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TRANSGENIC ANIMALS

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ABSTRACT

We examined the methods of creating transgenic animals, the reasons for doing so, and the resulting effect on society. The most common methods of making transgenic animals are described, and the transgenic animals created to date are categorized by purpose. Some myths about transgenic animals are dispelled, and the ethical criteria for determining whether such animals should be made are established. Three specific examples of ethical, difficult to determine, and non-ethical creations are analyzed for illustration.

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EXECUTIVE SUMMARY

In the interest of educating the public about transgenic animals and determining their effect on society, the methods of creating transgenic animals is discussed. The major types of transgenic animals are listed, with examples of each. Then common misconceptions will be addressed, and the ethics of transgenic animal creation will be discussed. In this manner, people who have never heard of transgenic animals might have a good idea of what they are, and can then form their opinions on fact and not on fear.

There are many methods for making transgenic animals, but all of them require making a transgene first. The transgene has three parts: a promoter to dictate the tissue in which the transgene is to be made, the transgene itself, and a termination sequence. The most common method of transgenesis is microinjection into the male pronucleus of a newly fertilized egg. This technique randomly inserts many copies into the male pronucleus with a micropipette. Another method is known as embryonic stem cell manipulation, in which the transgene is inserted into the stem cells of the blastocyst via microinjection, a virus, certain chemicals, or homologous recombination. The advantage of this technique is the cultured ES cells can be screened for uptake of the transgene, increasing the efficiency of the process. After the animals are born, they are typically screened for the transgene, usually via polymerase chain reaction (PCR), or a Southern blot analysis.

Transgenic animals can be divided into five major categories: disease models, transpharmers, xenoplanters, food sources, and scientific models. Disease models are

animals engineered to express the symptoms of a disease. Disease models that have been made include AIDS mouse, Alzheimer's mouse, oncomouse (a model for cancer), and Parkinson's fly. Transpharmers are dairy animals engineered to express a protein in their milk. So far, this process has been successful in mice, sheep, goats, and cows. Xenotransplanters are engineered to allow the transplantation of histocompatable organs into a human. Human trials have not been approved for this process, even though a xenotransplanter pig has already been produced. Food sources are food animals engineered to grow bigger, or simply more efficiently on less food. Superpig, one example, was a failure due to the pig's large list of health issues. Superfish is much more promising. Scientific models are animals with some transgene introduced to their genome for the purpose of studying a specific gene's, expression, or some biological process. ANDi is a famous example, and smart mouse, supermouse, youth mouse, and influenza-resistant mouse also belong in this category.

Is it ethical to create transgenic animals? It is impossible that everyone will agree with any one thing, so the issue is taken from an animal welfare standpoint, not an animal rights standpoint which would attempt to defend against the use of animals of any kind in research. The authors of this IQP argue that changing the genome is not wrong in itself, but specific applications can be wrong. Changing the genome for the purpose of creating art (such as the green fluorescent rabbits), and not to increase knowledge or save lives, is unnecessary. Even though some animals do die in most transgenic experiments, the numbers of human lives that can be saved far outweighs that suffering, however steps should be made to make the process more efficient so it results in fewer animal deaths, and oversights should be in place to minimize animal suffering. Instead of saying that all

transgenic animals are justified, each experiment should be taken on a case-by-case basis, like these three examples. Alzheimer's mouse experiences no pain because of its transgene, and the possibility of knowledge that could lead to a cure for Alzheimer's means that the creation was justified. The Beltsville pig, or "superpig" experienced an incredible amount of suffering for the possibility of very little chance of ending world hunger, so the experiment was justly terminated, and that path of experimentation discontinued. Oncomouse is on the edge because the mouse does suffer and eventually die from cancer, but the knowledge gained is so valuable in the fight against cancer. Therefore, the experiment is justified as long as the mice are kept in as much comfort as possible using painkillers or by sacrificing the animal before advanced tumor formation. Although animals have different physiology than humans, living disease models bring in much more knowledge than cadavers, so transgenic disease models are justified. Transgenic animals are not in danger of out-breeding all of their natural cousins if they escape, but they still should be kept secure. The Institutional Animal Care and Use Committee (IACUC) keeps scientists in universities and institutions from just creating transgenic animals at will, forcing scientists to justify animal use for each experiment. Some religious groups might be against the creation of transgenic animals as a violation of the creation, but most should have no problems as long as steps are taken to minimize animal suffering as much as possible, and to save human lives.

PROJECT OBJECTIVE

The objective of this IQP project was to examine transgenic animals and the societal issues that surround them. The report should explain to readers what transgenic animals are, how they are created, describe the types of transgenic animals created to date with examples, and discuss the bioethics behind transgenic animals. New transgenic technology has both positive and negative effects on the animals, as well as society, which is what makes the ethics issue so controversial. This paper should provide sufficient information to the reader so that they can make their own conclusions on whether or not to support transgenic animals.

CHAPTER 1: TRANSGENIC ANIMALS

DESCRIPTION AND CONSTRUCTION

A transgenic animal is an animal in which foreign DNA has been incorporated into its original DNA. Transgenic animals are altered so that their DNA produces chemicals that normally they would not produce. To gain a better understanding of this new technology, this first chapter will focus on describing a few of the more common ways that transgenic animals can be created.

There are numerous different ways to create transgenic animals. The most popular is microinjection of recombinant DNA into the male pronucleus of an *in vitro* fertilized egg. The second most popular method is embryonic stem cell transfer. Other methods include but are not limited to chemical or viral delivery into embryonic stem cells, or homologous recombination with embryonic stem cells.

Transgene Construction

Before these methods can be used to create a transgenic animal, the transgene must first be made. The transgene (Figure-1) is made up of 3 parts, the promoter, the transgene gene you want expressed, and the termination sequence.

