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TRANSGENIC ANIMALS

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ABSTRACT

This project examines the controversial topic of transgenic animals, and weighs its effects on society from both ethical and legal standpoints. It first focuses on the technology itself by describing how transgenic animals are developed, screened, and categorized in chapters-1 and 2. Then the transgenic controversy is discussed in Chapters-3 and 4 with ethics and legalities. From education, to medicine and industry, transgenic animal research has had an enormous impact on society. Based on the research performed for the project, the authors provide their own conclusions about this fascinating technology and whether it should continue.

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PROJECT OBJECTIVES

The purpose of this project was to investigate transgenic animal research and technology, as an example of the effects of technology on society. This report begins by describing transgenic animal methods of creation as well as their purpose, describes the main transgenic categories, and then discusses transgenic ethics and laws. The project aims to help educate the reader as to what transgenic animals are, their educational benefits, their applications (from medical to the industrial fields), and then finishes with the legal controversies that surround their patenting. This report provides ample information for readers to form their own conclusions as to whether they should, or should not, support transgenic animal research. We conclude that with sufficient oversight to help ensure the animal's welfare, transgenic research should continue.

CHAPTER-1: TRANSGENIC ANIMAL TECHNOLOGY

Richard Breault

Transgenic animals are a subset of genetically modified organisms that contain a foreign gene inserted in the genome that gives the animal a new property. Transgenic animals have become a major part of biomedical research, providing new disease models for screening therapies, new ways for manufacturing medicines, providing organs for transplant, or providing new food sources. The purpose of this chapter is to describe transgenic technology and how these animals are created which will serve as a background for later chapters discussing their ethics and legal issues.

What is a Transgenic Animal?

Transgenic animals typically are described as those containing a foreign gene(s) inserted into their DNA for the purpose of giving the animals new properties. These types of transgenic animals are referred to as "knock-ins" because a new gene has been inserted in the genome, and this usually increases expression of a particular gene. However, more loosely, transgenic animals sometimes include other categories of animals in which a particular gene has been removed (knock-out animals) or in which the expression of a particular gene is decreased (knock-down animals).

Chapter-2 will discuss the various categories of transgenic animals in detail, but transpharmers are one example. These animals have been engineered to produce human pharmaceuticals (hormones, antibodies, enzymes) in their milk or blood. In this case, the

animals serve as a type of living bioreactor to perform a complex biochemical synthesis. Generally the protein being produced is harvested from a secreting system (liver, kidney, or mammary glands) in a large mammal (e.g. goats, sheep, or cow). Milk is the preferred production fluid because the animal is not sacrificed or bled to obtain the product (Betsch, 1995). Human proteins often need complex chemical additions (such as glycosylation) to be fully active, and tissues like mammary glands are capable of performing these reactions. Mammals are constantly manufacturing their own proteins, so the foreign gene is expressed using the animal's own synthetic machinery.

A second type of transgenic animal example is Superfish (**Figure-1**). This is a type of genetically modified food source whose growth hormone gene has been placed under the control of a promoter that is always switched on (Devlin et al., 1997). This engineering allows the fish to continually produce growth hormone year round, unlike normal fish that produce it seasonally, so the fish grows to large size quickly and on less food. These kinds of examples will be discussed in detail in Chapter-2.

Figure-1: A Transgenic Animal Example, Superfish. The smaller fish in the front is a normal salmon, about 13 inches long weighing 2.8 pounds, while the Super Salmon in the back born at the same time grew to about 24 inches and 6.6 pounds (Anakupto, 2011)

How Are Transgenic Animals Created?

Transgenic animals are created using genetic engineering technology developed over the past several decades. Recombinant DNA (rDNA) technology allows scientists to excise specific DNA fragments or genes, amplify them, and insert them into other genomes. rDNA technology can be used to fuse different species' DNA together, allowing new DNAs to be created that did not exist in nature. The process is inefficient, and the offspring must be screened to identify which animals, if any, took up the transgene.

Generally, there are two main methods for creating transgenic animals, pronuclear manipulation, and manipulation of embryonic stem (ES) cells. These methods are both designed to get the same end result, but are done in very different ways, each with advantages and disadvantages. Pronuclear manipulation is much simpler, faster, and produces an animal with all cells containing the transgene, but the process is very inefficient, and the DNA is incorporated randomly. ES cell manipulation allows a pre-screening process to ensure the transgene is incorporated into the ES cells prior to implantation, and also allows targeting of specific areas of the genome, but it often produces a mosaic animal that must be bred to obtain full transgenics (Ceci, 2011).

Pronuclear Manipulation

Pronuclear manipulation was the first method used to insert foreign DNA into a host. In this process, the first step is to decide which DNA to insert. The inserted DNA usually has three parts: the transgene itself which encodes the desired new trait, the promoter which dictates which tissue will express the transgene, and the cloning vector which allows the DNA to be

amplified and purified. The cloning vector is usually plasmid or viral DNA. The vector allows safe passage of the DNA into the host.

Next, an egg is fertilized *in vitro*, and incubated. Before the male and female pronuclei fuse to make the zygote, a micropipette is used to inject the transgene DNA into one of the pronuclei (**Figure-2**). Because of its larger size, usually the male pronucleus is injected. The DNA is incorporated randomly into the genome, if at all, which can sometimes be unfavorable to the embryo. If the transgene does insert into the genome, all derived cells of the offspring will be transgenic. The injected egg is then incubated until about day-5 when a blastocyst forms, then is implanted into the uterus of a foster mother for development.

Figure-2: Microinjection of Foreign DNA Into a Pronucleus. This figure shows one of the two main ways for creating transgenic animals. Shown are a suction pipette (diagram left side) which holds the newly fertilized egg (diagram center) in place for microinjection, and the injection pipette (right side of the diagram). (DNA Microinjection, 2011)

After birth, the offspring are tested for the presence of the transgene. Only a small percent of the offspring will have taken up the transgene, and only a subset of those will be expressing it. Often, the offspring are mated with each other to select for another generation of transgenics with a higher level of expression (Kimball, 2011). Just like any other scientific experiment, there are drawbacks to this pronuclear microinjection method. Depending on where the transgene inserts in the genome, the position can affect the animal's survival. The site of integration can also affect the level of transgene expression; if the gene inserts randomly into an active area of the chromosome it can be strongly expressed, but if it randomly inserts into an inactive area of the chromosome it can be silenced. Many companies are trying to find new ways to minimize these problems (Krejci and Boccaccio, 2006).

