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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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ABSTRACT

This project investigated the use of transgenic animals in recent experiments and the effects of this novel technology on society. Using scientific journals and web resources, we examined the transgenic animals in existence, their uses, and the surrounding ethical and social issues. Recommendations were offered on which experiments involving transgenic animals should be continued and how to apply this new technology to the fields of science and medicine.

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EXECUTIVE SUMMARY

It is difficult to understand the emerging field of biotechnology without first discussing one very important molecule. Deoxyribonucleic acid, or DNA, is the basis for heredity in all animals, including humans. It is a double-stranded helical-shaped molecule found within the nucleus of almost every cell in the body. The sum of an organism's DNA, or genome, is like a complete map of all the proteins that give an organism its characteristics. The discovery of such a molecule, and the ability to manipulate it, has incomprehensible consequences for the human race.

Biotechnology is the field that studies how DNA functions and how it can be manipulated or controlled in organisms. One very important application of this technology is the creation of transgenic animals, animals that contain a foreign DNA sequence. This new sequence of DNA can have profound effects on the animal, including a change in its physical characteristics, or the secretion of a new protein in its milk. The first transgenic animals served a variety of purposes. Many of them were used in medical research, such as "oncomouse," a mouse model that is highly susceptible to human breast cancer, and Alzheimer's mouse, a mouse that shows symptoms of human Alzheimer's disease. Others were used in transpharming, a concept where animals secrete an important protein in their milk (i.e. human insulin) that can be harvested from a continual supply. Through the development of the first transgenic animals, many different methods were discovered and used to manipulate their DNA and achieve the desired result.

The creation of a transgenic animal incorporates well known biological techniques that have been used in many other experiments. One of these techniques is superovulation, or the ability of a female to release multiple ova in a single cycle. These ova can then be collected, studied, and handled. In transgenic science, the goal is to introduce a new sequence of DNA into an embryo before implanting it into the uterus of a surrogate to grow and develop. To accomplish this, microinjection is the most commonly used method. It involves fertilizing an ovum *in vitro* and injecting the foreign DNA sequence with a tiny injection pipet before the sperm and eggs have completely joined. Other methods use various types of vectors to carry the new DNA sequence into the cell and insert it into the existing genome. Possible types of vectors include plasmids, retroviruses, and cultured sperm cells. Experimentation in using embryonic stem cells also seems promising. The result is the birth of an animal that displays a new characteristic for our benefit.

Transgenic animals have a variety of uses in many different contexts. Animals used in transpharming have been important sources of human proteins needed in medicine, such as anticoagulants, blood clotting factors and even drugs to combat harmful diseases. Animals have also been used to model diseases, with notable and novel results in Alzheimer's and Cystic Fibrosis research. Such disease models provide a critical link for the production of vaccines and other therapies. Transgenic animals are also seen as a potentially valuable source of food. Livestock that can be engineered to produce more milk, more meat, or be resistant to diseases that could boost profits for farmers as well as increase the global food production capacity. Other exciting new

experiments involve using these animals in organ transplantation, and as biomonitors to regulate and study the environment.

Despite all the benefits that can be derived from transgenic technology, there are questions in a more social context that need to be considered. One major criticism of transgenic science comes from the animal rights front. They disagree with the way animals are used in experiments, and have qualms with our ability to control the manipulated DNA. Do animals have a moral right to be protected from transgenic research? Religious groups see this technology as "tampering" with God's work, or as an abominable use of sacred creatures. Does genetic technology constitute playing God? There is a fear that the continuation of transgenic research will put the human race on a slippery slope that could lead to dire consequences. Certainly, any new technology could be dangerous if used incorrectly. What should be done to limit what research is allowed? Should we use transgenic technology on humans? These moral and ethical debates are important to consider because of the social and legal consequences of biotechnology.

Social issues surround the incorporation of transgenic animals into mainstream medical and agricultural fields. The concern over food safety begs the question of whether transgenic foods should be labeled or regulated in a special manner. Patent law, and the ability to patent organisms, raises new issues over patentability and ownership. The role of the government in regulating is also under debate, as well as the ability to fund experimentation or limit it. Economic questions and social debate seem to be around every corner, and sometimes it becomes difficult to answer all of the public's fears.

In examining the benefits of transgenic animals, and their consequences, our project strived to find a common ground and to suggest some resolutions to the problems. In making our conclusions, we felt it was important that scientific research be continued, as the striking medical benefits cannot be denied. We decided that the positive uses of transgenic animals justify the use of transgenic science in some situations. However, we also felt that there should be limits imposed so that this technology is not abused. Transgenic animals hold great promise, and in some cases like food production vs. population growth, or spiraling medical care costs, the human race cannot afford to ignore this science.

PROJECT OBJECTIVE

The purpose of this Interactive Qualifying Project was to research the topic of transgenic animals, a new phenomenon within the field of biotechnology, and to investigate the various ways this novel technology affects society. Found in this document are the details of the techniques performed, and an explanation of such in layman's terminology. Also included is a description of some transgenic animals currently in existence and their potential scientific and medical benefits. The project investigated how this technology affects society by describing legal and ethical issues, and consequences. Based on the above information, conclusions and recommendations were proposed for the future use of transgenic animals.

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INTRODUCTION

Biotechnology continues to be one of the fastest growing fields in modern science. The media has focused on such novel accomplishments as cloning experiments, the increased use of advanced reproductive technologies, and the development of transgenic animals. The creation of the first transgenic animal in 1974 was soon followed by much scientific progress, as well as ethical and social debate. Transgenic animals are unique in that they contain a DNA segment from another organism. This piece of DNA, or transgene, causes the animal to exhibit traits not normally found within its species. The power of this new technology is made possible because of our recently discovered ability to manipulate and control the various components of DNA

In this project, we will follow the history of transgenic science and its progress including the many major advances and successful experiments performed in recent years. The studies done by Gregor Mendel in the field of genetics set the stage for a revolution in the biological sciences. With the discovery of DNA as the hereditary material, and the formulation of its structure and function by Watson and Crick, the field of biotechnology emerged with a new and exciting outlook on life. Later experiments gave scientists the power to predict, manipulate, and control heredity. This awesome tool soon led to the creation of the first transgenic animals.

By this time, scientists had discovered many new techniques for introducing genetic traits into animals as well as numerous applications for these animals in science and medicine. Each of the methods used to achieve genetic manipulation has its own advantages and disadvantages. Similarly, each potentially useful transgenic animal raised

a host of ethical and social questions. In this project we will discuss the current methods being employed in making transgenic animals, and present ideas for how to evaluate their usefulness. Through this we will encounter issues of consequence, which must be considered in light of current social trends and from various cultural perspectives.

In the end, we hope to draw some solid conclusions and make recommendations based on our research. We feel it is appropriate to focus our suggestions towards the national government and the global scientific community as the primary directors and supervisors of scientific research. We believe this project will be a useful source of information on transgenic animals for the general public. Our goal is to enlighten the layperson about the realities of transgenic technology, its applications, and its consequences. We hope that the text to follow will provoke some serious debate, and encourage you, the reader, to draw your own conclusions about the role of biotechnology in society today.

