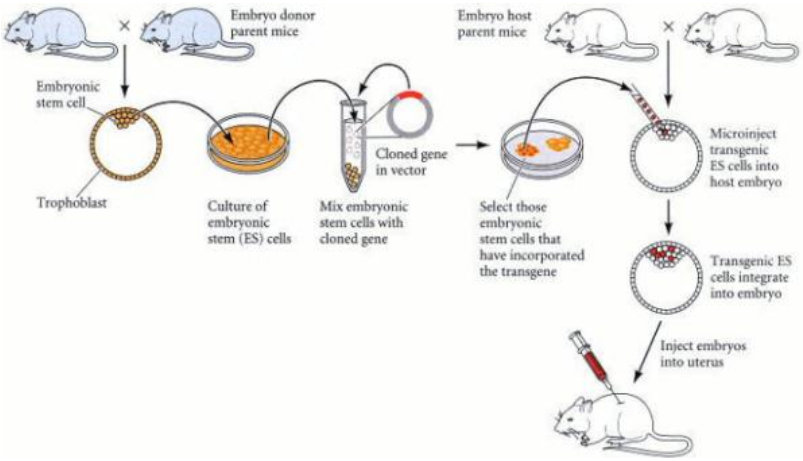

Figure 3: Schematic Showing the Process of Embryonic Stem Cell Manipulation. ES cells are isolated from a blastocyst (diagram left) and grown on a plate (orange). Cloned DNA (diagram center) is then transfected into the cells, and the modified ES cells are injected into a different blastocyst (diagram right). The modified blastocyst is implanted into a foster mother (diagram lower right). (Gilbert, 2006)

After the positive stem cells have been selected, they are injected back into the inner cell mass of the blastocyst (**Figure-4**). The blastocyst is then transferred to the uterus of a pseudopregnant female, and is allowed to develop normally to hopefully produce transgenic offspring.

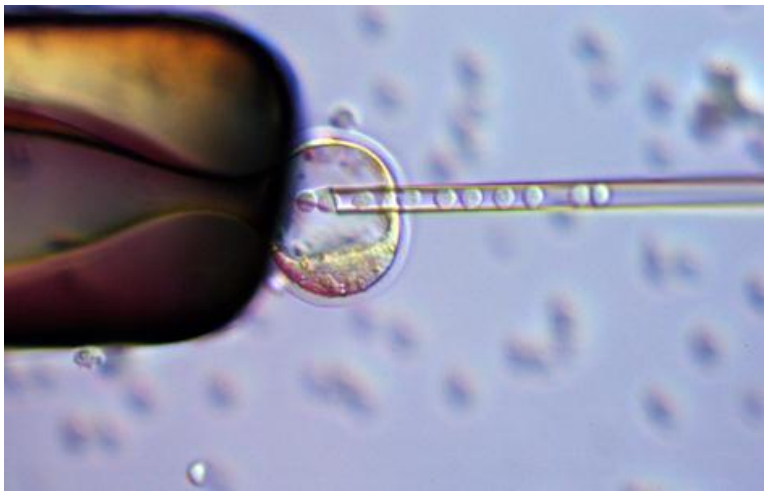

Figure 4: Injection of Genetically Altered Embryonic Stem Cells Into a Blastocyst. A suction pipette (left) holds a blastocyst in place (center) while a glass pipette (right) delivers several ES cells (small spheres) into the blastocoel cavity. (The University of Utah, 2009)

Technical Difficulties in the Production of Transgenic Animals

Although the production of transgenic animals has been performed many times, it is still a very inefficient process that is not fully understood. For mice, by far the best understood and used animal in transgenisis, the rate of gene-injected embryos that develop into viable expressing transgenic offspring is only 1-4% depending on the method used (Niemann, 2000). The efficiency decreases the more complex the animal is, so for example the efficiency is 4.4% for rats and only 0.7% for cattle. The various efficiencies of transgenic livestock production are shown in **Table I**. What causes this poor embryo survival rate is not fully understood, so much of the current transgenic research is targeted at increasing efficiencies.

Table I: Transgene Integration Efficiencies in Various Animals (Wall, 1999)**.**

Species	Injected & transferred embryos (No.)	Studies[a] (No.)	Offspring[b] (No.)	Transgenic animals produced: Per Offspring (%)	Transgenic animals produced: Per embryo injected & transferred (%)	Refs.
Mice	12,314	18	1847	17.3	2.6	(63)
Rabbits	1,907	1	218	12.8	1.5	(28)
Rat	1,403	5	353	17.6	4.4	(45)
Cattle[c]	1,018	7	193	3.6	0.7	(30)
Pigs	19,397	20	1920	9.2	0.9	(53)
Sheep	5,424	10	556	8.3	0.9	(53)

[a] Number of experiments, which in most cases was equivalent to number of different gene constructs tested.

[b] The value for cattle includes both fetuses and live born calves.

[c] Eleven thousand two hundred and six eggs were microinjected and cultured. One thousand and eighteen developed to morula or blastocysts and were transferred into recipient cows.

Screening for Positive Transgenics

Because not all of the embryos treated to take up the transgene actually incorporate it, it becomes necessary to screen the offspring once they are born to see if they carry the desired gene. This can be done by taking small blood or skin samples from the animal in question, extracting the DNA, and running tests to verify the presence of the gene in question. The most common techniques for doing this are PCR and Southern blot tests. In PCR screening (Mullis et al., 1986), the DNA sample is analyzed in a reaction tube containing short synthetic primers flanking the transgene. If the animal's DNA contains the transgene, the primers will anneal to the host DNA, and over time an amplicon representing the transgene presence will be produced, indicating the target gene is present in that sample and the transgenesis was successful.