Embryonic Stem Cell Manipulation

The second method for creating a transgenic animal is to manipulate embryonic stem (ES) cells. ES cells are the inner cell mass of the 5-day old blastocyst (**Figure-3**), and are relatively undifferentiated pluripotent cells that will eventually form all types of cells in the adult body. Advances in stem cell research have allowed ES cells to be isolated from blastocysts, and grown in culture to make ES cell lines (Martin, 1981). The ES cells can be treated with engineered viruses carrying transgenes to allow DNA integration, then can be selected in culture. The selection process often includes growing on a selection medium containing an antibiotic like neomycin; the transgene DNA can include a gene encoding antibiotic resistance, so only the cells containing the transgene will grow. Once the ES cell line has been shown to have incorporated the transgene, the ES cells are injected into a blastocyst, and the blastocyst is implanted into a foster mother as before (Boghossian et al, 2008). However, not all the cells of the blastocyst are

transgenic (only the injected cells were), so offspring created by this procedure are mosaics, with some cells being transgenic and others not. So after offspring are born, they are usually inbred to select for pure transgenics (Transgenic Animal Science, 2005).

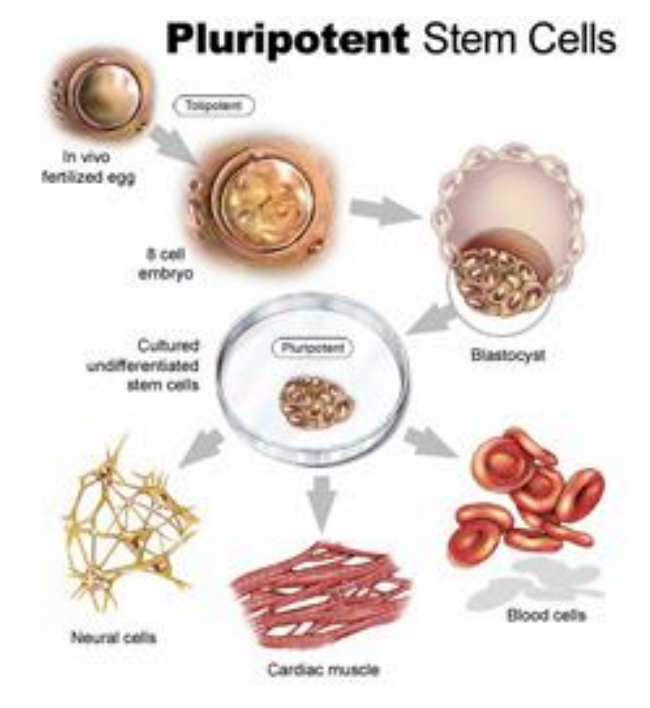

Figure-3: Diagram of Embryonic Stem Cells. This figure denotes the second main method for creating transgenic animals. Newly fertilized eggs (diagram upper left) are grown to the blastocyst stage (upper right), from which embryonic stems cells are isolated from the inner cell mass (diagram center). The ES cells can be grown into an ES cell line, which is then treated with engineered viruses carrying transgenes. The cells are screened to select for transgenics, then implanted into a new blastocyst. (Boghossian et al., 2008)

In addition to allowing engineered cells to be screened for the presence of the transgene prior to injection, the other main advantage of this technique over pronuclear microinjection is it allows the use of homologous recombination. In this process, the transgene DNA contains large portions of host chromosome that recombine with their respective areas of the host DNA

replacing it (Bronson and Smithies, 1994). This allows the foreign transgene to be inserted into a specific site in the host DNA to avoid activating any deleterious genes, or to avoid insertion in an inactive area of the host DNA.

Screening for Transgenic Positives

As mentioned above, the production of transgenic animals is not an efficient process, so offspring must be screened to identify positive transgenics. Screening can be done a variety of ways; Southern blots or PCR can be used to identify animals that incorporated the transgene into their genome, while Northern blots or RT-PCR can be used to identify animals expressing the transgene. Southern blots (**Figure-4)** are used to identify a transgenic DNA fragment from within a mixture of genomic fragments (Southern, 1975). The animal's DNA is isolated, then cut with restriction enzymes to fragment it. The DNA fragments are then separated by size using electrophoresis, and the pattern of fragments is blotted to a membrane. The membrane is then hybridized to a radioactive probe complementary to the transgene. If the animal contains the transgene, its fragment will be present on the membrane, and it will hybridize to the probe allowing a visual signal. In order to see whether the radioactive probe hybridized to any fragment on the membrane, the membrane is exposed to x-ray film. The presence of radioactive signal is seen as black bands on the film (McGraw Hill, 2011).

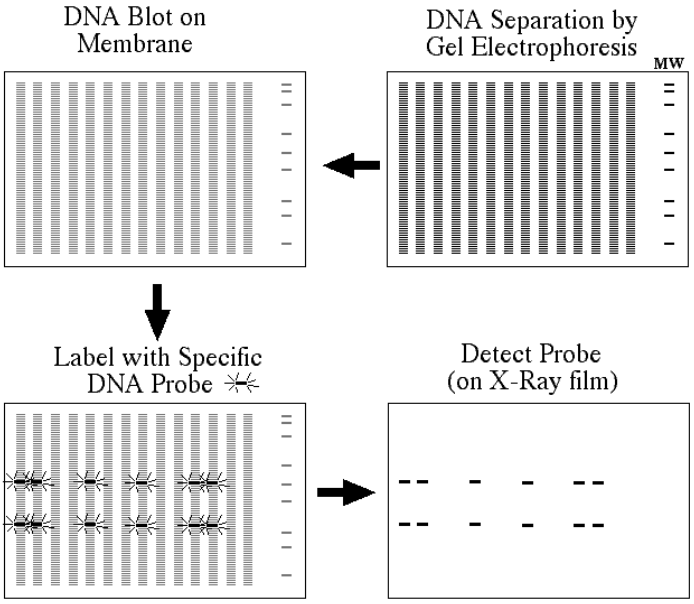

Figure-4: Diagram of a Southern Blot Used to Screen Transgenic Positives. In this process, DNA is cut by restriction enzymes, then separated by size using electrophoresis (upper right). The pattern of DNA fragments is blotted to membrane (upper left), and then hybridized with a probe for the transgene to allow it to base pair with a complementary transgene DNA fragment if present. (Southern Blot Methods, 2001)

The second main method for detecting the presence of the transgene is polymerase chain reaction (PCR) (**Figure-5**). PCR is used mainly to amplify specific regions of DNA located between two primers. If PCR primers are used that flank a particular transgene sequence, that set of primers will amplify the transgene if it is present in the animal's genome. A PCR reaction contains the target DNA (in this case the animal's DNA), the two types of primers (in this case for the transgene hopefully inserted in the transgenic animal), Taq polymerase (a heat stable enzyme used to replicate DNA), and DNA nucleotides (used as precursors to incorporate into the growing DNA chains. The temperature of the PCR reaction is controlled by a programmable

thermocycler. The reaction is slowly heated to about 93°C to separate the double-stranded template DNA. After strand separation, the reaction is cooled to around 60°C to allow the DNA primers to bind their complementary regions. After the primers bind, the reaction is warmed to about 72°C, the optimum temperature of Taq polymerase, to allow DNA synthesis from the primer sites. This creates two newly synthesized strands of target (transgene) DNA (PCR, 2007). The cycle of strand denaturation, primer annealing, and DNA synthesis is repeated about 30 times to amplify the region of DNA located between the primer sites. The so called amplicon (amplified DNA) is then visualized by electrophoresis, or can be seen in real time using fluorescent nucleotides. This technique not only allows us to determine whether an organism contains the transgene, it also allows amplification of that DNA for further manipulation if needed. Because the target gene is copied so many times, it is easy to visualize.