1. Background and History

1.1 Background Information

1.1.1 DNA

DNA, **deoxyribonucleic acid**, is the basic genetic material through which **gen**es are passed from one generation to the next. The many genes that make up the DNA strand encode the entire genetic makeup of the organism. The existence of a genetic material that is responsible for the passing of genetic information from one generation to the next was known as early as 1865. In that year, Gregor Mendel concluded from his numerous pea plant experiments, that there was some unit of heredity, which is now known as genes (Cooper, 1997).

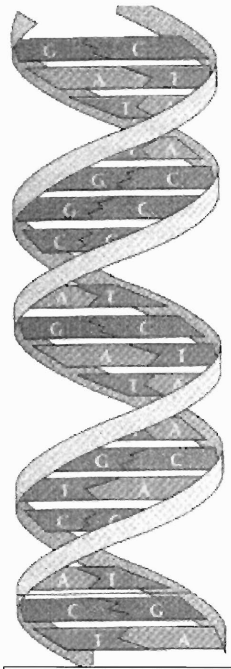

Figure 1.1: The DNA Double Helix. This is the structure of DNA proposed by Watson and Crick. (Cooper, 1997)

It was not until 1944, with the experiments performed by Avery, MacLeod, and McCarty that DNA was discovered to be the transferring agent of genes. Nine years later, in 1953, Watson and Crick discovered the structure of DNA, finding it to be in the shape of a **double helix** (See Figure 1.1). They also discovered that DNA, previously known to be composed of four different **nucleotides** (adenine, guanine, cytosine, thymine), is governed by specific pairings of the bases. The order of these nucleotide bases on the DNA strand makes up the genetic code. The pairing of **complementary** bases holds the two strands in the DNA double helix together. For example, adenine on one strand will always pair with thymine on the opposite strand. Likewise, cytosine will always pair with guanine (Cooper, 1997).

1.1.2 Replication and Expression

From their newly discovered structure, Watson and Crick postulated a method by which DNA could replicate and thus be passed on to the offspring. This method was based on the convenient pairing of complementary bases. Watson and Crick hypothesized that DNA could replicate by splitting the double helix into two complementary DNA strands (See Figure 1.2). Through **semi-conservative replication,** one strand would serve as a template for a new strand of DNA to be formed. Pairing bases with those on the complementary template strand would create the new strand. In fact, this method of DNA replication is now known to be the actual process by which DNA duplicates itself. Through this process, the offspring receive a copy of the parent's original DNA composition.

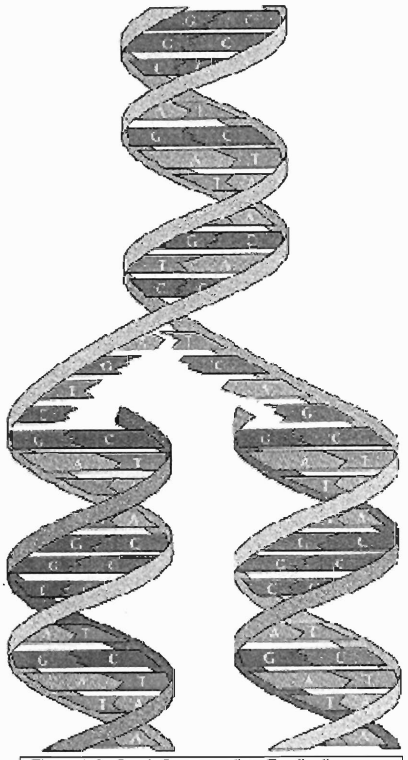

Figure1.2: Semi-Conservative Replication
The DNA strands split and are copied to produce two identical molecules. (Cooper, 1997)

It is important for the offspring to acquire a complete **genome** since DNA is the basis for the control of all cellular functions. This control comes about as a result of the proteins encoded by the DNA and produced by a process known as protein synthesis. In this process genetic information flows from DNA to RNA (**Ribonucleic acid**) to proteins. This pathway is known as the **central dogma** of modern biology. Proteins are responsible for displaying the visible traits of an organism encoded by its DNA. When proteins are correctly produced, the resulting trait(s) is/are said to be **expressed** by the DNA. This knowledge of how DNA is replicated and expressed is the foundation on

which all modern biotechnological techniques and experiments, including the production of transgenic animals, are based.

1.2 History of Transgenics

To fully understand the basis behind current transgenic techniques and ideas, one must first examine how this science has developed over the years. Originally, biology was considered to be one science, with no distinctions or specialization in various fields. This, however, was changed due to an increasing number of new discoveries. As a result, fields of specialization emerged. Transgenic animals, their production and function, fall mainly under the category of biotechnology and resulted from developments in cell biology.

1.2.1 Prior to the First Transgenic Animal

Biotechnology began slowly, but has picked up rapidly since the creation of the first **recombinant-DNA** molecule by Paul Berg (Access Excellence, 1999) in 1972. The following year, Cohen, Chang, and Boyer spliced sections of both viral and bacterial DNA (Access Excellence, 1999). By doing so, they were able to create a dual antibiotic resistant recombinant-DNA organism. The scientists proved that DNA from one organism could be recombined with the DNA from another organism. However, it took another year before they were able to demonstrate expression of the recombinant gene. The original organisms failed to survive and show signs of expression of the genes, but after incorporating the antibiotic resistant genes into *E. coli* bacteria, the experiments proved successful. Thus the stage had been set for initiating the transfer of foreign genes

into more advanced organisms.

1.2.2 The First Transgenic Animals

Now that DNA had been isolated and cloned successfully, the next step was to perform experiments involving animals. Rudolph Jaenisch and Beatrice Mintz, working at the Salk Institute in San Diego, decided to take on the task. They extracted and isolated the simian 40-virus (SV-40) gene from an ape, which they in turn incorporated into the DNA of a mouse through the use of retroviral vectors. The project proved to be successful and the two scientists were able to detect the presence of the SV-40 DNA in the liver and kidneys of 10 mice, which was coded for by the **transgene** (Jaenisch and Mintz, 1974). This was a huge step in the area of biotechnology because there was now evidence that a gene could be taken from one animal and integrated into another. The only downfall to this experiment was that the gene was not passed on to the offspring of the recombinant mouse. Further research was needed to discover a way to have the trait passed on to subsequent generations.

Genentech Incorporated, in California, performed the next major successful experiment in biotechnological research in 1977. Genentech researchers were able to produce human growth hormone in bacteria. Somatostatin, a factor that inhibits the release of growth hormone, was the first human protein produced by another organism (Access Excellence, 1999). With this experiment came hope of producing any type of therapeutic proteins human beings might need. This paved the way for the 1978 announcement of insulin producing bacteria. Once again, Genentech stunned the world by producing a bacterium capable of synthesizing human insulin (Access Excellence,

1999). This protein is currently being used for treatment of diabetes. Recombinant DNA technology had come a long way in only a few years, and began to grow even faster as company after company strived to produce organisms capable of making human health related drugs.