In Southern blot analysis (Southern, 1975), the animal's DNA is cut with restriction enzymes which cut DNA at specific sequences, then run through an agarose gel to separate the fragments by size. Once the DNA fragments have separated, they are transferred to a nitrocellulose membrane which is then washed with a solution that contains a labeled probe complementary to the transgene. If the transgene is present in the sample, the probe hybridizes to it creating a signal.

Establishment of a Transgenic Strain

Once you have confirmed that the transgene was successfully inserted into the animal, the next step is to use traditional breeding of the positives to select for animals that are more fully expressing the transgene. Although there are some exceptions, the vast majority of transgenic animals produced using the methods mentioned above do not contain the transgene in all of their cells. Instead, the animals are said to be *chimeric* for the transgene, containing it in only part of their body. In order to get purely transgenic strains of the animal, selective breeding is required. This process is based on the simple idea of Mendellian genetics, and has been used by farmers for millennia. The process is simple enough (**Figure-5**), mate the chimeric animals with normal animals to produce offspring that are heterozygous for the gene in question (diagram upper). Then mate two of the heterozygous animals together (diagram center), and about one fourth of the offspring from this generation will be homozygous for the transgene. The process of mating the homozygous animals with each other can be continued until all of the offspring contain the transgene in every cell.

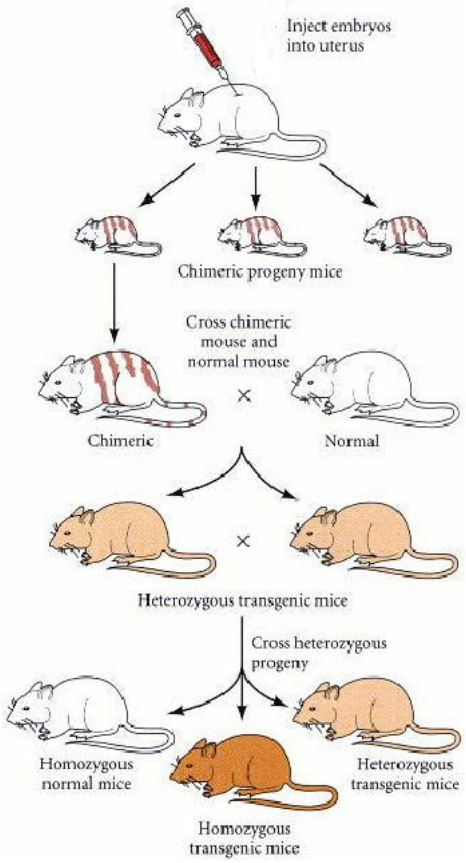

Figure 5: Breeding Scheme for Obtaining Homozygous Tansgenic Animals. (Gilbert, 2006)

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CHAPTER-2: TRANSGENIC APPLICATIONS

Arno Vandebroek

The focus of this chapter is to describe and categorize the various transgenic animals that have already been made and their purpose. This information will serve as an introduction to later chapters on transgenic ethics and legalities, where an understanding of each transgenic *benefit* is critical for the discussion. Transgenic animals can be classified into five main categories: Disease Models, Transpharmers, Xenotransplanters, Transgenic Food Sources, and Biological Models.

Disease Models

Transgenic disease models are animals that have been genetically altered in a way that makes them initiate a human disease or show symptoms similar to specific human diseases. These animals are used to study the disease in the interest in lieu of working directly with humans for the purpose of finding a cure. Animals generally do not contract human diseases, and so a transgene specific to the disease must be inserted in the animal to allow the animal to at least mimic some aspect of the disorder. Disease models are used to test new medicines so that we do not have to test them on people. If the medicine looks promising in the pre-clinical animal testing, it is then tested on human cells, and eventually in humans themselves in clinical trials.

AIDS Mouse

The first disease model we will discuss is AIDS mouse. Animals are not normally infected by HIV. Monkeys can be infected with siminian immunodeficiency virus (SIV) (the monkey equivalent of HIV), but are not easily infected by HIV. And much has been learned

about retroviral disease progression by studying SIV infection of monkeys. In labs, Chimpanzees can sometimes be infected with HIV, but the infection is not reliable, and monkeys are extremely expensive, so a cheaper rodent model is highly desirable.

Because mice are not normally capable of supporting HIV infection, they must be engineered to contain genes that encode proteins that allow HIV to attach to the cell surface (CD4 and CKR5) and must also express certain human cellular proteins that help support HIV replication inside the cell. The world's first HIV mouse was not transgenic; it was a mouse that contained transplanted human tissue which allowed HIV to replicate (Namikawa et al., 1988). This mouse also lacked an immune system (SCID mouse) so it would not reject the transplanted human tissue. An early transgenic mouse model contained the gene for HIV tat, a protein needed for viral replication (Vogel et al., 1988). This animal showed some signs of Kaposi's sarcoma similar to HIV patients.

More recently, an HIV mouse received the transgene that encodes the genome of type 1 HIV. Since mice also lack the two receptors needed to infect cells with HIV, the mouse was also genetically modified to have the gene for human CD4 and CKR5 co-receptor gene. The genes were inserted into a mouse zygote which was then grown to the blastocyst stage, then placed into the uterus of a female mouse. The pups from the litter which contained the three transgenes were then used for further research. The presence of the co-receptors in the mice will allow HIV to infect them which in turn allows them to create all the necessary proteins needed for the HIV to further infect the mouse.

Mice are not the only animals used in HIV research, other animals used for AIDS models include rats (whose cells contain host proteins that act like human proteins to support HIV replication), rabbits, *Drosophila*, and cats. Mice, however, are the most desired experimental

model since they are cheap. Many varieties of mice with certain genes knocked out already exist, which would help identify key host proteins needed for HIV replication. The outcome of using these models so far is that we have gained valuable knowledge into the life cycle of HIV and the human host factors that play vital roles. This knowledge will help us in the future to find potential therapeutic targets.