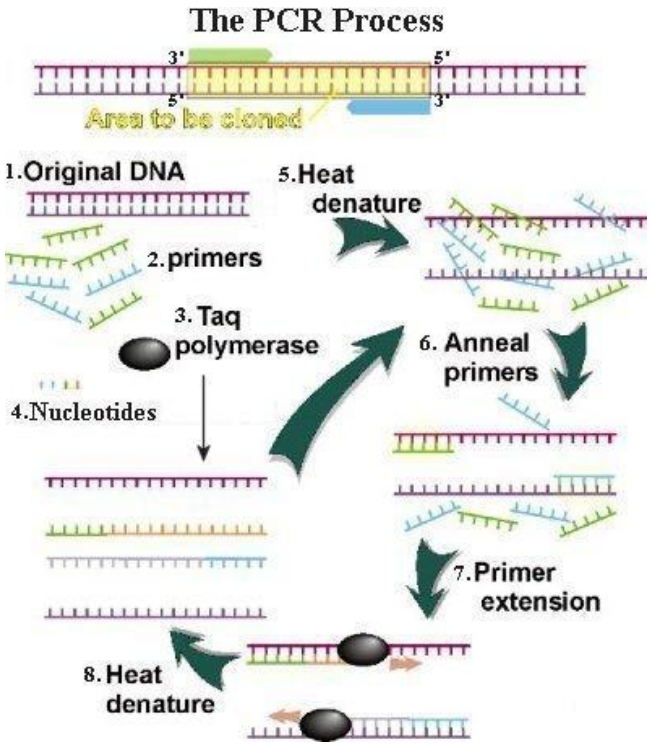

Figure-5: Diagram of PCR. This process is used to amplify a region of target DNA (in this case the transgene) to determine whether it is present in the transgenic animal. The process involves repeating a programmed cycle of DNA denaturation, primer annealing, and DNA elongation. (PCR Process, 2010)

Breeding Transgenics

Transgenic offspring are often bred with each other to create transgenic lines that increase expression of the transgene, or (in the case of chimeric animals) that contain a higher percent of cells containing the transgene. Transgenic animals tend to breed normally. Sometimes, chimeric animals are bred with wild type animals to produce heterozygous offspring. If these are then bred to each other, there is a 25% chance that two chimeric animals will produce a fully transgenic animal. After a few generations, animals are created in which all cells contain the transgene.

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CHAPTER-2: TRANSGENIC APPLICATIONS

Tania Emmanuelle Torchon

Transgenic animals are genetically engineered animals that express new, desired properties of a foreign gene inserted into their genome. Because of transgenic technology, the scientific and medical benefits to society appear endless. These animals can be altered to produce life-saving pharmaceuticals in their milk; they can be engineered to produce organs for transplant; or they can be used as disease models to help understand disease progression and cures. Their genetic codes can be adjusted to enhance their size, or they can be altered solely to study the function of a newly discovered gene. This chapter will discuss various transgenic categories, and provide examples of societal benefits within each category as a prelude to the next chapter on transgenic ethics.

Disease Models

Aside from studying infected human patients, one alternative to studying diseases is to use live animal models. In fact, human clinical trials often cannot proceed unless new drugs or treatments are first tested on animals. But some human diseases are not carried by animals, so to study these types of diseases new animal models must be created. Transgenics takes care of this problem by allowing human genes to be inserted in an animal's genome.

Alzheimer's Mouse

Alzheimer's disease (AD) is the most common type of neurodegenerative disease, and its patients are expected to drastically increase in the new few decades with our ageing population.

The complete cause of AD is unknown, however scientists have some understanding of genetically inherited early-onset cases. The brains of AD patients become riddled with senile plaques (composed of amyloid-beta protein) (Aβ) and neurofibrillary tangles (composed of abnormal tau protein). While these toxic plaques and resulting tangles occur at low frequencies as a result of old age in those not afflicted with AD, researchers have found that AD patients develop both abnormal structures very rapidly and primarily in areas responsible for memory (Alzheimer's Association 2004).

Aβ is a neurotoxin that kills neurons. This protein is formed from amyloid precursor protein (APP) on the surface of neurons and glial cells. Early-onset AD patients often have APP mutations that lead to the formation of more Aβ neurotoxin (Goate et al., 1991). Animals do not normally get AD (except for some great apes which are not a convenient model to work with), so to mimic AD in animals, scientists inserted human genes with mutated APP into mice.

The world's first true working model for AD was created in 1995 by Professor David S. Adams of Worcester Polytechnic Institute and his fellow researchers from the former Transgenic Sciences Incorporated (TSI), who successfully created mice expressing mutant forms of human APP (Games et al., 1995). After about 6 to 9 months, these AD-mice develop toxic Aβ and senile plaques, and show considerable brain damage in the hippocampus; however neurofibrillary tau protein buildup within the neurons was not observed (Games et al., 1995). This significant observation eventually led to the finding that although Aβ initiates AD, and by itself initiates brain damage, the production of abnormal tau is a required downstream event for making a more comprehensive model of the disease (Access Excellence, n.d.).

The creation of this AD-mouse line, opened many doors for screening drugs to block Aβ formation, and was directly used by Elan Pharmaceuticals (South San Francisco) to create the

world's first AD vaccine. The vaccine cleared Aβ from the brain (Schenk et al., 1999). The vaccine proved successful in decreasing the concentration of the plaques in older subjects, while mice treated at a young age showed no signs of Aβ or senile plaque formation. Subsequently, the FDA granted a fast track designation to the most effective of Elan's vaccines, and in December 2007 human trials began (Elan Corporation, 2009).

Oncomouse

The OncoMouse is another very successful transgenic animal. With funding from Dupont, this mouse line was created by Harvard and National Institute of Health (NIH) researchers Timothy Stewart and Philip Leder in the 1980s (Stewart et al., 1984). As the name would suggest, OncoMouse is a genetically engineered laboratory mouse designed to have an increased susceptibility to developing cancer, which can be used for studying cancer formation or for screening anti-cancer drugs. The original OncoMouse, also known as the Harvard Mouse, contained somatic cells and germ cells with a *myc* oncogene under the control of a mouse mammary tumor virus (MMTV) promoter. This promoter is switched on in mammary tissues, so oncomice develop mammary tumors, usually adenocarcinomas (Stewart et. al., 1984; Bioethics and Patient Law, 2006). The creators of the original OncoMouse eventually patented their method of engineering cancerous transgenic mice and the OncoMouse trademark name, with DuPont as the owner of the rights to the invention (Leder and Stewart, 1984; DuPont Technology, 2008). So these mice became the world's first patented animals, which will be discussed in Chapter-4.