Early in the 1980's, the United States Supreme Court ruled that patents could be granted for genetically altered life forms (Diamond v. Chakrabarty, 447 U.S., 1980). Shortly after this ruling, Exon Oil Company patented an oil-eating microorganism, which would aid in the cleaning of oil spills (Access Excellence, 1999). Also, in 1980, Kary Mullis and Cetus Corporation invented the **Polymerase Chain Reaction** (PCR) technique, which allowed scientists to amplify a specific sequence of DNA from a genome (Access Excellence, 1999). Also in the early 1980's, John Gordon and Frank Ruddle discovered a new transgenic animal producing technique. While working in their laboratory at Yale University, the pair produced the first transgenic animal by **pronuclear microinjection** that contained the DNA sequence for the thymidine kinase gene in a bacterial plasmid (pBR322). This mouse was the first to survive after incorporating this experimental technique (Gordon and Ruddle, 1981). Shortly thereafter, Ralph Brinster's experiment successfully showed the expression of the thymidine kinase gene in an animal created using pronuclear microinjection (Brinster *et al.*, 1981).

1.2.3 Further Experiments in the 1980's

The following years brought many additional advances in biotechnology. In 1982, Richard Palmiter produced a transgenic mouse containing a gene encoding rat

growth hormone within its DNA (Palmiter *et al.*, 1982). The mouse grew to an enormous size and with that, became the first transgenic animal to display a visible **phenotypic** change. This mouse earned the title of "Supermouse" because of its size, but was of no real use experimentally because not much could be done with an oversized mouse. That same year also brought the Food and Drug Administration into the field of transgenics. From the 1978 experiment, Genentech received approval to market the genetically engineered insulin produced by bacteria. (Access Excellence, 1999)

Over the next couple of years, not too much advancement occurred in the field of transgenic animals. It was not until 1984 that the next major successful experiment took place. In this year, Stuhlmann used retroviral vectors to introduce specific foreign DNA sequences into animals (Stuhlmann *et al.*, 1984). With this experiment came various **vector** production techniques for transgenic animals. A couple of years later in 1986, Gossler discovered yet another technique to produce a transgenic animal. His method is known as the embryonic stem cell mediated technique. In other words, he hoped to incorporate the DNA sequence into the animals' germ line cells. He had successfully isolated 11 mice with the transgene in the germlines of the animals (Gossler *et al.*, 1986). With this new technique came the hope of passing the traits on to the animals' offspring.

Two new mouse models came into the picture in 1988. Phillip Leder and Timothy Stuart became the first scientists to patent a genetically altered animal. The patent was granted to recognize "**oncomouse**," a mouse model highly susceptible to human breast cancer (Access Excellence, 1999). This mouse is valuable to study since it grows human cancer tumors all over its body. The second patented model was "SCIDHU mouse." SyStemix Incorporated received a patent for this animal after demonstrating its

immunodeficient characteristics. This mouse was used as a subject for AIDS research since it displays similar traits expressed by an immunodeficient AIDS patient. (Access Excellence, 1999)

1.2.4 Experiments Spanning into the Present

With successful animal models being produced, many companies raced to be the first to produce additional characteristics not normally seen in that species. GenPharm International Incorporated was one of them. In 1990 they became the first company to produce a transgenic dairy cow. It secreted human milk hormones in its milk. This experiment has been repeated, and the milk is used as infant formula for mothers who prefer not to breast-feed (Access Excellence, 1999). Also in 1990 a study by John Wilson explained why the transgene was not always successfully incorporated into the host animals' genome. He used the theory of **positive-negative selection** (Zheng and Wilson, 1990). This theory is important when using the embryonic stem-cell transfer technique, as will be discussed in the next chapter.

Over the next three years many experiments produced human proteins that could be used as nutrients, medicines, etc. All were excellent advances in the biotechnology field and will be extremely useful if they can be produced on a large scale without interference. With that in mind, the FDA performed numerous tests and finally, in 1993, declared that genetically engineered foods were "not inherently dangerous," (Access Excellence, 1999). Because of this conclusion, the FDA ruled that they did not have to be regulated by special guidelines and could be produced and sold just as any other food product.

Beginning in 1994, the world was introduced to additional mouse disease models, which became extremely useful. The first was one in which researchers were able to incorporate the human **Cystic Fibrosis Transmembrane-conductance Regulator** (CFTR) gene into the mouse's DNA (Access Excellence, 1999). With the incorporation and expression of the CFTR gene, studies could now be performed on Cystic Fibrosis without having to use human subjects since the mouse showed all symptoms of the disease. In 1995, Smith and colleagues produced "Down-Syndrome mouse," (Access Excellence, 1999). As with the previous model, it displayed all the characteristics of a typical affected human and was perfect for research. Also in the same year, "Alzheimer's Mouse" came into existence. Doctor David S. Adams at Worcester Polytechnic Institute and colleagues at TSI Corp. produced this model (Games *et al.*, 1995). The mouse displayed human Alzheimer's disease symptoms including neurodegeneration and the production of neurotoxic plaques in its brain due to an overproduction of amyloid. This mouse model was later used in the summer of 1999 to produce the world's first Alzheimer's vaccine (Schenk *et al.*, 1999).

In the last couple of years, Scientists at Gala Design created **"transgametic technology"** for the production of cattle (Gala Design, 1998). Also, in 1998, two different research teams succeeded in growing the first human embryonic stem cells (Access Excellence, 1999). In the same year, came the birth of the world's first transgenic female calf through nuclear transfer (Pharming Group NV, 1999).

The field of biotechnology has come a long way in only a short time. Every day new experiments are performed and new discoveries made which advance the field and drive its continual progress into the 21st century. A lot has been done, but there is still an

infinite amount of research and work that needs to be completed before scientists can fully understand and appreciate the technologies at hand. More tests need to be performed and ideas need to be brought forward to show everyone that techniques performed years ago aid in saving lives today.

2. Current Methods

The production of a transgenic animal incorporates many different biotechnological ideas and procedures (See Figure 2.1). There are many procedures specific to the creation of a given animal, including the species, the vector method, and the characteristics of the transgene, which all play a large role in the process. However, there are three major steps common to the creation of any transgenic animal: Superovulation, Gene Transfer, and Embryonic Implantation.