Figure-1: Transgene Structure. The promoter (blue) dictates in which tissue the transgene gets expressed. The transgene (red) contains DNA sequences encoding the protein to be expressed, for example a human therapeutic protein to be produced in the animal's milk. The termination sequence (yellow) dictates the termination of transcription. http://www.agresearch.co.nz/scied/search/biotech/gene_gmomaking_animal.htm)

The promoter sequence is in the transgene to be sure that the transgene functions correctly and in the correct tissue of the animal. The promoter sequence is designed specifically for the transgene. For example, a casein promoter would ensure the downsteam transgene would be expressed only in the animal's milk for transpharming. The gene following (downstream of) the promoter sequence is the desired transgene. (e.g. insulin or a clot dissolver drug). The termination sequence or poly A sequence signals the end of the transgene to the animal (Making a Genetically Modified Animal, 2001). The DNA construct is considered transgenic since it contains a mixture of DNAs, usually human for the transgene and promoter, packaged into a bacterial vector DNA like plasmid DNA.

Microinjection into the Male Pronucleus

Microinjection into the male pronucleus is the most popular way to make transgenic animals. Eggs must be collected by super-ovulating female animals and these eggs must be fertilized *in vitro*. After fertilization the egg is held stable by a microtube suction device (Figure-2, left side), while a solution containing many copies of the transgene is injected using a micropipette (right side of the figure).

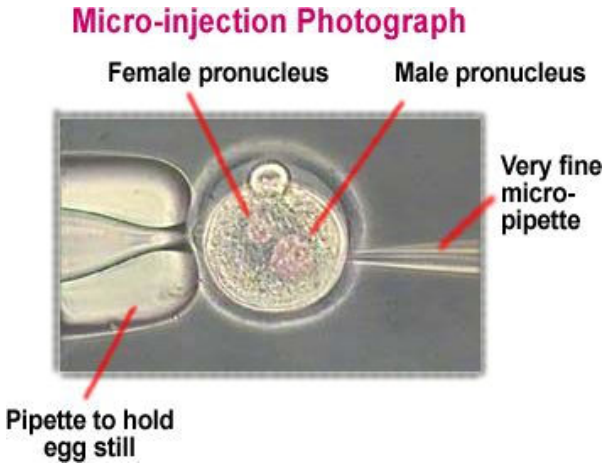

Figure-2: Picture of Microinjection into the Male Pronucleus. A microtube (left side) holds an in vitro fertilized egg (center) while a micropipette (right side) injects the transgene solution into the male pronucleus (large pink sphere) (http://www.agresearch.co.nz/scied/search/biotech/gene_gmomaking_animal.htm)

The transgene is injected into the male pronucleus because it is larger than the female pronucleus. This process is also depicted in the diagram in Figure-3. When the solution is injected into the fertilized egg it contains 200-300 copies of the transgene. Unfortunately, the percentage of animals that are transgenic (can pass this gene on) and or express this gene strongly is small, adding to the ethical considerations for making such animals since large numbers of eggs are destroyed (Making a Genetically Modified Animal, 2001).

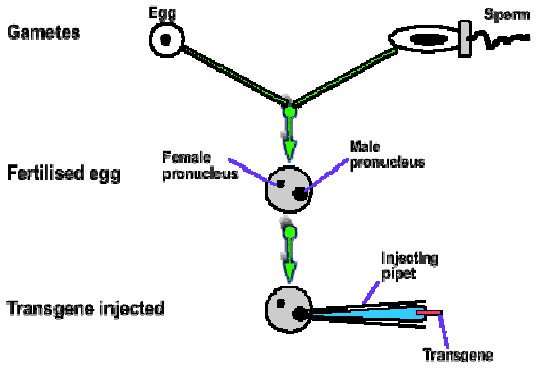

Figure-3: Diagram of the Steps Leading to Microinjection Into the Male Pronucleus. The *in vitro* fertilized egg is shown in the center of the diagram. (http://www.agresearch.co.nz/scied/search/biotech/gene_gmomaking_animal.htm)

Creating Transgenic Animals by Embryonic Stem Cell Manipulation

A second method of creating transgenic animals is by manipulating embryonic stem (ES) cells. This method is used mostly when trying to target a transgene to a specific site in the genome. ES cells are stem cells that are derived from the inner cell mass of a blastocyst prepared by *in vitro* fertilization or collected from female mice. The *in vivo* fertilized embryos can be collected by giving a mouse a steroid preventing implantation, or they can be collected a few days after the embryos have fertilized. ES cells have the ability to differentiate into any of the three main germ layers, so if the transgene is incorporated into an ES cell that cell can be injected into a blastocyst embryo to create a transgenic animal (Figure-4). Once the transgene is incorporated into the ES cells, those cells can be left to divide *in vitro*, or they can be injected into a blastocyst and implanted into a host's uterus to grow normally (Transgenic Animals, 2003).

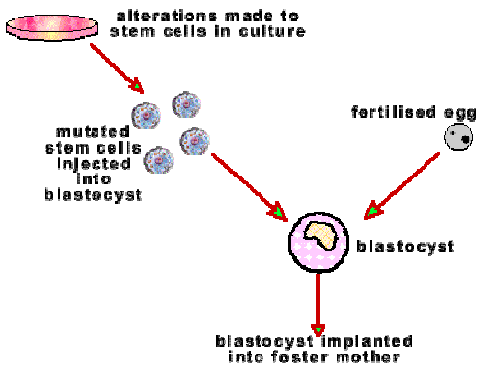

Figure-4: Diagram of the Steps for ES Cell Manipulation. Embryonic stem (ES) cells are injected or transfected with the transgene (upper left), then injected into a blastocyst (center) for implantation into a host uterus. (http://www.agresearch.co.nz/scied/search/biotech/gene_gmomaking_animal.htm).

There are four approaches to using ES cells to create transgenic animals. The transgene can be microinjected into the ES cells, or can be introduced by a virus, chemical, or by using homologous recombination. During homologous recombination (Figure-5) sister chromatids pair (left side). Sometimes DNA exchange occurs between paired sister chromatids (middle) resulting in recombined sister DNA (right side).