Since the creation of the first cancer-susceptible mouse, many other methods of inducing cancer in mice have arisen. For example, if mice are generated as either homozygous or

heterozygous for knocking out tumor suppressor *p53*, as the mice mature, because their p53 is not present to help correct for DNA mutations that can lead to tumor formation, the mice are highly susceptible to developing malignant lymphoma and osteosarcomas, respectively (Harvey et al., 1993). As another example, if mice are made heterozygous for a nonsense mutation in their *Apc* genes, they become increasingly more susceptible to intestinal adenomas (Jacoby et al., 1996). Yet another type of transgenic Oncomouse contains the v-Ha-*ras* oncogene driven by the MMTV promoter. These mice become increasingly susceptible to benign hyperplasia and develop tumors in mammary, lymphoid, and salivary tissues (Sinn, 1987).

Transpharmers

Transpharmer animals are another major success of transgenics. These animals allow scientists to produce life-saving drugs in the milk of farm animals. This is done by engineering the gene encoding the protein drug to be under the control of a milk protein promoter, like casein or lactoglobulin, so the drug is expressed in the milk. The protein can then be purified directly from the milk without sacrificing the animal (Biotechnology Information Series, 1995; Gillespie, 2010). This process is not harmful to the animal, and gathering the protein is simple, the animal need only be milked (Walsh, 2007).

Before transpharming, human therapeutic proteins were either isolated from large quantities of donated cadaver organs, or were produced by microbial bioreactors using recombinant DNA technology. Microbial production does not work for complex proteins that require post-translational reactions such as glycosylation to yield a biologically active drug (Janne et al., 1992). Although the use of the microbial bioreactors was relatively inexpensive, the

process sometimes produced inactive drugs. Mammary tissue is capable of the full range of post-translational processing steps.

*Transpharmer Goat*s

A very notable transgenic goat was announced to have been created in May of 1999 by Genzyme Transgenics Corporation (Framingham, MA), Louisiana State University, and Tufts University School of Veterinary Medicine (Grafton, MA). Human Antithrombin III (ATIII) is a protein found in human plasma that helps prevent blood clotting. In October and November of 1998 at Genzyme Transgenics' farm in Massachusetts, three healthy identical cloned female goats were born containing the human ATIII gene under control of a milk protein promoter, of which one produced ATIII in its milk (Genzyme Transgenics, 1999).

In 1999, the transpharmed ATIII protein was in phase-III clinical trials to be evaluated as an effective anticoagulant for patients following cardiopulmonary bypass surgery. In February of 2009, the FDA approved ATIII for those with hereditary antithrombin deficiencies (HD) undergoing high risk surgeries to prevent the patients from having peri-operative hemorrhaging, during operation, and post operation thromboembolic events. In Europe, transpharmed ATIII was approved for surgical use in HD patients by the European Commission as early as 2006 (ATryn, 2008).

Transpharmer Sheep

Human factor–IX protein is very important in blood coagulation, and if it is deficient (as in hereditary bleeding disorders such as hemophilia-B) it renders blood unable to clot. The only treatment for this disorder is to administer factor IX protein to patients. However, before

transpharming, the only method for obtaining the necessary factor was the extraction from human plasma, which can contain HIV or hepatitis C virus, and blood screening increased costs (Schnieke et al., 1997).

In 1989, scientists designed a hybrid gene encoding human factor-IX under the control of a milk protein promoter, and inserted it into sheep. Two ewes were designed to carry 10 foreign copies of the gene. Researchers found that each ewe successfully secreted factor-IX in their milk, deeming the experiment a success (Clark et al., 1989).

Xenotransplanters

Xenotransplantation is the implantation of cells of one species into another species. Xenotransplanters are donor animals that are genetically manufactured to prepare their organs for transplantation into human recipients. The front runners considered for these xenotransplants are pigs, as scientists say that pig organs are better than those of chimpanzees or baboons because their size is better suited for the human body, pig physiology is similar to humans, and pigs are already routinely slaughtered for food (Fabregas, 2006). Although this field of transgenics is relatively new, the use of pigs to help save human lives is far from new; pig heart valves have transplanted to diseased patients for years (Catez, 2005). Presently, in the United States, ten patients die each day waiting for organ transplants (U.S. Food and Drug Administration, 2009). The need for organs far outweighs the availability, which may be why scientists have begun looking to xenotransplantation as a viable option.

Pigs produce sugars called alpha-1,3-galactose on the surface of their cells that human immune systems recognize as foreign, causing immuno-rejection (Pearson, 2001). Scientists have successfully identified two gene alleles that are responsible for the production of these

sugars, and have managed to knock out one of them. But they still have to knock out the other allele. When the University of Pittsburgh Medical Center (UPMC) and Revivicor Inc. came together on a farm in Blacksburg, Virginia, they engineered 200 pigs to lack alpha-1,3-galactosyltransferase, the enzyme that adds galactose onto the cell surface which is viewed as foreign by humans. In 2002 and in 2003, some of these organs were transplanted into baboons (Pearson, 2003). The baboons received the genetically altered pig organs and survived nearly six months. The problem was formation of tiny blood clots that worsened over time. Now these scientists are putting human anticoagulant genes into the pigs as a method of solving the problem.

One potential problem associated with intermingling species is the possibility of spreading diseases. Some viruses, such as swine flu, can jump between species causing pandemics. So scientists argue pigs used for this purpose should be screened for known viruses. The US FDA required Revivicor to screen all pigs for a myriad of bacteria and viruses (Fabregas, 2006).

Transgenic Food Sources

To better accommodate food for an ever expanding population, some animals have been genetically modified to allow them to grow larger and faster. These experiments failed with mammals, but have shown some success with fish (Harper, 2006).

Super Fish

In aquaculture, trout and salmon have been genetically modified to grow larger than their normal non-transgenic counterparts. To test whether science could increase the rate of growth to

help production rates, Rainbow trout eggs were microinjected with DNA construct "OnMTGH1", containing a salmon gene that over-expresses growth hormone under the control of a metallothionein promoter that is always switched on. This allows the trout to produce growth hormone year round instead of seasonally. The results were successful, as the injected trout matured much faster than the wild type; however in this particular experiment the trout did not surpass the wild type in size (Devlin et al., 2001). Unfortunately, the animals died before reaching sexual maturation, and were observed to have both cranial abnormalities and reduced viability (Devlin et al., 2001).

In a second experiment, Pacific salmon were engineered to contain a Chinook salmon growth hormone gene driven by a Pout antifreeze protein promoter. These salmon grew 11 times faster than their wild type counter parts and were larger (Harper, 2006). Four out of five of these fish reached sexual maturity, and were able to pass the transgene onto offspring (Devlin et al., 1995). Unlike the Rainbow trout experiment, the Salmon showed increased growth rates, improved flesh color, and increased disease resistance, while eating less food. They even survived even after their tank had been frozen (Devlin et al., 1997). These fish, marketed by Aquabounty Technologies (Waltham, MA), are close to obtaining FDA approval for human consumption, and if so would become the world's first approved transgenic animals for consumption (Aquabounty Technologies, 2011).