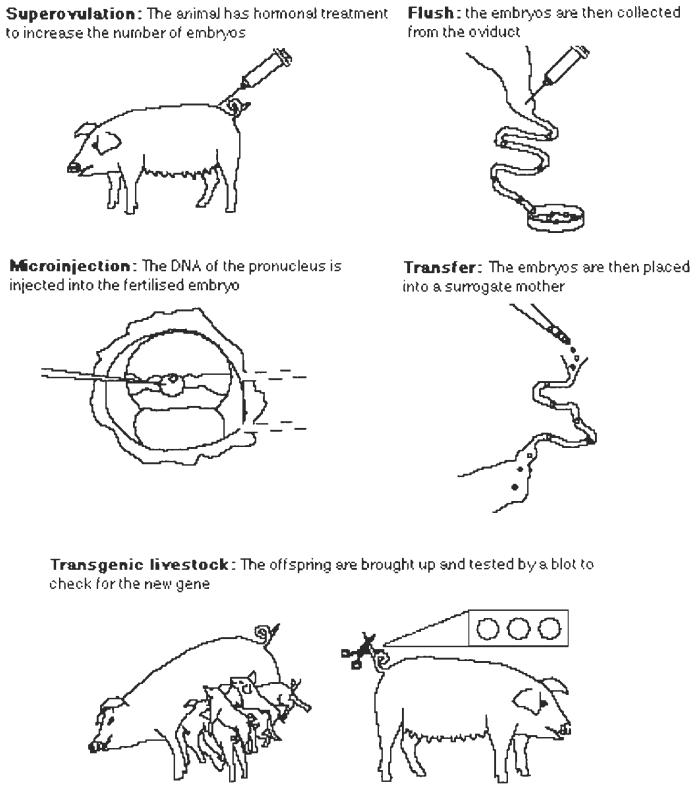

Figure 2.1: Transgenic Pig Production
Typical procedure for producing transgenic livestock by the microinjection of DNA. (Rexroad, 1996)

2.1 Superovulation

2.1.1 Choice of Parental Donor Strain

The choice of a **parental donor strain** is a top concern throughout most, if not all, laboratories. Each laboratory chooses a parental strain, which will best satisfy the topic the laboratory is attempting to study. Factors such as a specimen's response to **superovulation** and the frequency of survival after the DNA injection technique are also

taken into great consideration. If a specimen is unable to produce a sufficient number of ova during superovulation, the first process in the production of a transgenic animal, the organism will most likely be disregarded and another specimen will be used. This same concept follows with the mortality rate of DNA injected ova. If a certain strain is known to die after being injected with DNA, that strain will not be used as a source of study for that experiment. Another consideration during the parental selection process is the availability and cost of a particular donor strain. If the experiment is not carefully planned to take into consideration the specific characteristics of the specimen being studied, it may fail due to unforeseen complications.

2.1.2 Hormone Cycle

Any type of stimulation used to produce numerous ova from a single parental donor in a short time can be referred to as superovulation. This technique, however, is not specific to laboratories producing transgenic animals. Breeders of equines often use this technique to produce multiple ova from which they can select a certain trait or characteristic in a horse. Frequently this technique is used to produce racehorses, which have an ability to outrun other horses or have a greater agility. Superovulation is also used to produce Clydesdales, where a larger size is more favorable to showmen. People in the dairy field also use superovulation in conjunction with ***in vitro*** **fertilization** on a regular basis. Instead of equines, however, they breed certain strains of cows. This technique is used to benefit them in the long run because they breed cows which have an ability to produce an increased amount of milk, or bulls which can be used in rodeo shows. Aside from these non-transgenic means for the production of various strains of

animals, all transgenic laboratories use the technique to produce specimens for use in experiments.

As explained earlier, superovulation allows a laboratory to produce many ova from one donor at the same time. There are many hormones involved in a normal estrus cycle, which a scientist needs to be aware of before proceeding with superovulation (See Figure 2.2). FSH, or **follicle stimulating hormone**, is the first hormone to be released in the cycle. It is produced in the pituitary gland at the base of the brain, and it promotes the growth of the Graafian follicle on the ovary of a female after maturity. FSH is also involved in the production of estrogen. At the same time as FSH is released, the pituitary gland is also busy producing a second hormone known as **luteinizing hormone**, or LH. LH plays two major roles in the estrus cycle. Its first role is to promote ovulation, the release of the egg (ovum) from within the Graafian follicle. The second role of LH is to initiate the formation of the corpus luteum, which is a yellowish body, located on the wall of the ovary. Once the follicle ruptures, it releases not only the ovum, but also the next hormone in the cycle, **estrogen**. This hormone

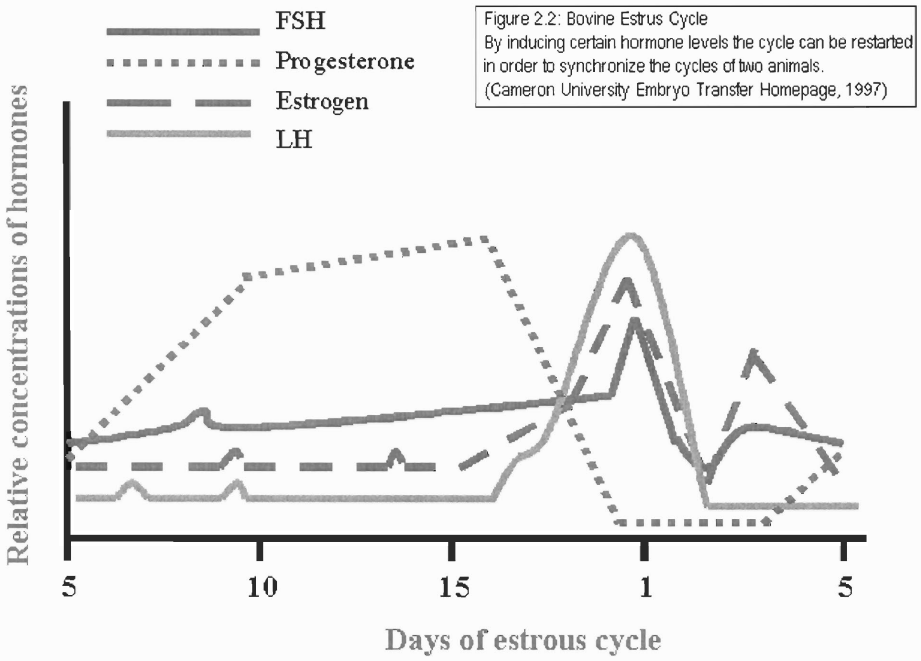

Figure 2.2: Bovine Estrus Cycle
By inducing certain hormone levels the cycle can be restarted in order to synchronize the cycles of two animals.
(Cameron University Embryo Transfer Homepage, 1997)

produces the actual onset of "heat." Estrogen is also responsible for stimulating the vascular tissue lining the wall of the uterus. When stimulated, the tissue increases in size and forms folds, which aid in "catching" the fertilized egg (zygote) after conception. This tissue is also the immediate nutritional source for the zygote. Ten to fifteen days after the formation of the corpus luteum, the fourth major hormone of the estrus cycle is produced. This hormone, **progesterone**, is responsible for the maintenance of pregnancy. If the zygote is not present within the 10-15 days following the onset of heat, the uterus will secrete the estrus-ending hormone, **prostaglandin**. This hormone causes the corpus luteum to regress and the estrus cycle begins again.

2.1.3 Methods of Superovulation

There are two ways in which superovulation can be artificially accomplished. The first procedure commences any time between day 6 and 15 of the female's estrus cycle. It begins with an injection of follicle stimulating hormone (FSH) which is performed twice daily for about four or five days. Estrus is induced on the third or fourth day by an injection of prostaglandin given at the same time as the 6^{th} or 7^{th} FSH injection. Estrus should occur on the fifth day. To avoid overstimulation, the ovaries should be palpated daily via the rectum. If overstimulation occurs, the ovaries could sustain irreversible damage causing them to ovulate improperly or not at all. This would result in the animal being unusable for ovulation purposes in the future.