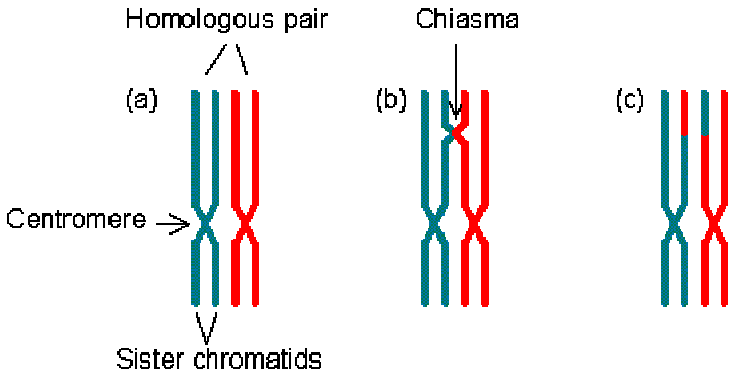

Figure-5: Diagram of Homologous Recombination. Two sister chromatids pair (left). Sometimes recombination occurs between two chromatin strands (middle), resulting in exchange of sister chromatid DNA. This process can be exploited when microinjecting a transgene flanked by chromosomal DNA to ensure the transgene is incorporated into a desired chromosomal site. (http://www.web-books.com/MoBio/Free/Ch8D1.htm)

This naturally occuring homologous recombination process can be exploited when making a transgenic animal. When DNA strands with the transgene on them flanked by host chromosomal DNA are introduced into the ES cells, crossing over or homologous recombination can occur between the flanking DNA and the corresponding host chromosomal DNA, incorporating the transgene into a specific site dictated by the choice of flanking DNA. If these ES cells are then injected into blastocyst embryos in and implanted into the uterus of a foster mother they will grow normally. As with microinjection into a fertilized egg, the percentage of offspring expressing the transgene is usually low, however in some cases PCR can be used to screen which ES cell has taken up the transgene prior to implanting that ES cell, greatly improving efficiency.

Chemical and viral delivery can also be used to introduce DNA into fertilized eggs or ES cells. Certain chemicals such as calcium phosphate or rubidium chloride and viruses will help carry the transgene into the cells. But similar to microinjection, the transgene will be placed randomly in the genome.

Screening for Transgenic Positives

Following the birth of the transgenic pups, they are typically screened by polymerase chain reaction (PCR) or by Southern blot analysis. Depending on the site of integration of the transgene, some transgenes may not be expressed if integrated into a transcriptionally inactive location. It is common practice to do further animal breeding to obtain maximal expression.

CHAPTER 2: TRANSGENIC ANIMAL CLASSIFICATION AND EXAMPLES

The purpose of this chapter is to discuss the types of transgenic animals that have been created to date, with particular attention paid to their benefits to mankind, which will facilitate the transgenic ethics discussion in Chapter-3. Transgenic animals can be divided into five major categories: disease models, transpharmers, xenoplanters, food sources, and scientific models. Disease models are animals that have been modified to exhibit the symptoms and progression of a particular disease, so that treatments for that disease can be tested on them. Transpharmers are animals modified to express a particular protein or suite of proteins in their milk to avoid animal sacrifice when obtaining the drug. The proteins can be purified to produce medicines and hormones to treat humans, or can possibly be administered as medicinal milk itself. Xenoplanters are animals that have been engineered to not express the foreign antigens that normally prevent the transplantation of their organs into humans. Food sources are animals that grow bigger or faster to produce more food in a shorter amount of time with fewer resources. Scientific models are animals producing more or less of a particular protein than usual, letting us observe that protein's purpose in biological mechanisms or development, which can in turn be applied to humans.

Transgenic Disease Models

AIDS Mouse

One good example of a disease model is AIDS mouse. AIDS mouse was created in 2001 at the University of Maryland by microinjecting the genome of HIV-1 into fertilized mouse eggs. The transgenic genome does not include the two genes that cause the virus to become infectious (Reid et al, 2001), which makes the animals relatively safe to handle while still allowing a study of HIV biology. The HIV-1 mouse cannot give the virus to humans. This model allows researchers to study early-onset symptoms to better diagnose the disease in humans. It also allows researchers to track chronic conditions associated with AIDS and test various treatments in an attempt to cure HIV (Kohn 2001). Previously, chimpanzees were shown to be capable of supporting HIV replication, but no inexpensive animal developed the virus (Bunce and Hunt, 2004). The one original female mouse that possessed the modified viral gene has been bred to healthy male mice to produce HIV gene-bearing offspring. One observation that was seen during the development of AIDS mouse is skin lesions that resemble Karposi's sarcoma, often seen in AIDS patients. This indicates that HIV may indeed be a cause of cancer (Vogel et al, 1988). The AIDS mouse is a large step towards finding treatments to prevent, ease the symptoms of, and perhaps even cure the disease.

Alzheimer's Mouse

Another important disease model is Alzheimer's mouse. The progression of Alzheimer's has been linked to the formation of beta-amyloid plaques in the brain, places where fibers have developed tangles that can block and degrade neurons. The Alzheimer's mice overproduce a protein that forms these amyloid plaques, and the mice display both the symptoms and diagnostic tell-tales of Alzheimer's disease (Duff et al, 1996). The first true Alzheimer's mouse was created in 1995 by a joint effort at Worcester Polytechnic Institute and Transgenic Sciences, Inc.

(which became Athena, then Exemplar Corp, then Elan Pharmaceuticals). This mouse line overexpresses a mutation that causes an aggressive early onset form of Alzheimer's disease (Games et al, 1995). Researchers studying Alzheimer's have been desirous of an animal model for some time before this breakthrough. A vaccine was tested on this line of mice that almost entirely prevented the creation of amyloid plaques in young mice, and even reduced the damage of the plaques already allowed to develop in older mice (Schenk et al, 1999). This was the first Alzheimer's vaccine. This vaccine moved to human clinical trials in 2000 (Jones 2000), and was cancelled in 2001 due to brain inflammation in a minority of patients; however a second generation vaccine by the same company is already in Phase II human clinical trials with no deleterious side effects observed. So far, a vaccine has not yet been FDA approved for the widespread treatment of Alzheimer's, but thanks to the mouse model, researchers are on the right track to preventing and curing Alzheimer's entirely.