Super Pig

The goal of the Superpig experiment was to create a pig that grew larger and faster, but ate less food. A DNA cassette containing an ovine growth hormone gene under the control of a metallothionein promoter (always on) was microinjected into 400 zygotes, and 15 transgenic

pigs were born. The liver, kidney, adrenal, thyroid, carcass fat and subcutaneous fat of all transgenic littermates were larger and thicker than their non-transgenic littermates (Pursel et al., 1997). Another type of Superpig was genetically modified in 1989 in Beltsville Maryland, containing the human growth hormone gene instead of the ovine version (Miller et al., 1989). Unfortunately, the Superpigs suffered from a number of terrible side effects, including kidney and liver problems, uncoordinated walk, thickened skin, ulcers, joint disease, heart disease, bulging eyes, and pneumonia, so the animals were euthanized (Rollin, 1996). This series of mammalian growth hormone experiments failed, which resulted in a voluntary suspension by scientists of all transgenic growth hormone experiments on farm animals.

Transgenic Scientific Models

This category of transgenic animals includes animals constructed to provide information on the function of specific proteins *in vivo*. Some of these animals are engineered to over-express certain proteins, while others have the genes encoding the protein knocked out to eliminate its expression. Some successful examples in this category include Doogie the Smart Mouse, ANDi the Monkey, and AlphaMUPA mice that eat less and live longer. All of these transgenic animals have opened many doors for further study.

For example, Doogie the Smart Mouse was engineered to over-express the gene encoding NR2B, a subunit of the NMDA receptor that predominates when the brain is young, and can presumably help the animals learn faster (Tang et al., 1999). The gene also controls the brain's ability to associate different related events, one of the foundations of learning (Harmon, 1999).

ANDi was the world's first transgenic primate, and he was engineered to contain a jellyfish gene encoding green fluorescent protein (GFP) (Chan et al., 2001). Although he was

transgenic, containing the GFP gene, he did not express it so his cells did not glow fluorescent green. But he proved transgenic technology can be applied to primates, so this technology might be expanded to primates in the future (Vogel, 2001).

AlphaMUPA mice were engineered to express urokinase-type plasminogen activator in the brain. These mice eat 20% less and live 20% longer than wild type mice, however their legs had tremors. The animals had much more plasma corticosterone (a stress hormone) when young, but a significantly reduced amount when old. These little fountains of youth offer scientists a method of studying delayed aging at the systemic and single-cell levels (Miskin et al., 1999).

Transgenic Applications Conclusion

The transgenic animals discussed above were created with different goals in mind for benefitting society. Because of these animals, more is known about Alzheimer's disease and cancer, while some serve as bioreactors producing life-saving medicines. Some transgenic animals may make it possible to help prevent human starvation, while others teach us about how the brain learns new information. This chapter served to introduce the reader to the various types of transgenic animals, and serves as a prelude to facilitate the project's later discussions of transgenic ethics and laws.

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CHAPTER-3: TRANSGENIC ETHICS

Tania Emmanuelle Torchon

A chimera is a mythical creature composed of parts of various animals. Human imagination has enabled chimeras to live on for centuries without truly inhabiting the earth. Some of the most famous range from the monstrous fire-breathing Greek Chimera that was composed of the body of a lion, head of a goat, and tail of a snake, to the Hindu and Buddhist Garuda that was half man and half eagle (Kimbrell, 1994). From a genetic point of view, a chimera is an organism containing altered DNA from two or more genetically distinct organisms; nowadays, these creatures are known as transgenic animals. Although chimeras were once thought of as fantasies, transgenic sciences have opened the doors for unimaginable fiction to become fact. However unlike the mythical chimeras, the purpose of creating transgenic animals is for society's benefit. The purpose of this chapter is to discuss the topic of whether transgenic animals *should* be created.

Framing the Transgenic Ethical Question

Transgenic ethics is a balancing act between the benefits to society versus the potential harm to the animals. Some of these transgenic animal designs are brilliant because modern science has invented ways to allow genes to be mixed between species. However brilliant they may be, society still holds many qualms about this research as some of the animals endure much pain. From the DuPont OncoMouse that can suffer with advanced tumor formation, to transpharmers and ANDi the monkey that do not suffer at all, transgenic ethics has proven to be impossible to cover with one blanket policy. And some animals provide strong medical benefits,

while others were constructed simply to see whether that species could be made transgenic. Consequently, strength of the benefits to society versus the suffering of the animals can be a gauge as to whether certain transgenic experiments should continue. This chapter will discuss the ethical status of specific transgenic examples, while providing author conclusions on which experiments should continue.

Disease Models Ethics

A disease model is an animal genetically altered to carry and express a disease gene normally found in humans, as previously discussed in Chapter-2. These animals are necessary to disease research to help screen treatments and test cures before implementing such treatments in humans. The question stands as to whether inflicting these animals with deadly diseases (or portions of the disease process) is worth the benefit to society (Christiansen and Sadoe, 2000). Chapter-2 discussed two very successful but very different transgenic animals: the Alzheimer's mouse and the OncoMouse.

Alzheimer's Mouse Ethics

The first example of a disease model to discuss is the Alzheimer's mouse. Alzheimer's disease (AD) cannot be prevented, cured, or slowed, and it ultimately leads to death. It is the sixth leading cause of death in the United States, and there are an estimated 5.4 million people currently suffering with it (Alzheimer's Association, 2011). The sum of the direct (patient care) and indirect costs (caregiver loss of work hours) of Alzheimer's disease and its related dementias is $183 billion dollars as estimated for 2011 (Alzheimer's Association, 2011). In the US, every 70 seconds someone develops Alzheimer's disease (Elan Corporation, 2009).

As previously mentioned, the AD mouse was created in 1995 (Games et al., 1995) and mimics the early stages of AD as seen in humans. The mouse line develops neurotoxic Aβ plaques and shows damage in its hippocampus, an area of the brain related to memory. So the mouse is useful for screening drugs and developing vaccines to block the early stages of AD. However, the mice do not get the complete disease; unlike humans, the mice show no mutated tau protein buildup that normally form neurofibrillary tangles (Games et al., 1995). The South San Francisco branch of Elan Pharmaceuticals used this mouse model to develop the world's first vaccine for AD that showed great promise in the mice (Schenk et al., 1999), and is now in human clinical trials.

With respect to the mice, there has been no report of any measurable pain or suffering endured by the AD mice. The mice play normally, eat normally, and reproduce normally. Their reduced memory would surely play a factor if they were competing for survival in the wild, but they live in laboratories with few to no survival skills required. The mice do not appear to suffer by any measurable criteria, they have increased our understanding of AD initiation, and they served as a required step for developing potential AD treatments.

The proof of their value lies with the billions of research dollars currently being spent by various pharmaceutical companies on inhibitors and vaccines whose purpose is to decrease the formation of toxic Aβ and remove existing senile plaques from neural tissue, based on data obtained from AD mice (Elan Corporation, 2009). Substantial medical advancements have been made possible with AD mice, with the animals' welfare intact, so where's the harm in continuing experiments on this disease model?