The second method used for superovulation is through an implant in the specimen. Generally, implantation is done with norgestamet or progesterone. Simultaneously with the implant, an injection of progesterone and 17B estradiol is given.

This can be done on any day of the female's estrus cycle, causing the cycle to begin again. From here, superovulation should commence within four to five days, and the first method explained is then implemented as described for that process. The dosages of each of the above methods will depend entirely on the animal being used, its weight, and age.

2.1.4 Flushing

Once superovulation has been successfully completed, the next step is to obtain the ovum from the female. This is done in either of two ways. The first method includes surgery. If the scientists believe the animal is expendable and will serve no purpose for a later experiment, they will euthanize the creature and extract the ovum from the euthanized animal. To do this, the surgeon makes an incision along the stomach of the animal through which the abdominal cavity is entered to search for the **oviducts** containing the ova. The ova are generally brought out in clumps through the process of flushing or dissection. Once removed from the animal, the ova are placed in a sterilized buffered medium. The ova are held together by **follicular cumulus cells**, which must be removed by treatment through a series of microdrop solutions. The first drop is with a solution containing the enzyme hyaluronidase, which is then followed by two or more wash drops. To transfer the ovum, a heat-pulled tapered micropipet is used. It is controlled by mouth suction and this process is done repeatedly until the ova are free from cumulus cells, debris, and enzyme. Once separated, the pure ova are transferred to a petri dish, which contains a pool of medium, so the ova do not dry out. This pool is covered with a layer of sterile-filtered, autoclaved mineral oil to prevent contamination from microorganisms and debris. This oil also inhibits the evaporation of the medium

and subsequent pH changes, which could kill the ovum. All media used in the above process include a buffering system (bicarbonate or HEPES) and a protein source (bovine serum albumin) to prevent ovum from adhering to dishes and pipets. In addition to these, the media may also contain antibiotics (penicillin and/or streptomycin) and a heavy-metal chelating agent (EDTA). (Cameron University Embryo Transfer Homepage, 1997)

The second method of retrieving the ovum is the more practiced method by those who consider the parental donors essential for future work. This process, referred to as transcervical uterine lavage, involves removing the ovum without euthanizing the donor. All the equipment to be used during the collection process needs to first be autoclaved or sterilized with ethylene oxide. Once ready to begin, the first step is to evacuate the feces from the rectum of the animal. For this, either an enema can be used or the fecal matter can be removed manually. Once evacuated, the perineal region must be cleansed with a mild detergent (i.e., ivory dish soap) and rinsed thoroughly with water. Once dry, the operator is ready to begin the catheter insertion. After applying a sterile lubricant to the catheter, the operator inserts it into the vaginal opening and through the cervix into the uterine body. A special type of catheter known as a French Foley (two-way flow catheter) is used for this (See Figure 2.3). The balloon cuff on the French Foley is inflated with air or a saline solution and once inside is pulled back against the internal cervical opening to prevent leakage of medium through the cervix. The next step in the process is the lavaging, or flushing, stage. During this time,

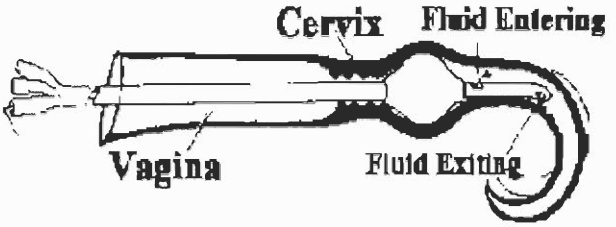

Figure 2.3: French Foley Catheter
Diagram of catheter used to flush out the ovum for collection.
(Cameron University Embryo Transfer Homepage, 1997)

a solution of pre-warmed phosphate buffered saline (DPBS), containing antibiotics (penicillin and streptomycin), is pumped into the uterus. This is done three to four times, each time allowing the uterus to drain out through the catheter and into an ova filter or collecting receptacle. After the first lavage, the uterus is massaged in subsequent lavages, via the rectum, which should aid in the recovery of more ova by suspending them in the DPBS. Once the process of lavaging is completed, the animal is given an injection of antibiotics and prostaglandin. The former is used to prevent any form of infection while the latter prevents pregnancy from any ovum not recovered during this process. (Cameron University Embryo Transfer Homepage, 1997)

The fluid passing through the filter should be collected in graduated cylinders to monitor the amount of fluid recovered. The majority (>90%) of the DPBS solution infused into the uterus should be retrieved. This fluid should be free of blood and cellular debris. Cellular debris present in the recovered solution could be evidence that the donor had an active endometritis at the time of recovery. If massaged too vigorously in the previous step of the process, the uterine lining could begin to bleed causing the fluid obtained to have a reddish tint. The balloon of the catheter could also lead to internal bleeding of the walls of the uterus if it is rotated or moved too much during fluid recovery. Once the flush is completed, the fluid is then poured into a sterile search dish and the filter is rinsed with DPBS solution to ensure adequate recovery of all ova. The dish is then examined to locate any ova, which were flushed out, under a stereomicroscope. Once identified, an ovum is washed by transferring it through three 1-ml drops of DPBS and finally placing it in a small petri dish containing additional DPBS solution. The medium used for holding the ovum is sterilized as well as the semen-

freezing straws, capillary pipets, and French open-ended tomcat catheters. The latter three are instruments used for transporting and handling the ovum. When using any of these instruments to handle an ovum, air is drawn up after the ovum (and solution) to prevent it from being accidentally pulled out should the tip touch an absorbable area in the workstation. (Cameron University Embryo Transfer Homepage, 1997)

2.2 Methods of Gene Transfer

2.2.1 Obtaining Recombinant DNA

Prior to the insertion of the DNA into the **pronucleus** of the cell, the DNA itself must first be obtained. Primarily, the transgenic DNA is engineered in the laboratory with the goal to achieve predictable expression in the mammal. By the use of **restriction enzymes** and **ligases**, different functional regions of genes can be isolated and recombined to form a transgene construct (See Figure 2.4). At the end of this construct, polylinkers, which are specific sites for restriction enzymes, can be added so that the construct will be incorporated into a variety of DNA strands.