Oncomouse

A landmark mouse disease model is oncomouse, which models cancer. Because there are many ways to cause and develop different kinds of cancer, there are many ways to make a model for cancer. One way is to create mice that do not contain the p53 allele, crucial in the process of checking the uncontrolled growth characteristic of cancer. This leaves the mice susceptible to many types of cancer, most frequently lymphoma (Harvey et al, 1993). The very first oncomouse was created in 1984 by replacing the normal mouse *myc* gene with a virus tumor promoter/myc fusion transgene. The mice and their offspring developed carcinomas. This mouse was made at the Harvard Medical School in Boston for DuPont (Stewart et al, 1984). They applied for a patent on the process of creating the animal, and on the mouse itself, and

received it in 1988 (Leder and Stewart, 1984) making the world's first patented animal. This caused considerable stir in the scientific community, which will be discussed in detail in chapter-4, since in order to study this cancer model, or to create a new one using Harvard's process, a laboratory was required to get a license. DuPont argued that the patent covered any transgenic animal predisposed to cancer (Marshall 2002). Since then, the company has allowed researchers working with the U.S. National Institute of Health to work on the mouse for research not intended to make a profit (Smaglik 2000). Experiments continue to be done on oncomouse that may lead to preventing and curing multiple forms of cancer.

Parkinson's Fly

In Harvard Medical School in 2000, a *Drosophila* fly was developed as a model for Parkinson's disease. The fly contains a mutation of the α-synuclein gene linked to inheritable Parkinson's disease. Parkinson's fly shows the characteristic loss of motion control and loss of dopamine neurons seen in humans with the disease. The fly's much more simple genome serves as an excellent model for learning about Parkinson's from a genetic level (Feany and Bender, 2000). Also, it allows scientists to study the previously unobservable progression of the disease. By the time the symptoms of Parkinson's are visible in humans, it is estimated that 60 to 80 percent of dopamine nerve cells have already died (Vatalaro 2000). The fly allows scientists to study early-onset symptoms, which could lead to earlier diagnosis of the disease in humans and eventually, a cure for it.

Many, many other disease models have been made and continue to be made. The most popular subject for transgenesis is a mouse due to its relatively short generation time and ease of laboratory manipulation, however, a growing number of researchers favor pigs, for their anatomical and physiological similarities to humans (Kragh 2006). Inflammation, heart disease,

Lou Gehrig's disease, sickle cell disease, and many others can be studied in these models like they cannot be in humans.

Transpharmers

Transpharmers are engineered to overexpress a particular gene in the mammary gland (so that the milk contains the desired protein). This was first done in an animal's blood, however since then the trend has shifted to milk, since it is easier to acquire the drug from milk, and proteins expressed in milk are less likely to affect the animal's physiology than in the blood. Mice are commonly used to test the transpharming transgene first (to make sure it encodes a functional protein) before it is built into larger animals like goats and cows, which are significantly more difficult for performing *in vitro* fertilization and surrogate motherhood. The transgenic procedure is promising, but very expensive, and still has a low success rate (Houdebine 1994), especially for larger farm animals.

The first transpharmer, of course, was a mouse engineered in 1987 to express the clot dissolver drug tissue plasminogen activator (tPA) (Gordon et al, 1987). In 1990, human alpha-antitrypsin, a type of inhibitor used to treat emphysema, was produced in the mouse's milk as hoped (Archibald et al, 1990). The same thing was done with rats in 1997 at the YS New Technology Institute to allow them to secrete human alpha-lactalbumin in their milk (Fujiwara et al, 1997).

As useful as mice and other rodents are for the lab, the truth of the matter is that they do not produce nearly enough milk to be useful as anything more than models. Larger animals like sheep, goats, and cows are the targets for large-scale transpharming. A team created 6 transgenic lambs for Roslin Institute in 1997. The lambs produced a human clotting factor in their milk (Schneike et al, 1997). The first transpharmer goats were produced in 1991 at the Tufts

University School of Veterinary Medicine to produce tissue plasminogen activator, a clot-dissolving drug (Ebert 1994). Another variation of transpharmer goats were produced in 1999 using the then new process of somatic cell nuclear transfer (SCNT). These goats not only overexpressed the intended gene but also passed down that transgene to their offspring (Baguisi et al, 1999). These goats contained high levels of human antithrombin III, a kind of anticoagulant. These proteins have no effect on the animals, and the goats themselves most likely do not even realize that their milk is different. On June 2, 2006, a committee of the European Medicines Agency said that they would recommend that ATryn, a human anti-clotting drug made by transpharming goats by GTC Biotherapeutics. The final European Commission decision is to be made three months from that date. However, if it goes through, ATryn will be the first approved drug made by a transgenic animal. The company predicts that it will apply for approval from the US Food and Drug Administration by 2007. Once the first one is approved, it will open the door for many more.

Gen Pharm International in California engineered the first transgenic cow, dubbed "Herman", and his first transgenic offspring were bred at Gen Pharm's lab in the Netherlands (Krimpenfort 1991). Two calves were produced by microinjection of DNA into embryos that were then implanted in surrogate mothers and born alive. One of these cows was female, but in her, the transgene rearranged itself so that a portion of the lactoferrin cDNA was deleted. The other calf was male, later called "Herman." He and his offspring contained the correctly arranged gene for human lactoferrin, the source of iron for newborn babies. Cows' milk does not naturally contain lactoferrin; human babies have to rely on their mothers' milk and formula and cannot live on cows' milk (Biotech Notes 1994). Milk with lactoferrin would be a large improvement over formula for mothers who cannot breastfeed, for one reason or another. The

milk is pending a safety notification before being produced for sale. Keeping transgenic cows is expensive, due to tight rules on their captivity to make sure they do not escape and have undocumented and uncontrolled offspring. Because the cows have not yet been able to fully return the investment put into them, the company considered putting them down to cut their losses. However, many Dutch citizens and businesses (most notably a funeral insurance company called Yarden) raised funds to keep Herman alive until he dies a natural death (Cho 2002).

Xenotransplanters

During an organ transplant procedure, a donor's organ (for example a liver) that is determined to be histocompatible with the patient is transplanted to take over the function of the diseased organ. Because only a small percentage of donated organs are histocompatible with any given patient, there is an extreme shortage of such matched organs, so more often immuno-suppressive drugs are given to the patient to lower their immune response. However this lowering of the immune system leaves the patient open to infections.

To solve this organ shortage, xenotransplanters are being engineered to provide animal organs that are histocompatible with humans. Normally, the host rejects organs that come from other tissue types, not to mention other species. This is because of antigens on the organ's surface that tell the host that the organ is not itself. The host's own immune system attacks the transplanted organ, causing a whole range of problems, most notably blood clotting, particularly dangerous to an already weakened patient. Xenotransplanters are animals engineered so they do not express those antigens.