Oncomouse Ethics

In 2010, an estimated 1.5 million people worldwide, and a half a million Americans, were diagnosed with cancer (American Cancer Society, 2010). On the complicated end of disease model ethics lays the Harvard and Dupont OncoMouse. This model was originally created in 1982 (Stewart et al., 1984) containing a *c-myc* oncogene under the control of a mammary tumor virus promoter. The promoter drives the expression of the oncogene in mammary tissue, so the mice are prone to mammary adencocarcinomas. This mouse model can be used to screen new treatments for cancer, just as the AD mouse does for AD. The model has taught scientists a great deal about cancer and why tumors form. However, unlike AD mice, Oncomice can indeed suffer, depending on the stage of tumor formation prior to sacrifice.

Yes, animals are unable to clearly communicate their levels of pain and suffering, but why would their tolerance levels not be analogous to those of human patients suffering from cancer's afflictions? This is a valid reason for stopping experiments if possible prior to advanced tumor formation by euthanizing the mice. However, the 1.5 million people suffering from cancer, and their families and friends, are strong benefiters of the information learned from this mouse line. Institutional Animal Care and Use Committees (IACUCs) should use strong oversight to mandate euthanasia before unnecessary pain and suffering develops. These mice have provided scientists with an excellent opportunity to learn about tumor formation and to develop treatments, so the experiments should continue with strong oversight.

Transpharmers Ethics

A transpharmer is an animal engineered to express a foreign gene (usually a gene encoding a desired human drug) in their mammary glands. The drug is produced and secreted

into the milk without needing to sacrifice the animal. Transpharmer species produced to date include mice, goats, sheep, and cows. Using these animals allows for easy purification of the drug, low manufacturing costs, and the wellbeing of the animal is kept intact.

Before transpharming, therapeutic proteins were often obtained through cadaver organs (always in short supply) or produced cheaply but inefficiently with microbial bioreactors. But microbes are unable to produce active complex protein drugs because they lack some of the necessary post-translational processing capabilities (Janne et al., 1992). Mammary tissue is able to complete these complex post-translational processing steps and has allowed several drugs to be manufactured.

For example, transpharmer goats were created in 1999 that can produce human anti-thrombin blood thinning protein ATIII in their milk. ATIII is currently used in Europe on antithrombin-deficient patients undergoing surgery to minimize clotting (ATryn, 2008). This drug was also the first FDA-approved transpharmed product in the US. There have been no reports of transpharmers suffering (for those that survive the transgenic process), yet they produce lifesaving medicines, therefore these experiments should continue.

Xenotransplantation Ethics

In the United States, currently there are 111,812 people awaiting an organ donation. Eighteen people die each day waiting for an organ, while one organ donor can save 8 lives (U.S. Department of Health and Human Services, 2011). These numbers become of even greater concern when considering that in 2010 there were only 14,502 organ donors in the United States (Donate Life, 2011). Although 8 lives are saved with one organ donor, some of the donors aren't compatible with the patients, so the desired organ is rejected.

Xenotransplanters are transgenic animals engineered to produce organs histo-compatible with humans. Their ability to mass produce organs could give hope to those awaiting transplants. Surprisingly, pig physiology is very similar to that of humans, so pigs are preferred for xenotransplantation (Fabregas, 2006). Caution must be used however, as pigs can carry viruses that infect humans. But this could be minimized by screening the pigs for known viruses. Considering the chaos caused by the H1N1 influenza virus that once affected swine, cross species infection should be intensely supervised.

Should pigs be raised for organ donation? The author of this chapter says yes. Pig heart valves have been transplanted into humans for years, and pigs are already routinely slaughtered for food, so harvesting their organs in addition to using their meat is not that different. Swine organs are usually thrown away after slaughter, other parts are fed to pets, and other meat is used for human consumption. Why not use all of the pig rather than just a portion? If a pig is to be slaughtered for consumption, why not collect it's organs for transplantation also?

Transgenic Food Sources Ethics

In 2009 there were 50.2 million Americans living in food insecure homes with 17.2 million of these Americans being children (Feeding America, 2011). Supply and demand is the economic system that governs the world; as demand increases without supply increasing, prices increase, and the poor are left to fend for themselves. Transgenic food sources are genetically enhanced plants and animals that mature at a more rapid rate and are larger in size compared to their wild type counterparts. When discussing transgenic animal food source ethics, Superpig and Superfish, as previously mentioned in Chapter 2, are great examples of both unsuccessful and successful transgenic food source animals, respectively.

Superpig Ethics

The goal of superpig was to create a pig that provided more meat, in less time for the increasing food demand. Superpig was created in 1989 in Beltsville, Maryland, and contained a human growth hormone gene under the control of a strong always-on promoter (Miller et al., 1989). However, the first super pig was a disaster and the animal suffered serious health problems, including kidney and liver problems, uncoordinated walk, thickened skin, ulcers, joint disease, heart disease, bulging eyes, and pneumonia, so the animals were euthanized (Rollin, 1996). Due to this experience, scientists proposed a voluntary moratorium on performing growth hormone experiments in mammals.

Aside from Superpig's suffering, the animal may not have been a good food source anyway. Unhealthy animals that can barely stand should not be released to the public. These animals spread diseases and are routinely euthanized for human's sake, and to protect their herds. The author is against performing mammalian growth hormone experiments. Yes hunger worldwide is increasing, but consuming unhealthy food is not the answer.

Superfish Ethics

The goal of Superfish was similar to that of Superpig, grow a fish larger in less time on less food. As mentioned in Chapter-2, the Super Pacific Salmon grew 11 times faster than their wildtype counter parts and were less susceptible to diseases. These fish were so superior to their wildtype, they were able to survive even after being frozen (Devlin et al., 1997). And unlike Superpig, these aquacultures of Superfish seem to have exhibited no pain or side effects. Because of the superb progress made with SuperFish experiments, the FDA is close to approving the world's first transgenic animal manufactured for human consumption (Aquabounty

Technologies, 2011). In addition, Aquabunty's Superfish were engineered to be sterile, so they cannot mate with wildtype fish if they escape their aquafarming cages. The author is in favor of Superfish experiments continuing, so long as no health effects to the fish are noticed.

Transgenic Scientific Models Ethics

The transgenic scientific models discussed in Chapter-2 are engineered to over-express or eliminate expression of specific proteins. The examples discussed were Doogie the Smart Mouse (that overexpressed the NR2B subunit of the glutamate receptor), ANDi the not so fluorescent monkey (who was supposed to express green fluorescent protein), and the long living AlphaMUPA mice (who over-expressed urokinase). These three animal examples were all normal with no reports of pain, yet they increased our knowledge of the function of specific proteins. The ability of scientific models to expand our knowledge is incredible. Some of the animals were even superior to wild type counterparts. The author of this chapter is generally in favor of this category of transgenic animal, based on the knowledge gained.