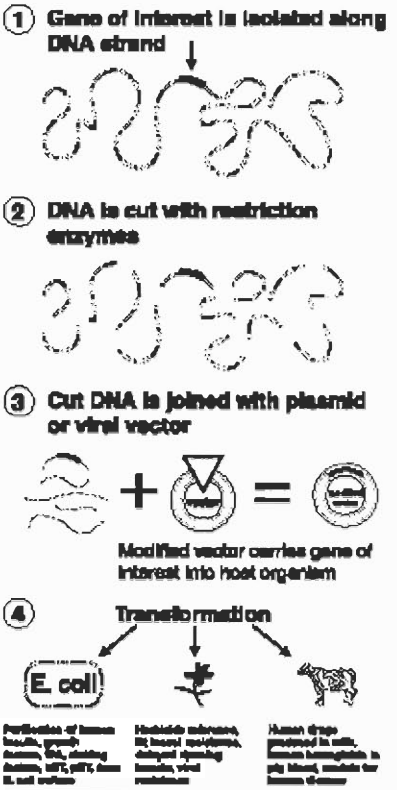

Figure 2.4: Restriction Enzymes
The DNA is cut with restriction enzymes to isolate the gene of interest and join it with the plasmid vector. (Betsch, 1995)

Large quantities of the transgene constructs are inserted into a **plasmid vector** for growth in *E. coli* bacteria (See Figure 2.5). The bacteria containing the plasmid are selected by using antibiotics to which the plasmid is resistant. With each bacterial reproduction, more

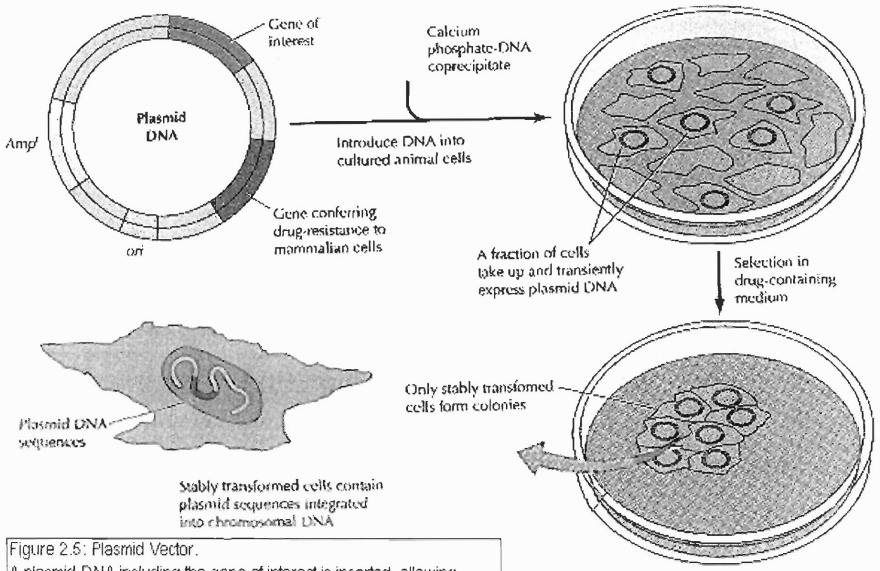

Figure 2.5: Plasmid Vector.
A plasmid DNA including the gene of interest is inserted, allowing some cells to incorporate the DNA and be transformed.(Cooper, 1997)

copies (sometimes several thousand per cell) are made. These surviving bacteria are grown in a nutrient medium overnight. Several million plasmids are removed from the bacteria the following day. Restriction enzymes are used to isolate the fragments of DNA encoding the transgene from these plasmids. Upon removing the fragment containing the transgene, the ends of the DNA plasmid are removed to prevent interference with expression of the transgene. The DNA is transfected into other cells to test whether or not the gene is capable of integrating itself.

Promoter/enhancer constructs control the expression of a gene. Thus promoters/enhancers are carefully chosen to allow expression of a transgene only in a specified tissue. Another major use for the promoters is to examine the effects of overexpressing and misexpressing endogenous or foreign genes at specific times and locations in the animal.

Many factors influence whether a promoter/transgene construct will be expressed (produce the appropriate protein) in transgenic animals. The promoters that are used must be known to function appropriately ***in vivo*** (***in vitro*** function does not always guarantee this). Transgene constructs may have accumulated mutations during cloning.

Perhaps the most important consideration has to do with the transgene's insertion site in the DNA. At many chromosomal locations, transgenes are not expressed. At others they may be expressed, but with a tissue and temporal specificity that is not identical to what has previously been seen with the same promoter construct. The intrinsic ability of a promoter construct to drive transgene expression reliably and with faithful tissue specificity also varies from promoter to promoter, for reasons that are not well understood.

Once the ova have been extracted and separated from the cumulus cells and the transgene has been isolated, they are ready to undergo the actual process of DNA transfer. DNA can be introduced into the ovum by retroviral vectors, microinjection, the introduction of genetically engineered embryonic stem cells, or by using sperm.

2.2.2 Retroviral Vectors

Out of the various methods for genetic transfer, retroviral vectors have an advantage of being a simple and effective means of integrating the transgene into the chromosome of a recipient cell. Since retroviruses have the ability to infect cells in this manner, they are commonly used as vectors for the transfer of genetic material into the cell. The retrovirus genome consists of little more than the genes essential for viral replication. Retroviral vectors are produced simply by replacing the viral genes necessary for replication with the genes to be transferred (See Figure 2.6).

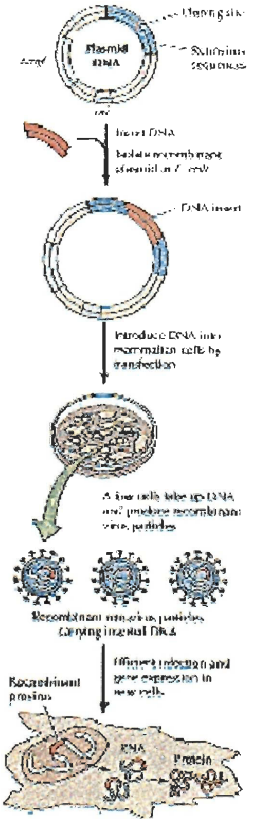

Figure 2.6: Retroviral Vectors
The transgene is inserted into the plasmid DNA and retroviral vectors are produced. (Cooper, 1997)

The transgenic DNA can be manipulated to meet a variety of needs allowing multiple applications and the design of very elegant vectors. Retroviral vectors are produced through packaging cell lines, which contain the genes for the creation of retroviruses. Following insertion of the desired gene into in the retroviral DNA vector, and maintenance of the proper packaging cell line, it is now a simple matter to prepare retroviral vectors. A process known as transfection is implemented where DNA is mixed with calcium phosphate to form a precipitate, which is absorbed by the packaging cells. This incorporates the retroviral DNA vector. After a series of tests determine that the packaging cells produce a retrovirus (containing the transgene), these packaging cell lines are used to infect specific types of cells with the transgene construct. These viruses are used to infect the embryos at the 8-cell stage and incorporate the transgenic construct into all 8 cells. After the infection of the embryos by the retrovirus, the embryos are implanted in a recipient female.

There are several advantages to the retroviral system. For example, the system is very well studied, there is a high efficiency for the infection of embryos, and the integration of the DNA into the embryo can be sustained into subsequent generations. It also allows the DNA to integrate itself into the germ line cells and if a mistake is made, it too is permanent. They also have the drawback of only being able to transfer small sequences of DNA, which, because of the size constraint, may lack essential adjacent sequences for regulating the expression of the transgene.