Alzheimer's Mouse

Alzheimer's disease (AD) is another disease modeled, in part, in animals. This was accomplished by Professor Adams of WPI and his colleagues at the former Transgenic Sciences Inc. by inserting the gene encoding a mutant version of human Amyloid Precursor Protein (APP) in mice, under the control of a PDGF promoter that ensures APP expression in the cerebral cortex and hippocampus that are strongly affected in AD (Games et al., 1995). Mutant APP is cleaved on the surface of neuronal cells to produce highly neurotoxic amyloid-beta (Aβ). Humans containing this mutant APP gene are at increased risk for early onset Alzheimer's. Because these engineered mice showed signs of neurodegeneration by 8-13 months, they helped prove the hypothesis that the synthesis of Aβ is necessary for the onset of the disease. The mice also develop memory deficits similar to Alzheimer's patients (Moran et al., 1995). Interestingly, the mice did not develop neurofibrillary tangles (another hallmark of the disease), which led to a complete rethinking of the pathology of the disease in which the tangles were a result of the disease and not a cause (Alzheimer's Breakthrough, 2005).

The Alzheimer's Mouse was subsequently used as a model to test the world's first vaccine for Alzheimer's disease. The mice were vaccinated with Aβ, which caused antibodies to form against Aβ. The antibodies surprisingly crossed the blood brain barrier to enter the brain,

where they bound toxic Aβ to help prevent neurodegeneration and memory loss (Schenk et al., 1999). The process worked so well in the mouse model, that human clinical trials were initiated by Elan Pharmaceuticals. The phase I and II tests appeared safe, but a few patients in the phase III test developed encephalitis, so the phase III was halted, and a second phase III test is currently underway. The vaccine is still under further development.

Oncomouse

Oncomouse is a transgenic mouse that has been genetically altered to exhibit symptoms of specific types of cancer. It was created by Philip Leder at Harvard University (Stewart et al., 1984), and it is the first animal ever to be patented (Leder and Stewart, 1984). The original oncomouse's somatic and germ cells contain human *myc* oncogene introduced to the animal at a very early embryonic stage. Later models also incorporated the *ras* oncogene, driven by the mouse mammary tumor virus (MMTV) promoter (Sinn, 1987). Both ras and myc proteins promote cellular growth, and the ras gene has been known to cause tumors when mutated. Oncomouse has proved an extremely valuable model for screening anti-cancer drugs. With these mice we can investigate how a tumor forms, and test potential therapies at a faster rate than if we tested on humans. Because oncomouse is the world's first patented animal, it will be discussed again in Chapter-4 on Transgenic Legalities.

Parkinson's Fly

Parkinson's disease (PD), like Alzheimer's disease (AD), is a neurodegenerative condition. But unlike AD, PD affects the substantia nigra area of the brain. This area of the brain synthesizes dopamine, a neurotransmitter involved in neuro-muscular control. Without

dopamine, or dompaminergic neurons, the patient eventually loses muscular control. The AD mice were made earlier than PD mice because for a long time we did not know which genes were involved in the onset of PD. It was only after studying an Italian-Greek family in which Parkinson's was inherited did scientists finally determine that the disease can be caused by a mutation in the gene that codes α-synuclein. Individuals with this mutation show increased neurodegeneration in the substantia nigra area of the brain. These findings were used by scientists Feany and Bender (2000) to create a PD model in a *Drosophila* fly. The fly expresses the mutated human α-synuclein gene in all of its nervous cells. The experiment was a success, and the fly exhibits many symptoms of PD. Researchers are now trying to make a Parkinson's mouse, but so far have had only minor success.

Transpharmers

A transpharmer is a transgenic animal that has been engineered to produce a human pharmaceutical in their blood, eggs, or milk. The process of creating a transpharmer is called Gene Pharming (Gillespie, 2005). The pharmaceutical protein encoded by the transgene is engineered with a secretion leader sequence to ensure the protein is secreted into the blood, milk, or eggs. The drug can then be collected and purified without harming the animal. The most common site for transpharm expression is the mammary glands, as milk is easy to obtain yet it produces complex glycosylated proteins if needed. Animals that have already been made into transpharmers include cattle, sheep, goats, chicken, rabbits, and pigs (Gillespie 2005).

The best method to create a transpharmer is to use a promoter from a milk protein gene, such as casein or beta lactoglobulin, to express the transgene in milk. The best example of a transpharmer is Herman the Bull. Herman was made by Gen Pharm International of Mountain

View, CA (Krimpenfort et al., 1991) and expressed lactoferrin using a beta casein promoter (Biotech Notes, 1994). The gene for lactoferrin, an iron-binding protein required for normal infant development, was inserted into an IVF embryo. Herman's female progeny produced lactoferrin in their milk (Biotech Notes, 1994). Cow's milk, increasingly consumed by babies, does not contain lactoferrin. Although the milk of Herman's offspring contained lactoferrin at such low levels it was never commercialized, Herman proved that transgenic technology could be applied to cattle.

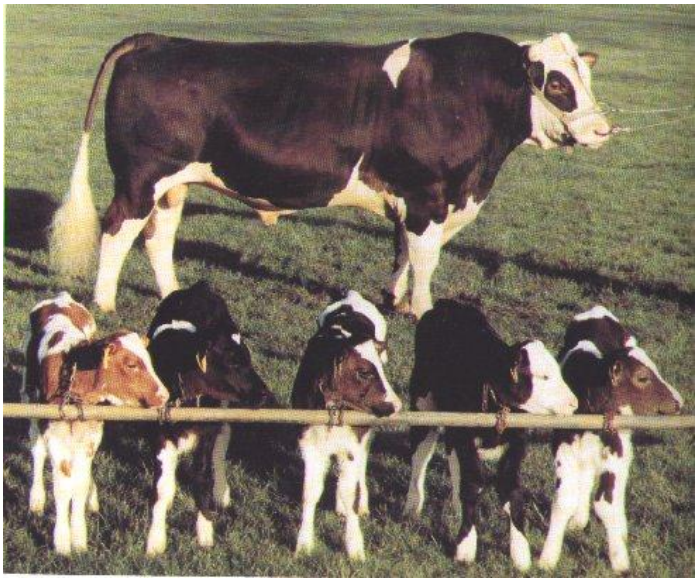

Figure-1: Picture of Herman the Bull and His Offspring.
(Anth.org, 2010)