Chapter-3 Conclusions

This chapter discussed the ethical concerns of the five different categories of transgenic research: Disease Models, Transpharming, Xenotransplantation, Food Sources, and Scientific Models. It discusses how some cases of transgenic animal research should continue to open doors to some of the vital problems that plague society, while others should not be pursued at the expense of another living creature's wellbeing. It also discusses how one policy should not govern all experiments, as the benefits to society and effects to the animals vary considerably.

Although some animals appear to suffer a great deal (SuperPig and OncoMouse), some don't suffer at all (Alzheimer's mouse, transpharmers, Doogie the Smartmouse), therefore a blanket policy on all transgenic animals is both unrealistic and unfair to the potential breakthroughs that this technology has to offer.

With respect to disease models, the author of this chapter believes the experiments with Alzheimer's mouse should continue (there is no pain to the animal, yet the medical benefits have been strong), and experiments with Oncomouse should be strongly overseen by IACUC committees to minimize animal suffering by either using painkillers or by early euthanasia. The author is also generally in favor or transpharmers (no animal suffering while saving lives with their medicines), Xenotransplanters (pigs are already being slaughtered for food so why not get their organs too), Scientific Models (we have too much to gain by creating them), and Food Source sources like Superfish (but not Superpig).

As previously mentioned, transgenic ethics is a balancing act between the benefits to society versus the potential harm to the animals. As demonstrated in this chapter, the balance should be weighed on the case by case basis, and not with one blanket utilitarian policy.

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CHAPTER-4: TRANSGENIC LEGALITIES

Richard Breault

Because the use of transgenic animals is controversial, laws have been enacted to regulate transgenesis and the production of these animals (Ladas, 2003). In addition to these rules, some animals have been patented, which raises the question of whether such patents should be allowed. This chapter will discuss the patenting of transgenic animals.

US Patent Requirements

According to the US Patent and Trade Office (US PTO, 2005) to patent something the inventor must demonstrate three basic requirements: novelty (the invention must be something new, not previously invented), non-obviousness (so an expert in the same particular field of study would not easily come up with the same idea), and utility (there must be a purpose to the new invention). The law states "a claimed invention is deemed useful if… it is capable of providing some identifiable benefit. The benefit must be specific, substantial, and practical." (US PTO, 2005).

In addition to these requirements comes a set of applications and paperwork. If approved, the inventor has rights to the patent for 20 years (US PTO, 2005), so other people do not have the right to manufacture, sell, import, or use the same invention without direct compensation (Garza, 2007). The PTO states that an inventor "who invents or discovers any new and useful process, machine, manufacture, or composition of matter… may obtain a patent therefore, subject to the conditions and requirements of the [US PTO]" (BitLaw, 2000).

The First Patented Organism

Prior to patenting animals, a legal precedence was required for patenting any life form. This precedence was the case of Diamond v Chakrabarty, 1980. In this case, microbiologist Chakrabarty engineered a *Pseudomonas* bacterium containing two plasmids carrying genes for metabolic enzymes that allowed the microbe to digest oil. The microbe was to be used to digest oil slicks (**Figure-1**). The patent was initially filed in 1972, but it was challenged because the patent examiner concluded the patent could not be granted because microbes are natural, living organisms, not "compositions of matter". There was no precedent for patenting life.

Figure-1: Advertising Poster for Chakrabarty's Oil-Eating Microbe.
This picture was posted in China to inform people what bacterium was cleaning up their BP oil spill (Palomi, 2010)

In the appeal, Chakrabarty argued that the engineered bacterium was novel and did not naturally exist in nature, so it should be classified as a "composition of matter". This specific ability is not possessed by any other organism known to man and therefore is a creation by man. Using this logic, Chakrabarty appealed to the PTO Board of Appeal and eventually won the acceptance of his patent in 1980, so the world was given its first patented organism, described as

a “live, human-made micro-organism” (Diamond vs. Chakrabarty, 1980). This landmark case opened the door for the further patenting of transgenic animals.

United States Oncomouse Case

In 1984, four years after the acceptance of Chakrabarty’s patent, Philip Leder and Dupont attempted to patent a transgenic animal known as Oncomouse (**Figure-2**). Oncomouse was originally created for cancer and tumor research (Stewart et al., 1984), and contained a myc oncogene under the control of a hormonally inducible mouse mammary tumor virus promoter. The promoter caused expression of the oncogene beyond its normal levels in mammary tissue, causing mammary adenocarcinomas. The mouse is used to study cancer formation by oncogenes, and to screen anti-cancer drugs (WIPO, 2006).

Figure-2: The Harvard Oncomouse.
(Harvard, 2007)

For four years, the issues of whether animals should be patented, and the benefits to society versus animal suffering were argued. While the Oncomouse case was under consideration, in 1987, another case involving polyploidy oysters was considered which affected the outcome of the Oncomouse case. The case considered whether the polyploidy oysters were

patentable subject matter. The case ended by the US PTO announcing that "non-naturally occurring, non-human, multicellular living organisms, including animals, are patentable subject-matter within the scope of the Statute" (Schutt, 2004), which opened the floodgates for animal patents including the Oncomouse (although the animal cases caused more arguing with animal rights activists, farmers, etc).

After much debate, in 1988 the US PTO granted patent #4,736,866 to produce non-human transgenic animals with oncogenes inserted into their DNA (filed as Leder and Stewart, 1984). Leder went on to claim additional patents on preparing cell cultures from non-human transgenics (Leder and Stewart, 1992), and on the testing method for mice expressing an oncogene (Leder and Stewart, 1999).

European Oncomouse Case

The European Patent Office (EPO) had a different stance on the patenting of Oncomouse. In this case, initially the EPO concluded the patent did not fully meet requirements of a patent therefore most claims were accepted except the mouse itself. The EPO initially stated that patents do not cover animals and that this patent was immoral (WIPO, 2006). But eventually the EPO decided to apply a utilitarian test to weigh the potential of the mice to benefit society via helping develop medical treatments for cancer patients versus the potential pain suffered by the mice. The EPO eventually concluded that the medical possibilities grossly outweighed the potential negative outcomes to the mice (WIPO, 2007), so finally in 1992, the EPO approved the patent for Oncomouse (Sharples and Curley, 2009). However, the patent was restricted only to mice.

Canadian Oncomouse Case

Besides filing patents in the US and Europe, Harvard also applied for Oncomouse patents in Canada and Japan (Check, 2002). Canada decided to go in the opposite direction as Europe and the US. They saw Oncomouse itself as uninventive. The claims to the techniques and means of making Oncomouse were accepted, but the mouse itself was not, as engineered animals was not included in the definition of an invention (WIPO, 2006).

Japanese Oncomouse Case

Japan followed in the footsteps of the US and Europe in being generally favorable to patenting animals. The Japanese Oncomouse patent was accepted much faster than the US and European patents, because Japan did not have a provision excluding biological inventions (unless they violated a morality clause). In Japan, as well as Europe, the animal itself, and the production of said animals is patented (Schutt, 2004).