2.2.3 Microinjection

Because of the disadvantages of using retroviral vectors as a way of DNA

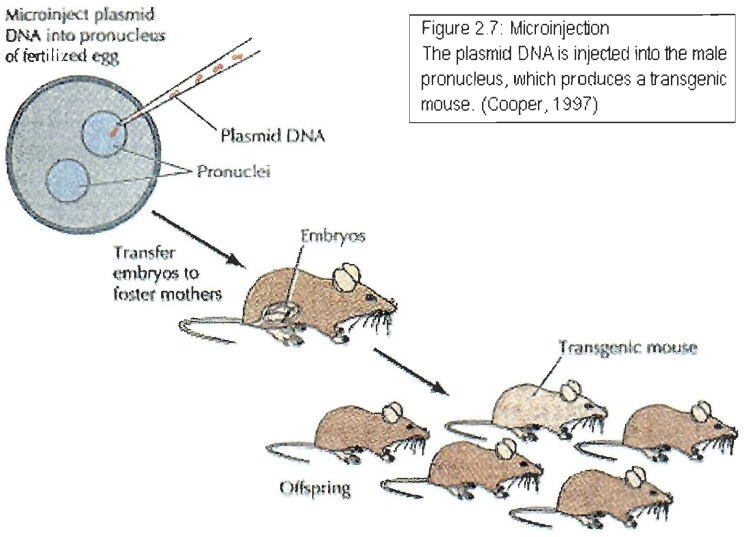

Figure 2.7: Microinjection
The plasmid DNA is injected into the male pronucleus, which produces a transgenic mouse. (Cooper, 1997)

transfer, microinjection of DNA is currently the preferred method for the production of transgenic animals. Unlike the problems with the size of the retrovirus, microinjection can be used to introduce larger segments of DNA (Greenberg *et al.*, 1991). In mammals, after entry of the sperm into the egg, both the sperm nucleus (male pronucleus) and female pronucleus exist separately for a few hours after the fertilization. The male pronucleus, which tends to be larger than the female pronucleus can be located by using a dissecting microscope. The zygote can then be held in place and microinjected (See Figure 2.7).

For the process of microinjection, the petri dish containing the embryos is placed into focus under a dissection microscope, and, at such time, any embryos that not have survived can be separated from the healthy embryos. The holding pipet is inserted into the medium, and the first embryo to be injected is gently sucked onto the end of the pipet and held in place. The tip of the ejection pipet is adjusted so that it is in the same plane of focus as the pronucleus to be injected. At this time, a small amount of DNA is released into the medium to insure the function of the pipet. The injection

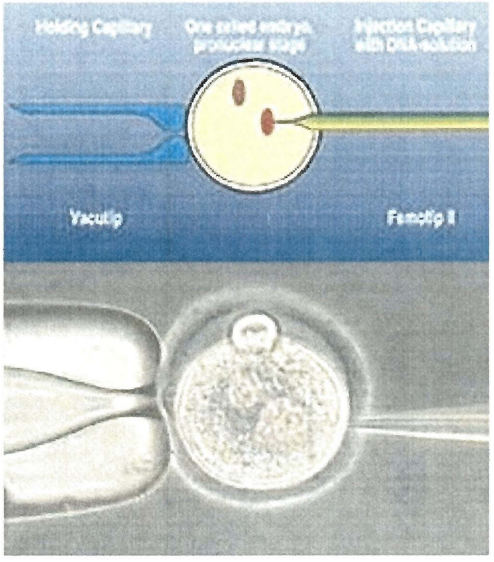

Figure 2.8: The Injection Pipet
Diagram and photograph of the inserted microinjection pipet. (Betsch, 1995)

pipet is inserted through the outer layer, the cell membrane, cytoplasm, and into the pronucleus in a single motion (See Figure 2.8). After the DNA is injected into the male pronucleus, the pipet is removed in a smooth fashion after which the embryo is relocated to the opposite site of the medium. The next embryo is then selected for injection and the process is repeated. After the group of embryos has been injected, they are transferred to a different dish to undergo observation. If a pronounced swelling has occurred in the male pronucleus, it is a good indication that the DNA has successfully entered into the pronucleus. The viable embryos are then transferred to a recipient female.

Microinjection requires a number of steps to be performed accurately. At best, only 5% of the inoculated eggs will develop into live transgenic animals. Since none of the steps are perfectly efficient, many eggs must be used, in order to ensure that at least some of the offspring are transgenic. Only 66% of the eggs survive the injection sequence. About 25% of the implanted eggs develop into viable offspring and about 25% of these offspring are transgenic (Glick, 1994). Sometimes the genes will incorporate themselves into random sites and do not get expressed. Therefore, even after genetic tests confirm that the animal has integrated the DNA, it may not show any signs of gene expression. In addition, the number of times this gene integrates itself in the cell may be excessive and lead to overexpression, which could cause serious problems for the animal. Notwithstanding the overall inefficiency, it has become routine to use microinjection, which is the most applicable and effective technique for most animals.

2.2.4 Embryonic Stem Cells for Injections

An alternative to DNA microinjection is the use of the embryonic stem cell

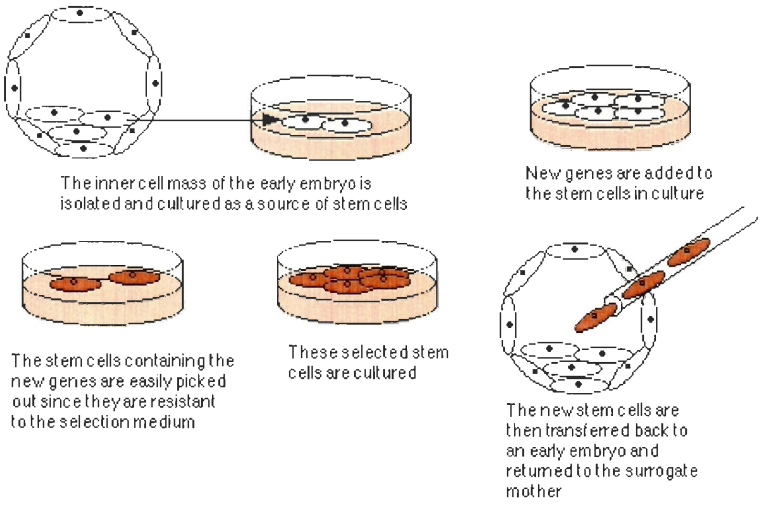

Figure 2.9: Producing Embryonic Stem Cells
ES cells are cultured before adding the new gene. The cells containing the transgene must be selected. (Rexroad, 1996)

method. In this method, cells from the blastocyst stage, a very early embryonic stage of development, can grow in a cell culture and still retain the capability of changing into other cell types. These cells are called embryonic stem (ES) cells (See Figure 2.9). Gossler first implemented this technique in 1986 (Gossler *et al.*, 1986). ES cells must be maintained on a layer of feeder cells, typically mouse embryo fibroblasts that have been irradiated to prevent them from dividing. ES cells must be **passaged** every 2-3 days to keep them from differentiating.