Xenotransplanters

Xenotransplantation is the process of transplanting an animal organ into a human. Xenotransplanters are animals that have been, through the use of genetic manipulation, better prepared to donate organs. The organs of the animal are modified in a way that lowers the chance of rejection by the host. If the body rejects the organ, the immune system will attack the cells of the organ, which can be life threatening to the organ receiver. Before transgene

technology was developed, doctors would occasionally use primate organs when there were not enough human donors, but due to availability and threats of extinction, the primates are no longer used as donors, so pig organs are sometimes used. The physiology of a pig is very similar to that of a human, but pigs have specific sugars (alpha-1,3-galactose; αGal) on the surface of their cells that can cause very strong immune responses in humans (Pearson, 2003). The enzyme that creates αGal is alpha-1,2-galactosyltransferase (GGTA1). Researcher David Ayares found that organs that had been genetically altered to knock out this enzyme had a much lower rate of rejection than organs that had not been modified (Pearson, 2003). While xenotransplantation has many risks, the need for organs is very high (US Transplantation Data, 2010), and if this technology were perfected the organ list could decrease.

Transgenic Food Sources

Animals that have been modified to make them grow bigger are being created as potential food sources. These animals are made by adding a gene for a growth hormone into the animal's genome. Animals that have this gene inserted into their genome grow faster and have a higher disease resistance (Devlin et al., 1997). In cattle, bovine growth hormone (BGH) is used to make the cattle grow bigger than their relatives. In mice, human growth hormone (HGH) was used to make the world's first *expressing* transgenic animal (Palmiter et al., 1982) (**Figure-2**). In salmon, a salmon growth hormone (SGH) gene was engineered to be switched on at all times causing the salmon to grow much bigger than its siblings (**Figure-3**). Growth hormone experiments in mammals have generally produced severe debilitating symptoms, so these experiments have been discontinued in mammals. But growth hormone experiments in fish have

been so successful that Aquabounty is near to getting FDA approval to market their transpharmed salmon (Aquabounty, 2009).

Figure 2: Comparison of Mouse with HGH (right) versus a Normal Mouse (left) (Palmiter et al., 1982).

Figure 3: Genetically Modified Salmon vs. Normal Salmon (Ariel Schwartz 2010)

Transgenic Biological Models

A biological model is an animal that has been genetically modified to test what the modification will do to the animal. Animals can be engineered to over-express a newly discovered protein to see what effects the extra amounts will have, or knock out its expression to observe the effects of no expression of the protein. Animals created for this purpose include ANDi the monkey, Supermouse, Smart mouse, and Youth mouse.

ANDi the World's First Transgenic Monkey

ANDi is the first primate to successfully have a gene from a different species put into his genome (Chan et al., 2001). The gene encodes Green Fluorescent Protein (GFP), a protein produced by jellyfish that glows green under special light. The GFP gene was chosen since scientists would be able to easily see the effects of the green protein under the microscope, but GFP produces no other physiological effects, so it only serves as a transgenic marker in this first experiment. While samples of tissue and hair from ANDi showed the presence of the GFP gene, the amount expressed was too small to glow. The gene was inserted into ANDi's genome using a virus. The team infected 224 primate eggs with the virus, and ANDi was the only live monkey who had assimilated the GFP gene into his genome.

Although ANDi showed no strong expression of his GFP transgene, the fact that a foreign gene was successfully inserted into a primate opens up a whole new horizon for disease research. Mice are not the best disease models as they are very different from humans. Primates are most closely related to humans, so using them as models could potentially provide much more information on diseases and how to treat them.

Supermouse

Supermouse was engineered to over-express the gene encoding the enzyme phosphoenolypyruvate carboxykinases (PEPCK-C) (BBC, 2007). These mice are able to run five to six kilometers at a speed of twenty meters per minute on a treadmill for six hours without stopping (BBC, 2007). They are also able to breed much later in life, and live longer than a normal mouse. The muscles of the supermouse have ten times the mitochondria of a normal mouse (BBC, 2007). A drawback of the supermouse is that the animals were more aggressive than their normal relatives, but if this technology can be perfected, human diseases that cause muscle degradation could be treated.

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CHAPTER-3: TRANSGENIC ETHICAL CONSIDERATIONS

Neil Crawford

The focus of this chapter is to describe the ethical issues that arise when considering the use of transgenic animals. As can be expected for any controversial technology, arguments can be made both for and against transgenic use. In deciding whether transgenic animals should be made, one must weigh the benefits the animal confers to society, balanced against any detriment to the animal or any risk the animal may pose to the environment or humanity.

Disease Model Ethics

Transgenic animals have been used for years now to help fight disease. The benefits to society of this type of transgenic animal is undeniable, as these animals help lead to the discovery of treatments for many of the diseases afflicting the world's population. But the ethical question that arises from this class is whether it is acceptable to purposely create animals with debilitating diseases. To determine the level of animal suffering, one must look at the animals on a case-by-case basis.

In specific cases involving less severe diseases, where the animal suffering is mild or nonexistent, it can be easily argued that the medical knowledge gained from that experiment is worth their minor suffering. An example of this can be drawn from the case of the Alzheimer's mouse mentioned in the previous chapter. The Alzheimer's mouse (Games et al., 1995), apart from being a little slow cognitively, suffers no other measurable health defects, while the benefits provided by this model include the ability to screen drugs for treating Alzheimer's,

which today afflicts approximately 5.3 million people, costs billions in dollars annually, and is one of the most emotionally devastating diseases for the families of the affected (Alzheimer's Association, 2011). This mouse model has already been used by Elan Pharmaecuticals Inc. to develop a vaccine for removing the amyloid-beta neurotoxin that causes neurodegeneration (Schenk et al., 1999). Any progress that the Alzheimer's mouse can provide for treatments appears to far outweigh the mild cognitive impairment of the animal.