Current FDA Laws on Transgenesis

The US FDA oversees "articles (other than food) intended to affect the structure or any function of the body of man or other animals" (FDA, 2009), so the FDA watches over every part of transgenesis, from the initial construction of the recombinant DNA, to how the animals are maintained and transported. According to the FDA the only difference between a "normal" animal and a genetically engineered (GE) animal is the presence of rDNA which provides a new trait to the animal. Animals that obtained their rDNA in the embryonic stage, can breed and pass on their GE DNA because it resides in all cells including their gametes. Based on feedback from

scientists and opponents, the FDA created new streamlined regulations for these animals and their products (FDA, 2009). In order to patent an animal or transpharmed drug, they must pass through seven categories:

1. Definition- what are claims made, and what is the novel drug.
2. Construct- how was the rDNA made.
3. Lineage- how has a strain of the described transgenic performed over generations.
4. Phenotype- provide evidence that the animals are in good health.
5. Durability- prove there will be no change in production of drug over time.
6. Safety- provide evidence to the safety of the environment and, if used for food, to the consumer.
7. Validation- provide evidence the final product does what you say it does.

Even though some scientists view the FDA as sometimes detrimental to science because they can slow the process of patenting, with the creation of the new streamlined guidelines for filing transgenic patents, the hope is to increase both ease of the process and the safety to animals and the consumers.

Should Life Be Patented: Pros and Cons

Many good things have come for society from the patenting of organisms. And even though transgenics can aid medical research they are still changing the natural genetic code of these animals. The patenting of these animals allows the creator of to maintain legal rights to his/her creation for 20 years to attempt to sell it to make money. For the patented period, any scientist wishing to use the invention/creation must get approval from, or compensate the

inventor. In general, this is a good thing because it protects the inventor from losing credit for his/her invention, and allows money to flow into the company to stimulate further research.

However, in some cases, the licensing fees were so high that some scientists feared only the wealthy labs could afford to work with the animals, leaving out the smaller labs. This was the case with Oncomouse when Harvard and Dupont first applied their licensing fees. DuPont wanted to charge for the use of Oncomouse in drug screening and company-related experiments but allowed free research licenses for "noncommercial studies". So all entities had to register and comply with basic licensing terms, but the license was free for non-profits. Some groups argued that these fees could slow down testing and that they marred science as a whole. Some even argued that the patent would not hold up if challenged in court, but no such claims have been filed to date. Although the price of a license is a major factor, the mere fact that the patent covers such a broad spectrum of transgenic animals is more of a problem. Either way, DuPont still has rights to Oncomouse and any cancer-induced transgenic mouse (Marshall, 2002).

Also on the negative side of allowing transgenic patents are the concerns of environmentalists who are worried that transgenic animals may escape into the wild to breed with their wild type counterparts spreading the transgene. In the case of Aquabounty's Superfish, who grows to a large size in aqua-farming (Aquabounty Technologies, 2011), environmentalists were worried the large salmon would escape and outbreed normal salmon. However, Aquabounty engineered the fish to be sterile, so in this case the argument is minimized.

Also on the negative side are animal rights activists who argue scientists should pay closer attention to animal suffering. Animal rights activists say that the pain an animal must endure must not be overlooked, even with extreme advancements in medicine to help minimize

the suffering (Letterman, 2007). While some activists believe that animal pain should be minimized and weighed against the positives coming out of the research, other activists see only the negative side and want to stop all further animal testing. The welfare of the animals should be important to both "man-made" and naturally occurring animals. Some activists argue that simply giving these mice cancer is in direct violation of their rights, but the scientific governing groups of animal protection state that animal rights and animal welfare are not the same, and that they cannot support animal rights activists whose beliefs condemn the responsible use of animals for the betterment of human society (Arnold, 2001).

Since the original patenting of Oncomouse, the US PTO has approved hundreds of animal patents. Without a doubt, transgenic animals have benefited society, and likely will continue to do so in the future, however I agree that animal suffering should be minimized. Institutional IACUC committees should provide strong oversight to ensure the least number of animals are used in testing, that animals are quickly euthanized if necessary, and that painkillers are used appropriately.

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PROJECT CONCLUSIONS

From purpose, to creation, to the many moral and legal issues surrounding transgenic animals to date, this IQP report covers it all. Transgenic animals are genetically engineered animals that contain, and may express, foreign genes that have been inserted into their genome. They can be created by a number of methods and have various applications. However, as beneficial as research on these animals may be to scientific growth and to society's health, a number of ethical and legal concerns surround these creatures.

Transgenic animals are typically created by either microinjecting the transgene into the pronucleus of a fertilized egg, or by manipulating embryonic stem (ES) cells. The former usually creates animals in which all the cells of the body are transgenic, while the latter technique produces chimeras that need to be bred to make pure transgenics.

Transgenic applications have five primary classes: Disease Models, Transpharmers, Xenotransplanters, Food Sources, and Scientific Models, all discussed in chapter two. The disease model class of transgenic animals enables researchers to study the progression of human diseases on animals to help find cures. Transpharmers produce lifesaving medicines in their milk that are otherwise produced inadequately by other technologies. One transpharmed drug ATryn has already been FDA approved to help prevent thromboembolisms in antithrombin deficient patients. Organs are a precious asset, spare organs are even more precious and very scarce. Xenotransplanter research gives hope to patients facing death with an exclusively engineered pig organ alternative that is less likely to be rejected by the host immune system. Fish designed to grow larger, faster, while consuming less food may make it possible to reduce

the human hunger statistic. Transgenic biological models offer scientific knowledge of specific protein function *in vivo* unattainable by other means.

Chapter three explores ethical issues related to transgenic animals. It leads with a statement outlining the debate as a balancing act between animal welfare and the medical, educational, and industrial advantages to society. The chapter poses important questions that encourages the reader to form their own opinions of whether the line between animal discomfort and society's benefit has been crossed in specific cases. Chapter four discussed some of the legal issues associated with transgenic animals, including the advantages and disadvantages of patenting animals.

As for the opinion of the authors, based on the research performed in this project, we believe that most experiments on transpharming, xenotransplantation, scientific modeling, AD disease modeling, and fish aquaculture food enhancement should continue, as these reportedly posed little to no harm to the animals, while providing strong benefits to society. However, the authors believe that other types of transgenic experiments, including Oncomouse experiments with advanced tumor formation (that involves pain to the animal) and mammalian growth hormone experiments (like Superpig) should cease until better methods are found, as these animals have been reported to suffer much pain. For Oncomice experiments, administering euthanasia prior to severe disease progression should be considered as an alternative, or even administering pain killers throughout the entire research process. However, because there is no direct way for the animals to report their levels of pain, and the integrity of the diseases' progression may be affected by outside medications, the best method is to completely suspend these types of experiments for now. With respect to Superpig experiments, the authors believe

that when the animals can barely move because of severe joint pain, all forms of research with the animals should cease and the affected animals should immediately be euthanized.

Overall, based on the findings of this project, the authors believe in general 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 or that involve animal suffering. 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.