The animal currently chosen for xenotransplant research is the pig since its physiology closely matches that of humans, and pigs are far cheaper than monkeys. In the pig's case, a kind

of sugar called alpha-1,3-galactosyltransferase, resides on the surface of the cells. In 2002, four pigs whose transferase genes had been "knocked out" (deleted) were produced in the Department of Animal Science of the University of Missouri (Lai et al, 2002). Nuclear transfer introduced one allele of the null gene into the pig embryos. Embryos from these adult pigs were then given a second copy of the blank gene by nuclear transfer, resulting in piglets without either copy of the gene that encodes the antigen. Previously, the organs from transgenic pigs designed not to trigger the immune systems of baboons were transplanted into baboon subjects and no rejection was observed (Logon and Sharma, 1999). Human trials have not yet been approved. The biggest setback is the fear that the pig organs will allow the crossover of viruses from animals to humans (zoonotic infection), especially to weakened humans like those waiting for a transplant. The most clear-cut example is influenza, which is often transmitted from pigs to humans even without organ transplantation (Carnell 2000). In England, scientists believe that human trials could begin as soon as 2008 (Fabregas 2006). It is an ongoing debate, because both sides feel very strongly about the issue.

Transgenic Food Sources

Superpig

When growing animals for food, it would be beneficial to society if they could grow more efficiently and with less food. One of these attempted food strains was dubbed "superpig," that is, a pig that would grow bigger and faster, thus producing a more efficient food source. Most of the transgenic superpigs were made by microinjection of the transgene for a growth hormone, whether porcine, ovine, bovine, or even rat (Pursel et al, 1997). The famous Beltsville pig was made in Beltsville, Maryland under the supervision of the US Department of Agriculture. These pigs expressed human or bovine growth hormone and expressed higher

levels of growth factors (Miller et al, 1989). Unfortunately, the Beltsville pigs had many health problems, the most commonly quoted one being arthritis (Connor 1999). Animal rights groups claim that the pig also was impotent and had ulcers, heart problems, lameness, kidney disease, and pneumonia (Animal Aid, 2006). The pigs were euthanized, and biologists imposed a voluntary moratorium on performing any further studies on mammals involving growth hormone.

Superfish

Another attempt at a more efficient food source was a fish. One species of these fish, the tilapia, was engineered by microinjection by the Centro de Ingenieria Genetica y Biotechnologia of Havana, Cuba to overexpress its own growth hormone. This animal was not transgenic, but it was genetically engineered. It showed accelerated growth, but it reached an adult size no larger than normal tilapia (Martinez et al, 1996).

Similar techniques have been used on salmon (Devlin et al, 2001). The transgenic salmon produce the growth hormone continuously, instead of turning it off depending on the season (Biotechnology, 2006). The eggs of a species of usually slow-growing trout were microinjected with the gene of a salmon that grew quickly after many generations of selective breeding (Devlin et al, 2001). Because of a concern over the escape of these fish into the environment, a very tight control is kept over transgenic fish farms (Stokstad 2002). The biggest fear is that these fish will breed out native fish because they would be able to out compete them for food. One of the companies involved in the farming of transgenic salmon state that the salmon have been raised on fish pellets and would not know how to forage for themselves in the wild (Biotechnology, 2006). There is considerable opposition to the creation and farming of

these “superfish”. But the transgenic fish look like a much more likely source of food than any transgenic animal species.

Transgenic Biological Models

Biological models are transgenic animals made with the aim of increasing knowledge of genetics and expression, or some natural process.

ANDi

One of these animals is ANDi, the first transgenic monkey, born in 2000, introduced in publications in 2001 (Chan et al, 2001). “ANDi” stands for “inserted DNA” spelled backwards. An engineered virus was used to insert the harmless gene for green fluorescence protein (GFP) into ANDi’s rhesus genome. He produces the mRNA for the protein, although he does not actually glow under UV light. Two of the other monkeys in the project, stillborn twins, glowed under UV light at their eyes and fingernails. The GFP gene was chosen for two reasons: it would have a very little effect on the monkey, and it would be very easy to detect if the transgene had been transmitted properly (Ackerman, 2006). ANDi (shown in Figure-6) is the only monkey of 40 fertilized eggs to be born alive expressing the gene. However, ANDi proves that transgenic primates can be created, and can express a foreign gene delivered into their genome. ANDi opened the doors for creating other primate biological models of humans for research.

Figure 6: The World's First Transgenic Monkey, ANDi. (Vogel, 2001; Chan et al, 2001).

Smart Mouse

Another biological model was made in 1999 by Princeton University. The "smart mouse" or "Doogie," was engineered to overexpress NR2B receptors in synaptic pathways. This makes the mice learn faster like juveniles throughout their lives. The "Doogie" mice do better on tests designed to test learning and memory (Tang et al, 1999). To test the memory of a mouse, two objects are presented to the mouse in a cage and the mouse explores them. Then researchers replace one object with another, and the mouse is again allowed to explore them. If the mouse spends more time paying attention to the new object (Figure-7), it is a good sign that it remembers the old one. If the mouse explores each equally, then it has probably forgotten that it already explored the old object already. The Doogie mice do consistently better on these tests as they age. It is entirely possible that in the future, this research may lead to improving learning and memory in humans and other animals, as well. The fact that this gene improves memory confirms an old theory about how mammals think and learn (Harmon 1999). Research on the

Doogie mice can provide valuable information on how humans develop, learn, and remember, as well.

Figure 7: Photo of "Doogie" the transgenic smart mouse involved in a learning and memory test (Princeton, 2006).

Supermouse

Another transgenic model is "supermouse", a mouse given the gene of a rat growth hormone in 1982 by microinjection into fertilized eggs. The mice grew noticeably larger than their littermates. These mice were the world's very first expressing transgenic animals, the first ones with an obvious phenotypical response to the transgene. Scientists hoped to use the mice to study the effects of growth hormone, accelerated animal growth, gigantism, and as a means of correcting genetic defects (Palmiter et al, 1982). This mouse also lead to the processes used when scientists started to pursue the accelerated growth of food producing animals. The most obvious application of these animals is in the possible correction of dwarfism.