On the other end of the spectrum of disease models are the animals given the most devastating and pain-inducing diseases. For these animals, sometimes painkillers can be used to diminish the pain, or they can be sacrificed early after being used for an experiment. But it is the horrific nature of their disease that makes their existence invaluable, because these diseases also afflict people who have to suffer their daily lives with the same pain and who would greatly benefit from any advancements made. One example of an animal of this type is Oncomouse, which is a mouse developed by Harvard University and Dupont that has a predisposition for developing cancer (**Figure-1**). Cancer is the second leading cause of death in the United States, second only to heart disease, and can cause excruciating pain. Oncomouse has already aided in the development of some improved cancer screening techniques and continues to be widely used in research today. In deciding whether it is ethical to create such animals, one must weigh the strong medical benefits against the suffering of the animal, and use all attempts to minimize their suffering.

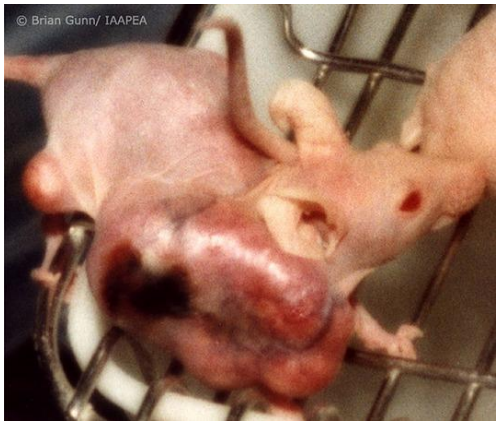

Figure-1: Picture of Oncomouse with Advanced Tumors. Courtesy of the International Association Against Painful Experiments on Animals (IAAPEA).

Transpharmer Ethics

Transpharmers are a class of transgenic animal that present less of an ethical problem than disease models, as these animals do not appear to be harmed at all. These animals are engineered to produce human pharmaceuticals in their milk, and do not appear to show any effects of the production. Usually, the human protein is secreted into the animal's milk which can later be used to isolate the product without sacrificing the animal. The only chance for harming the animal in this case occurs during their creation as extra embryos are destroyed. Although much is known about recombinant technology, many unknowns remain that can lead to unexpected problems, including random gene insertion which could cause a negative effect on the fetus. Also, the produced proteins could have unknown effects on the animal, although no such cases have yet been documented for production in milk. Both of these issues can be solved with better recombinant technology and better understanding of biological functions. The benefits to humanity are great, providing a cheap and effective alternative to the *in vitro* protein synthesis methods used today. Transpharmed therapeutic proteins have been used to treat several deficiency diseases, and are otherwise quite expensive. Once the model has been

created, they do not appear to be harmed, and the benefit to humanity is undeniable. So it is likely that the use of this class of transgenic animals should proceed with little opposition.

Xenotransplanters

The global demand for human organs for transplantation for the treatment of end-stage organ disease has greatly increased over the past few years. The creation of national organ donor programs has helped provide a slow stream of organs for transplant, but the requirement that a person usually needs to die to use their organs means there are few organs that actually make it to recipients, so the demand always exceeds the supply. Xenotransplantation offers a novel solution to this problem where animals are grown with human organs to be used for transplantation. These animals could provide a steady stream of organs for individuals awaiting human transplants, and the supply could easily be increased to meet demand. Ethical arguments surrounding this practice include whether it is ethical to sacrifice animals for this purpose, and whether it is ethical to create humans/animal cross species (Correa, 2001).

To this author, the idea that an animal would be sacrificed to save a person is not a novel idea, considered in light of the fact that thousands of animals are killed daily for human consumption. Some groups are opposed to any animal killing (for food consumption or otherwise), such as PETA (People for the Ethical Treatment of Animals) and ALF (Animal Liberation Front). These groups have for years battled against the brutal killing of animals for *any* reason, and have even put fort arguments against xenotransplantation:

> "The traditional sanctity-of-life ethic forbids us to kill and take the organs of a human being who is not, and never can be, even minimally conscious. At the same time, this ethic accepts without question that we may rear baboons and chimpanzees in order to kill them and use their organs. Why does our ethic draw so sharp a distinction between human beings and all other animals?" (Singer, 2000).

There is no real answer to this question, it lies as a matter of personal opinion, but it is widely accepted by the general public that the life of a human is above that of an animal, and the sacrifice of animals in xenotransplantation would no doubt lead to a longer healthier life for the recipient.

Another, more substantial concern regarding xenotransplantation is the risk that creating animal-human hybrids could aid in animal diseases transferring to humans. In a hybrid animal, it would me much more likely that an animal virus would also affect the transplant organ, and the consequences to the recipient could be devastating if the recipient is on immunosuppressive drugs. "Pig to human organ transplants are within the reach of scientists and could save thousands of lives... But the risks are enormous. If pig viruses attack human cells, they could unleash a new AIDS-type epidemic [AIDS is also a zoonotic disease originating in monkeys] against which we have no in-built defenses" (Bryan and Clare, 2001). Researchers have attempted to quell these fears by stating that any animals used in xenotransplantation would be kept in clean labs for the entirety of their lives, and would be screened for known viruses prior to transplant, but unknown viruses could be a problem. Due the potential for a zoonotic disease from xenotransplantation, government regulatory agencies should insist on strong oversights and rigorous pathological screening prior to the use of such animals.