Youth Mouse

Another model is "youth mouse", created in 1997 at the Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel. The mice overexpress the urokinase-type plasminogen activator, primarily thought to be a clot dissolver. The mice are smaller, eat less,

and live much longer than normal mice of their type, about twenty percent longer, in fact (Miskin and Masos, 1997). It is possible that the overexpression of the clot dissolver extends life by preventing atherosclerosis, a process that develops plaques in the arteries of an animal as it ages and can lead to clots, hemorrhages, and heart attacks. Of four transgenic lines of the transgenic mice attempted, only one autonomously ate less and lived longer, but also displayed infrequent muscle tremors. This line, dubbed Alpha MUPA, shows the same characteristics as normal mice on a restricted diet (Miskin et al, 1999). The "youth mouse" promises to be very useful in studying development and aging, especially in relation to diets.

Influenza Resistant Mouse

A subset of biological models (which are engineered to mimic some aspect of a disease for us to analyze) is the category of animals engineered for disease resistance (which directly helps the animal species). An example of this is the influenza-resistant mouse. The mouse overproduces Mx protein, known to act as an antiviral agent. These mice are significantly more resistant to influenza and other orthomyxoviruses (Staeheli et al, 1986). If this process can be applied to farm animals like pigs and ducks, that would lower the chance of avian strains of influenza and other viruses being passed from these animals to humans. It could also lower the rate of evolution of these viruses in the animal hosts, helping us retain immuno-protection against future outbreaks.

Chapter Conclusion

There are many different kinds of transgenic animals, made with many different purposes in mind. This chapter summarized the main categories of transgenic animals to aid our understanding of why such animals are made, and to facilitate the ethical and legal discussions

which follow in chapters 3 and 4. Most of the animals can be grouped into the categories explained above, but transgenesis lets scientists and research do things never thought of before. Such research does have its false starts and mistakes, but over all, these experiments can help raise the standard of living for all people, and for many animals as well.

CHAPTER 3: TRANSGENIC ETHICS

The purpose of this chapter is to explore the ethics of the production and handling of transgenic animals and the products that come from them. Examples from Chapter 2 will be used to help the reader focus specific ethical issues that apply to transgenic experimentation. Common concerns about transgenic animals will be examined, misconceptions will be identified, and suggestions made for improvements.

For some people, the word "transgenic" triggers confusion and mistrust. It may be that some experiments deserve this disapproval, but certainly not all of them, and not without education. The following are top ethical issues with transgenic animals. We have no right to meddle in the genomes of living beings, and for curiosity or novelty's sake create monsters. There is a high death rate when creating transgenic animals. Is it worth all that death just to have one successful animal? Animals that express the transgene are monsters. They either live a short life of suffering because of whatever gene they are given, or they unknowingly become a danger to creatures around them, and if they escape, to the environment as well. How can an animal so different from humans as a mouse is be an accurate model for a disease or condition in humans? Each of these concerns will be addressed in the following paragraphs.

Animal Rights Versus Animal Welfare

A distinction should be made between animal rights and animal welfare. From the PETA website, the difference is that "animal welfare theories accept that animals have interests but allow those interests to be traded away as long as the human benefits are thought

to justify the sacrifice, while animal rights theories say that animals, like humans, have interests that cannot be sacrificed or traded away to benefit others. However, the animal rights movement does not hold that rights are absolute—an animal's rights, just like those of humans, must be limited, and can certainly conflict.

Supporters of the animal rights movement believe that animals are not ours to use for food, clothing, entertainment, or experimentation, while supporters of the animal welfare movement believe that animals can be used for those purposes as long as 'humane' guidelines are followed" (PETA, 2006). Thus, according to those who believe in animal rights, the making of transgenic animals is wrong without room for argument. However, those who believe in animal welfare do condone the responsible and humane experimentation on animals, including the making of transgenic animals.

Tinkering with the Genome

Humans have been "meddling in the genomes of other animals" for centuries, possibly millennia. All the breeds of dogs seen today are results of selective breeding. The wanted traits were kept, and the unwanted traits were bred out. Modern day horses, cows, sheep, and many other species are very different than the original domesticated species. It is true that selective breeding deals with traits already present in the species, while transgenic animals are often implanted with traits from a different species entirely. However, most scientists see transgenesis as a logical step beyond selective breeding, a way to open doors past what we previously have known to cure diseases and possibly end world hunger entirely. In fact it could be argued that for some transgenic animals, the presence of the transgene confers less overall change to the animal than selective breeding. This would especially be the case for transpharmers that show no expression of the transgene outside the milk. It is current policy that experimenters must

predict as accurately as possible how the transgene will affect the animal and minimize suffering to the best of their knowledge.

Transgenic Art

Creating monsters simply because it can be done certainly should not be a motivation for scientists, and it almost certainly is not currently allowed by university animal care committees that oversee such research. Trusted scientific journals publish articles in order to increase biological and medical knowledge. There is little knowledge to be made from making monsters. However, there are some privately funded experiments done for "art's sake". Here I am referring to "Alba," the rabbit that glows under UV light, designed by and created for Eduardo Kac (Figure-8). The rabbit is part of Eduardo Kac's plan to create "transgenic art." This refers to animals and plants with a planned genome intended to express an artistic idea symbolized by the proteins they code for. With more species going extinct every day, Kac hopes that artists can add to the biodiversity by creating new species of their own design (Kac, 1998). Although Kac does speak of artists having the responsibility to take care of the new life they create, "transgenic art" is created largely for the sake of doing it, with no thought for medical advancement or saving lives. In plants, this can be interesting and compelling. However, in animals, it crosses the line of what is necessary.

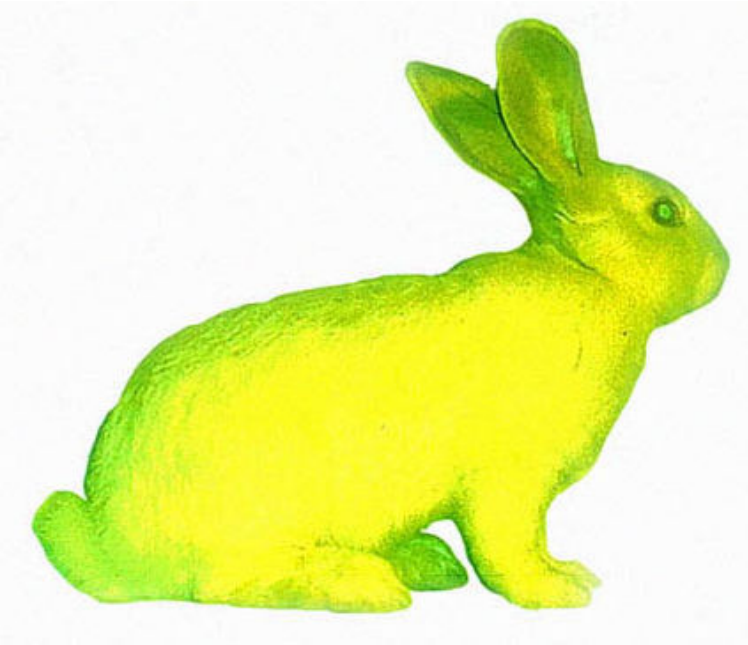

Figure 8: Alba, the "GFP Bunny" (Kac 2002). This animal glows green in the dark when illuminated with a certain light, but offers no new medical information, so the authors of this IQP are against this kind of transgenesis.

Animal Death Versus Human Lives Saved

It is true that there is still a low success rate in creating transgenic animals. For every success, there is a score or more of failures. In general, the higher the species of transgenic animal, the greater the cloning failure. These failures are transgenic animals who die before they are born, or animals that are born without the transgene. However, the number of failures continues to shrink as scientists become better at the various cloning techniques. Also, the prenatal deaths of a few animals mean saving the lives of potentially thousands of humans. The reward is very high, and the improvement of the process is continually reducing the cost.

Because the various cloning processes are not yet perfected, many attempted transgenic animals are born with a segmented, inactive transgene, or without the transgene altogether. These animals are often killed because they are experimental failures, although they are normal biologically, and companies do not want to pay to keep animals that do not further their work on transgenesis. This is a case of practicality and potential profit having a

higher priority than the well being of animals. However, just because the animals are not transgenic does not mean that they are useless. Particularly in the case of mice, laboratories use healthy, normal mice for tests all the time. With greater cooperation between experimenters, the normal mice dismissed as failures from the transgenic attempts can be used in other research, eliminating unnecessary animal death and waste of resources. This is one area in which significant improvement can and should be made. Instead of using it as an excuse to condemn and ban transgenic research, movements should be made to change the current practices.

Herman the Bull, a transgenic bull with the gene for lactoferrin, faced being put down when his days breeding the next generation of lactoferrin cows was over. With no further profit to be made for Pharming, the company that made him, his expenses were higher than that of a normal bull because of regulations concerned with keeping transgenic animals from escaping into the wild. Upon hearing this, many Dutch citizens and businesses offered to pay for the expense of keeping Herman alive, including a burial-insurance company called Yarden (Cho, 2002).

One of the biggest deterrents to improvements in efficiency and humane practices is funds. It is simply more expensive to take precautions that minimize suffering for the animals. Expense is what determines the decision to put down the animals that are not successfully given the transgene. The country's legislators need to approve more grants for researchers intending to improve the efficiency of the various techniques used to make transgenic animals. This will result in fewer failures and fewer deaths.

Transgenic Ethics Examples

If you find the idea of animal experimentation in the general sense to be acceptable, then each example of a transgenic animal should be taken in a largely case-by-case basis. What is the possible benefit of making the proposed animal? What are the possible risks? Is there any animal suffering? How can suffering be minimized? The authors of this IQP argue that if making a particular transgenic animal significantly increases the knowledge and well being of humanity and animals, and there is little or no suffering, then the experiment should proceed. But scientists have the responsibility to consider these factors before the experiment begins. With these things in mind, three cases are helpful for examples of good, bad, and more complicated experiments.

Alzheimer's mouse is a good example of a transgenic animal that does not suffer. The Alzheimer's mouse does poorly on maze tests, but does not feel any pain related to its condition by any standard used in laboratories. Because Alzheimer's mouse spends its days in a laboratory setting, any survival skills that would be hampered in the wild by its diminished memory do not come into play. And Alzheimer's mouse continues to provide significant information on Alzheimer's disease that could lead to a cure. In fact, the Alzheimer's mouse created in part here at WPI (Games et al, 1995) was used to develop a vaccine that lowers senile plaque burden in mice (Schenk et al, 1998), and is currently in phase II human clinical trials by Elan Pharmaceuticals. The benefits are large, and the animal suffering is nonexistent, so Alzheimer's mouse was a good idea.

The Beltsville Pig, also known as "superpig," turned out to be a very bad idea. The concept was to make a pig that grew more, leaner meat. The benefits would be to partially alleviate world hunger. A noble cause, but other sources could supply food just as well or

better. The pig turned out to have many health problems, the worst being arthritis so overpowering that the pig could not walk. Sometimes scientists cannot predict every problem that will occur from making a transgenic animal, as in this case. There was nothing to be done but put the animal under to stop its suffering. Scientists then willingly instituted a moratorium to stop any and all experiments with mammals and growth hormone. The ethical cost was too great for the potential gain in this case.

One more complicated example is oncomouse. Oncomouse develops fatal tumors over its lifetime that allow researchers to study the development of cancer. Cancer is such a widespread problem. In 2005, an estimated 570,000 people died of cancer in America alone (American Cancer Society, 2005), so a transgenic animal that increases our knowledge of this deadly disease has enormous medical benefit to society. However, as an oncomouse ages, it begins to suffer from the tumors just like humans with the disease do. So in this complex case, although the animal has enormous medical benefit, its use is accompanied by strong ethical constraints. Since most of the work on oncomouse focuses on early development of the tumors, we argue that perhaps the mouse can be put down before its suffering increases needlessly. Or in those cases where advanced oncogenesis needs to be studied, perhaps pain medication could be required by university animal care committees. In this case, it is more difficult to say that the creation of this animal was completely a good idea, since the mice do suffer and die. However, based on the overwhelming need for ways to prevent, relieve the symptoms of, and perhaps even cure cancer, most researchers believe that oncomouse is the best hope.

Are Mouse Disease Models Good Predictors of Human Success?