Transgenic Food Sources

With the world population now reaching near 7 billion, finding enough food to feed everyone has become one of the world's great problems. Today, it is estimated that nearly 1 billion people suffer from hunger worldwide. Transgenic crops have already been developed and

implemented to increase crop yield, and it is hoped that transgenic animals will also be used in this capacity. The idea behind the use of transgenic animals as food sources is that animals will be engineered to grow faster and convert feed to meat more efficiently. Transgenic livestock could also be altered so the meat derived from them is healthier.

But these food source benefits do not come without risks, including the possible release of the animals into the wild and animal welfare (Environment News Service, 2000). The potential for accidental release into the environment becomes much greater once it is moved from the laboratory to the farm, and this caution must be considered when using transgenic animals on a large scale (Kohler et al., 1992). New FDA regulations require that the environmental impact of such animals be assessed, including the "inadvertent release or escape of the genetically engineered animal and/or its products into the environment, and whether certain measures may mitigate any potential significant impacts that would adversely affect the human environment" (FDA.gov, 2009). The worry here is that the animal in question may escape into the wild and disrupt the natural ecosystem to cause unforeseen negative effects on the entire ecosystem.

With respect to food source animal welfare, while altering the animal's genetics to make it grow faster and have more meat, the animals could suffer. A prime example of this is Superpig. Although scientists successfully engineered a pig to grow faster and larger, the pig suffered from many diseases, including kidney and liver failure, degenerative joint disease, and heart disease (Rollin, 1996). Eventually the pig was euthanized, and scientists have imposed a moratorium on producing "super" mammals for consumption.

However, not all transgenic animal food source experiments have failed. Recently, a strain of genetically engineered salmon was submitted to the FDA for final approval, and it looks

likely to be approved (Aquabounty, 2010). These salmon show no adverse affects from their genetic modification, and would greatly increase salmon meat production. As researchers gain a better understanding of recombinant technology, the adverse effects of such modifications could be reduced.

Biological Models

Biological models are a class of transgenic animal used to increase our knowledge about the function of specific genes or proteins in animals. Examples in this class include ANDi the monkey and supermouse, but this class is much harder to justify because in most cases they are not directly used to save lives. No doubt, such animals increase our knowledge of how specific proteins function in a complex environment, and improve transgenic technology in general, but the potential for animal suffering is as prevalent here as in any other class. The value of the learned material is subject to question. The transgenic monkey "ANDi" is a prime example. Although he was the world's first transgenic monkey, and taught us that transgenic technology can be applied to primates, making a monkey whose cells glow under UV light can with difficulty be said to be helping humanity. Researchers argue that this is an important step in leading to other more useful recombinant primates such as disease models or xenotransplantaters. Whether this happens remains to be seen.

Ethical issues that arise from this class of experimentation raises questions about the sanctity of an animal's genetic code. Ever since the creation of recombinant DNA technology there have been those that accuse scientists of "playing god" with life's genetic code. Arguments can easily be made for both camps. Theologically speaking, many individuals believe the creation of life forms rest solely with God, and any attempt to improve his creations is akin to

blasphemy. But this is not the only argument. In the Old Testament book of Genesis, God gives dominion over the creatures of the earth to man. "...God said, “Let Us make man in Our image, according to Our likeness; let them have dominion over the fish of the sea, over the birds of the air, and over the cattle, over all the earth and over every creeping thing that creeps on the earth" (Genesis, 1:26). It could be argued that this biblical phrase grants man a godlike dominion over animals, and we may do with them as we please, including transgenesis if it saves human lives.

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CHAPTER-4: TRANSGENIC LEGALITIES

Arno Vandebroek

The creation of transgenic animals is a very controversial topic that requires legal policies to help ensure minimal animal suffering while maximizing the benefit to society. This chapter deals with laws overseeing the creation and use of transgenic animals. While on one hand, animal patenting offers incentives to the inventor and furthers biomedical research, on the other hand many protest the authority of the US Patent and Trademark Office to patent animal life. In this chapter both the positive and negative sides will be presented, along with a few milestone court cases and the differing views of the US, Canada, and Europe on this issue.

The US Patent Process

In order for an invention to be patented in the US, the Patent and Trademark Office (PTO) states that it must fulfill the requirements of novelty, utility, and non-obviousness (PTO, 1987, 35 U.S.C. § 101, § 102, § 103). The requirements do not state that the invention has to be non-living. Title 35 of the United States Code explains that "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title" (Bitlaw 2000). The only requirements for a patent are that the invention has to be new, useful, and not something that anyone could come up with based on obvious common sense. A transgenic animal, for the most part, fulfills all of these requirements.

Diamond vs. Chakrabarty

Biotechnology patent issues did not begin with animals, but with bacteria. In 1972, microbiologist Ananda M. Chakrabarty applied for a patent for genetically engineered bacteria that could break down crude oil. The bacterium was created by adding two plasmids to the original *Pseudomonas* bacterium that allowed for two different biochemical pathways to break down oil (**Figure-1**). The bacterium fulfilled all three patenting requirements, it was unlike any bacterium currently existing (novelty), it had the potential to treat oil spills (usefulness), and could only be made by a scientist in a laboratory (non-obviousness) (*Diamond v Chakrabarty*, 1980).

Figure-1: Photo of the Current Gulf Oil Spill by British Petroleum. The current oil spill would be a potential application of Chakrabarty's oil eating bacteria (Densley, 2010)

The patent officer initially rejected the patent on the grounds that transgenic microorganisms were products of nature, and therefore the bacteria were not patentable. But Chakrabarty appealed the decision to the Supreme Court. The court eventually decided that the claim met all three requirements under section 101. They granted patents for the

bacteria, as well as for the *method* of producing them, and the *carrier* material that was in the water with the bacteria (*Diamond vs. Chakrabarty,* 1980). The court also decided that the potential environmental hazards that could come from genetic research should be addressed by the Executive branch of the government, and that the Judicial branch's only involvement should be using the current legislation to determine patentability of future inventions. The interpretation of the judges in this case was that the microorganism was indeed a new "composition of matter". This case paved the way for subsequent animal patents.