One argument often quoted by animal rights activists is that animal models will always be different than humans, so different that the information gained by animal testing is not as useful as researchers claim. It is true that treatments for diseases that appear to work nicely in mice often don't work at all in humans. Pigs have anatomy and physiology that is very similar to us, and primates are our close cousins, but even they are not perfect disease models. Mice are cheaper and easier to handle in the lab, which facilitates the performance of complex experiments. Mice also have a much shorter generation time, which allows for rapid observations on heredity that simply cannot be done in humans. Experimentation is the key to increasing out knowledge about biology.

Assuming that the animal rights activists would not approve of testing on living humans, the only alternatives are human cadavers. It is true that much of our knowledge about human anatomy comes from the dissection of corpses. However, when the body shuts down, it is impossible to observe many normal biochemical and physiological interactions and responses to stimuli. Also, most of our lack of knowledge is centered on the beginnings of diseases, the slow development that takes place before any noticeable symptoms. It is strictly impossible to study that development on a corpse that has died from advanced disease processes.

However, if you know that an animal is going to develop a certain disease because it is genetically programmed to have that disease, then it is of considerable benefit to monitor as much about that animal as possible to be able to diagnose and cure humans with that disease. That benefit is lost without testing on transgenic animals, even if the animal is not a perfect model for the entire human disease process. A good example of this is AIDS mouse,

genetically engineered to express HIV co-receptors and other human host proteins needed for HIV to enter cells. The cells in this mouse can take up the HIV virus, allowing us to study this key event in the disease process, even though the animal itself develops few true AIDS symptoms. So although this is not a perfect AIDS model, we can still learn important facts about the cause of the disease.

Transgenic Animals and the Environment

One large setback to the making of new transgenic animals, particularly marine life and large farm animals is a concern for the environment. If any of these animals should escape, it is believed that they would breed out all the natural varieties due to their greater level of fitness and thus contribute to the decrease of genetic variability within that species. Also, people are concerned that if these animals produce undocumented offspring, people will end up eating the meat or drinking the milk from transgenic animals unknowingly. Transgenic animals are not "more fit" than their "normal" cousins. They are specialized, just like most domestic breeds. In the case of the transgenic salmon, the US Food and Drug Administration will not approve the use of transgenic salmon unless they prove to be sterile (Biotechnology 2006). This means that salmon cannot be responsible for breeding out the wild type. Other than the transgene, which is different in almost every case, most transgenic animals are just like the domestic breeds they came from. Until transgenic animals are better known and accepted, the fear of eating one unknowingly should be respected, even by those who think that there is nothing to fear. The authors of this IQP think that until transgenic animals become commonplace, they should be carefully and securely kept, if for no other reason than they embody a rather expensive investment.

Transgenic Oversight

Transgenic experimentation should be as humane as possible. The public often thinks scientists can create any transgenic animal they want. However, in the United States, federal law requires that universities and institutions that use laboratory animals for instruction or research must form an Institutional Animal Care and Use Committee (IACUC), which oversees all animal experimentation with a focus on humane practices (IACUC 2006). These committees must take special care to monitor transgenic experiments to make sure every effort is being made to improve the well being of the animals involved. They should expect researchers to propose steps taken ahead of time to minimize suffering as much as possible and hold them accountable.

Religions and Transgenic Ethics

Even if an individual scientist does not believe in a particular faith, it is the responsibility of the scientific community to at least consider the beliefs of the different faiths. For example, the Hindu faith holds that cows are sacred, and nonviolence should be a way of life. In fact, a large number of devout believers of Hinduism are vegetarians. *Ahimsa*, or nonviolence, is the Hindu tool used to judge all major ethical issues, including medical and scientific ones (Chandrashekhar 2002). So, in the case of the transpharming cows, can it be said that violence is done to the cow? Milking cows is not considered to be a violent act, and drinking milk is also nonviolent. So according to this thinking, taking and using a medicine produced in the cow's milk is not against the *Ahimsa* beliefs of Hinduism, although many Hindus argue that tampering at all with a cow, or interfering with its daily routines is to be avoided, so these Hindus may take issue with transgenic cows. Certainly,

the cow probably does not notice which proteins are produced in her milk, as long as it is not expressed in the blood, and does no harm to her calf. Such discussion needs to take place on these issues, even if the process is difficult, to promote greater understanding. In spite of the Hindu stance, the authors of this IQP argue that creating transgenic cows should be allowed when human lives are to be saved and no animal suffering occurs, especially in the case of transpharmers, although we are against any growth hormone bovine transgenesis.

CONCLUSIONS

This project explained many of the issues having to do with transgenic animals. The report begins with what a transgenic animal is, how one is created, the different transgenic animals that have been created to date, and extends all the way to moral and ethical issues pertaining to transgenic animals.

Chapter one described what transgenic animals are, and quickly jumped into the ways that transgenic animals can be created. Although earlier methods were inefficient producing many dead embryos for each experiment, as these ways of creating transgenic animals become finer tuned they have increased their efficiency.

Chapter two explained the different types of transgenic animals and how they can be used. The chapter starts with disease models which could be the single most important category. Transgenic animals such as oncomouse and Alzheimer's mouse enable us to study life threatening diseases for humans on animals. These animals are needed because new techniques and drugs, and their effects, cannot be tested on humans. Transpharmers, though very expensive to create at this point have a promising future because they provide important proteins in their milk, blood, or eggs. Animals who have specifically engineered organs for human transplants, xenotransplanters, are becoming more and more necessary with the shortage of donor organs. The organs are engineered without antigens so that human antibodies will ignore them so the host will not reject the implant. Hopefully this can take the place of the shortage of actual human organs available for transplants. Transgenic food sources, animals created to grow as large, or larger than normal animals with less food and less time, would be perfect for the growing society of the world today, especially transgenic fish that appear to tolerate the growth hormone

transgene nicely. Transgenic biological models are aimed at increasing our scientific knowledge of a natural process, such as memory.

The last chapter of the report is geared toward the moral and ethical issues related to transgenic animals. The two major factors that have to be weighed are how much does the creation of a particular transgenic animal benefit humans, and how much pain or discomfort does it cause the animal. The issue is where you draw the line between human benefit and animal discomfort. For example Alzheimer's mouse experiences very little pain or discomfort and the medical benefit for humans is enormous. On the contrary, superpig has little human benefit and experiences many medical problems, so the authors of this IQP agree with the current moritorium on growth hormone transgenesis in all mammals.

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