In 1987, the Patent and Trademark Office confirmed the decision of the Supreme Court in a statement to the Official Gazette: "The Patent and Trademark Office now considers non-naturally occurring non-human multi-cellular organisms, including animals, to be patentable subject matter within the scope of 35 U.S.C. s. 101." The animals must also be "given a new form, quality, property, or combination not present in the original article existing in nature, in accordance to existing law" (Patent and Trademark Office Notice, 1987). In light of this notice, new applications surged in for genetically modified organisms as well as a substantial amount of criticism for the patent office.

Animal Patents

The first animal patent was awarded in 1988 for the Harvard Oncomouse, just one year after the 1987 PTO stated that animals may be protected under patent law (originally filed as Leder and Stewart, 1984). As explained in Chapter 2, Oncomouse is a mouse genetically modified to be prone to developing tumors at a much higher rate than a normal

mouse. This court case was one of the most complex in US history, with several appeals, but eventually acted as a landmark case for all others.

Since the original 1987 PTO Notice allowing transgenic animal patenting, about 800 animals have since been patented (Wired, 2010), including cows, sheep, pigs, birds, fish, mice, cats, chimpanzees, and horses. In addition to patents for the animals themselves, patents have been awarded for the *methods* and *technologies* used to produce the animals. For example, a patent was given to Avigenics Inc. for a "Windowing Technology" which entails creating a hole through eggshells that allows the creation of transgenic chickens, which will be very important as a food source as well as in drug production (Avigenics Inc., 2000). As more discoveries were made in the field of transgenic animals, the patents rose exponentially.

Effects of Patenting Oncomouse

The Harvard Oncomouse was the first patented animal in the world. It received patent number 4,736,866 on April 12 of 1988 (filed as Leder and Stewart, 1984). This patent gives ownership of a species to a corporation for the first time, making it very controversial. Claim 1 of the patent was as follows:

> A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal, or ancestor of said mammal, at an embryonic stage (Leder and Stewart, 1984).

This initial claim was very broad, and allows the holder of the patent to lay claim to any non-human mammal containing the oncogene sequence of interest, as well as any

offspring of the animal. This means that DuPont, which now holds the patent, may legally challenge anyone who uses such an animal without their permission.

But problems arose in how to control who gets to use the mouse. Many scientists complained that with licensing fees so high, only large labs could afford to work on the animal which would hinder medical research. DuPont initially set up distribution of their oncomouse through Taconic, an international supplier of pathogen-free lab animals (Taconic, 1998). The company acquired the license to distribute the mice in the hope of offering easier access to the mice to researchers as well as offering an alternative to exposing normal lab mice to high amounts of carcinogens. But some scientists felt this was not enough, and in 2000, DuPont and the US National Institutes of Health negotiated a deal to give non-profit researchers free access to the mouse with the stipulation that any commercial use must pay for the mice (Smaglik, 2000). But even with this deal, many researchers who use the mouse feel that DuPont's requirements for free licensing (which include annual reports and force researchers to comply with a contract) are too strict. They also argue that if companies are forced to pay the licensing fee, it will create an economic burden that would hamper research (Marshall, 2002). Regrettably for the scientists, for now they have to follow DuPont's policies. Since it was the very first animal patent, Oncomouse had much wider terms than are awarded today. Nowadays when a patent is awarded, it applies only to one species of animal and one gene. This is done so that one company cannot gain unreasonable power as DuPont did, to promote competition between companies, and to reward creativity in the experiments.

Figure 2: DuPont's Logo (Ricketts, 2009)

Oncomouse in Europe and Canada

Europe and Canada each had their own policies concerning the oncomouse patent. In 1990, the examiner from the European Patent Office (EPO) initially rejected the patent on the grounds that patents on plants and animals are forbidden by the European Patent Convention. The discovery had not been shown to be reproducible, and the ethics of transgenic animals could not be overseen by patent law (Dickman, 1990). But the case was appealed, and the appeals board later reinstated the patent. DuPont successfully argued for the patent pointing out the benefits the European biotechnology market could get from the incentives. Until the patent was approved in Europe in 1992, DuPont protected itself with licensing agreements. The difference between U.S. and European patent law concerning this patent is that EPO restricted the patent twice, first in 2001, limiting it to only rodents not all animals, and then in 2004, limiting it to mice (Cyranoski, 2004). Both restrictions were in reaction to complaints against the initial patent.

On the other end of the spectrum from the U.S., Canada completely *rejected* the patent for oncomouse in 2002. The Canadian Supreme Court stated that "A higher life form is *not* patentable because it is not a new 'manufacture' or 'composition of matter'"

(Check, 2002), and this renouncement remains in effect today. But some Canadian biotech companies have complained, saying the important thing to consider is the effect of the decision on Canada's life-science research and biotechnology. BIOTECanada, a biotechnology firm, says that the ruling will discourage researchers from creating research models and transgenic animals, therefore Canada will lose the future benefits.

Positives of Patenting Animals

Making a transgenic animal is not easy to accomplish. It requires years of development, talented scientists, and a great deal of money. The scientists making these animals often find the money they need from corporations who provide financial support both up front and in the long run. Investing money into making transgenic animals is risky as it does not always succeed. Even in parts of biotechnology that have already been developed, such as xenotransplantation, there are many more aspects of the science that still must be developed before it can be used effectively. The best case scenario for a company that is investing would be a patent that would become a source of revenue to the company to help support the investment. It is for this reason that companies give millions of dollars to universities to fund research. Using the revenue gained from an initial patent, a company can invest further in transgenic animal research, and with this cycle the knowledge of biotechnology grows. Without patent protection, there would be no economic incentives to fund the research, and our knowledge of biotechnology as a whole would suffer. Since transgenic science has a huge potential to help human beings, there is no longer a question of whether we should pursue it, but rather how we shall pursue it.

Another advantage to patenting animals is that the more that you draw attention to the science of biotechnology the fewer secrets it has. By patenting animals the patent office creates an incentive for scientists to study biotechnology and even work together to discover new aspects to get their own patents and their own names out there in the scientific community. By patenting transgenic animals, you not only give an economical incentive but you also raise awareness about the science. This means that biotechnology will no longer be an obscure science, and will be drawn into the public eye where more support for the science will lead to more advances.

Negatives of Patenting Animals

One of the biggest objections to the PTO is they patented animals without taking into consideration whether any animals should be patented at all. Some complain that the PTO merely determined that novel transgenic animals meet the current standards of a patent, without expanding the debate to the more serious ethical issues. In response to this, some scientists say the ethics and morality of transgenesis should not be discussed by the PTO but rather by Congress (Walter, 1998). As the ethics of animal patenting has not yet been extensively debated, animals are freely patentable as long as they fulfill the three requirements of novelty, utility, and non-obviousness.

Some religious groups have objected to patenting animals, stating that putting a patent on an animal is the same as putting a price on it. According to them no form of life should have a price on it. For example, many Buddhists argue that all cows are sacred, so they are against any form of transpharming with cows (Dharma Discussions, 2003). Most

Buddhists also believe in the general principal of "doing no harm", so this would extend to many transgenic experiments (Keown, 2004).

Another concern is economic, that all the patents for transgenic farm animals will be held by a small number of corporations which will drive the family farm out of business. Without patents, the owners of the transgenic animals would only license their animals to people who are able to pay for them, the large corporations (Walter, 1998), which would be disastrous for small family farms, as the large commercial farmer would have an advantage. With patent protection, holders could offer their wares at a scalable price depending on the ability to pay, so that local farms may be able to afford them.

Then there is the slippery-slope argument, which asks the question could animal patents lead to human patents? It is already acceptable to patent human genes in animals, who knows how far this could go. No country currently allows human reproductive cloning, so laws are already in place internationally to prevent this practice. The PTO considers animals to be patentable, but they have not yet issued a statement stating the number of genes that could convert an animal into an animal-human chimera (Edwards, 2001). Congress itself has not decided one way or the other whether animals are patentable, it has only been in courts that have made rulings to allow it.

Chapter-4 Conclusions

It is the opinion of the author of this chapter that animal patenting should be allowed but strongly regulated, as the European Patent Office has done. The medical advantages to society of patenting animals strongly outweigh the drawbacks. The drawbacks should not be ignored but integrated into patent law, and strongly overseen by

Institutional Animal Care and Use Committees (IACUC). If a patent is too broad, restrict it. If a corporation is being too restrictive and overbearing with their patent, force them to allow broader access to it. With this method we can gain the benefits of animal patenting with very few of the drawbacks.

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PROJECT CONCLUSIONS

A transgenic animal is a special type of animal engineered to have a foreign gene inserted in its genome. Expression of the foreign gene imparts new characteristics to the animal not normally found in nature, such as the ability to produce human therapeutic proteins in milk, or the ability to serve as a human disease model. There are two main ways of constructing a transgenic animal, either by manipulating the pronuclei of newly fertilized eggs, or by manipulating embryonic stem cells. In either case, the gene of interest (transgene) must first be cloned, usually by polymerase chain reaction (PCR). The cloned gene is then inserted into a vector, such as a virus or plasmid, that contains regulatory sequences for controlling expression of the transgene. Transgenic technology is not efficient, and many embryos are wasted attempting to create a positive animal. Transgenic pups are usually screened by PCR or by Southern blots to detect the presence of the transgene in the animal's DNA.

Transgenic animals can be divided into five main categories: disease models (that mimic specific aspects of a human disorder), transpharmers (that produce human pharmaceuticals in blood or milk), xenotransplanters (that produce organs for human transplants), food sources (for consumption), and biological models (that study the effects of specific proteins *in vivo*). Each transgenic class has its own ethical considerations that weigh their benefits to society versus the detriments to the animal or the environment. In the cases of disease models, transpharmers, and xenotransplanters, the medical benefits are quite strong, and in those cases where the animals can suffer (Oncomouse), strong regulations must be followed to minimize animal suffering, including using painkillers and euthanizing the animals prior to advanced tumor formation. Although some animal welfare groups are against xenotransplanters since the animals would be

sacrificed to obtain their organs, their numbers pale compared to the millions of animals sacrificed daily for normal human consumption. Transgenic animal food sources are one of the most controversial classes, as they would be consumed by humans. "Super animals" that grow faster on less food have already been created, but super-mammals like Superpig developed extremely serious side effects of the transgenesis requiring its euthanasia. However, Superfish like Aquabounty's salmon and trout had no observable deleterious effects and soon will be approved by the FDA for human consumption.

As is typical for any controversial technology, laws have been enacted to control transgenesis. The world's first patented *life form* was Chakrabarty's bacteria, engineered to consume oil slicks. This was a very difficult patent to obtain, but its passage eventually lead to the award of the world's first *animal* patent, for Oncomouse. The issue of whether life should be patented is highly controversial, and Canada to this date does not allow it. The potential benefits of patenting animals include protecting a company's profits which will increase medical research by allowing the profits to be applied to other experiments. However, patents can have a down side if the fees are so high they discourage smaller labs from performing research.

Based on the findings of this project, the authors of this project believe that all five major classes of transgenic animals should be continued, but with caution for those types of experiments that have no strong medical benefits to society. And in all cases, every effort should be made to minimize any animal suffering if it occurs. The authors also believe that transgenic fish should be approved by the FDA to help fight world hunger, but agree that any "Super mammals" (such as Superpig) should be disallowed. In all cases, strong legislative oversights should be followed to help ensure that any experiments gone wrong lead to immediate animal euthanasia.