In order to allow the ES cells to receive the transgene, the DNA must first be incorporated into the cell (See Figure 2.10). The transgenes are incorporated by the use of calcium phosphate mediated transfection, in a similar process to that used in retroviral vectors. After the insertion of ES cells in culture, some cells will have DNA integrated at non-target sites. In other cells, integration will correctly occur at the

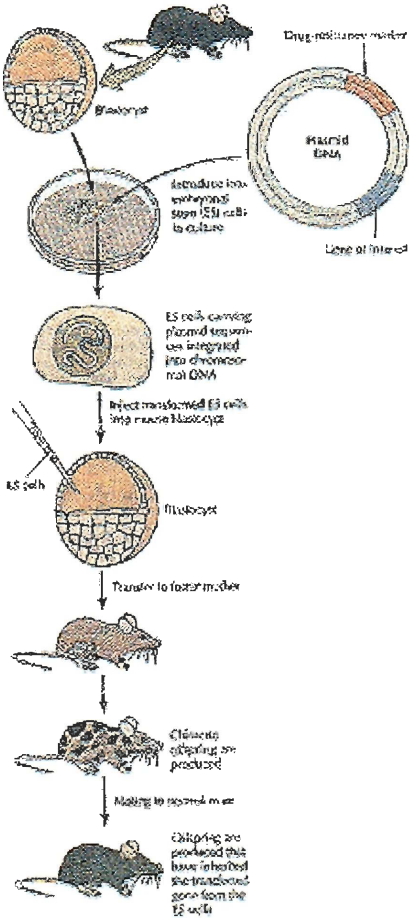

Figure 2.10: Embryonic Stem Cell Method
Plasmid DNA is added to ES cells, producing transgenic mice. (Cooper, 1997)

target site. Moreover, in most of the ES cells, the input DNA will not be integrated at all. A major advantage of using ES cell transfer is that one can determine whether or not the specific cells have the transgene integrated in them prior to inserting them into an animal (Gossler *et al.*, 1986).

To determine the cells with DNA integrated at the target site, a procedure called positive-negative selection is utilized (See Figure 2.11). For the process of positive-negative selection, the objective is to use a vector that contains the transgene and two other important components. The first is a resistance gene (Neo^r) to the compound G418 (neomycin). The genes for the transcription of **thymidine kinase** are the second component. The vector is designed such that if the DNA is integrated at the target site correctly, the Neo^r gene will be incorporated but the thymidine kinase gene will not (See Figure 2.11). The cells are cultured on a medium containing the compounds G418 and gancyclovir, a compound that is toxic in the presence of thymidine kinase. The positive selection is the test for the presence of the Neo^r gene. If a cell does not contain the Neo^r gene it will not be resistant to G418. This cell will die and is thus, positively selected. The negative selection is the test for the absence of the thymidine kinase gene. If the

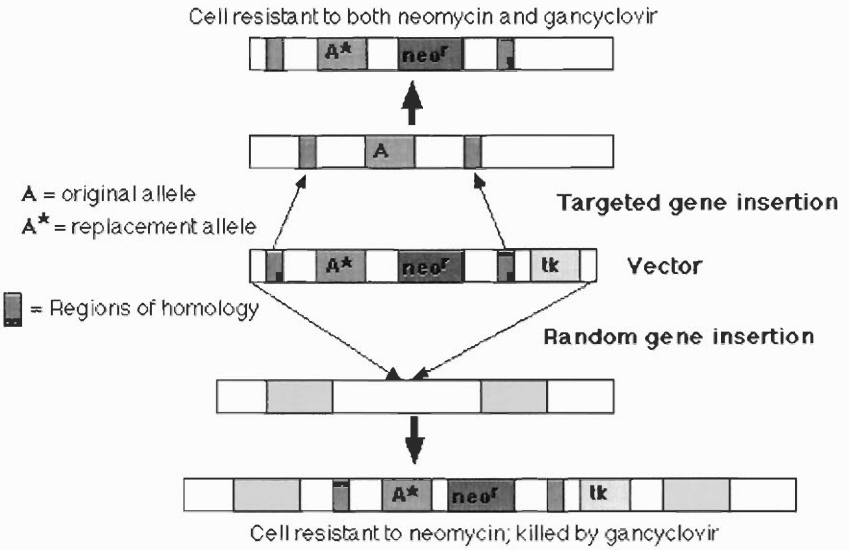

Figure 2.11: Positive-Negative Selection
The ES cells are enginneered so that only the cells integrating the transgene will survive. (Kimball, 1999)

integration occurs at a non-target site, the genes for thymidine kinase will be included in the integration and thus, when gancyclovir is added to the culture, the cells will die. Hence, these cells are negatively selected. Only cells that express the Neo^r gene, but not the thymidine kinase gene, will survive. These cells, which have correctly integrated the transgene, are positively selected (Zheng and Wilson, 1990).

A more direct way to detect ES cells that carry a transgene at a targeted chromosomal site is to use the polymerase chain reaction method. As mentioned before, PCR amplifies a specific sequence of DNA. In this case the vector transgene is amplified and detected to determine which cells have correctly taken up the new DNA. In that way, clusters of cells containing the gene can be established (Zimmer and Gruss, 1989). These embryonic stem cells that are carrying integrated transgenes can be cultured and inserted into recipient females.

The aggregation method for generating recombinant embryos, as opposed to the microinjection technique, does not require an expensive microinjection apparatus or sophisticated manipulative skills, and utilizes embryos that are easier to obtain in numbers. The ES cells are introduced into the developing embryo by the adherence of the cells to an eight-cell stage embryo. Aggregation and culturing of the embryos is performed in microwells prepared in a plastic tissue culture plate using a darning needle. The **zona pellucida** is removed by adding acid Tyrone's solution and M16 (solutions which dissolve the zona pellucida) to the embryo culture plate. With the absence of the outer coating, the embryos tend to be difficult to handle and may become trapped in the insertion pipet during embryo handling; therefore, the pipet needs to be coated with silicon. After the removal of the zona pellucida, the embryos are transferred to each well

in the culture plate and placed in an incubator. A trypsin solution is then added to the embryonic stem cells to prepare them for mixing with the embryos. The ES cells are gently pipetted until suspended cell clumps are formed. These clumps are transferred into the wells with the embryos so that the embryos will incorporate the ES cells into them.

Using the ES cell transfer method, a functional transgene can be effectively integrated at a specific site within the genome of the ES cells as a result of the positive-negative selection process. The target site should be located in a section of DNA that encodes no essential products so that, after the integration of the DNA, there is no interference with any developmental or cellular function. Moreover, it is essential that the transgene be integrated into a site that does not prevent it from being expressed. The genetically engineered cells can be selected, grown, and used to produce transgenic animals. In this way, the randomness of integration that is inherent with the DNA microinjection and retroviral vector systems is avoided. Unfortunately, the site targeting is not entirely accurate and there exists no mechanism to ensure integration into a specific region of the existing DNA.

Embryonic stem cells, however, have only been isolated in a few species such as mice, pigs, and humans, and thus far, ES cell transfer techniques are only successful in mice and pigs. Since there are so few species with embryonic stem cells available and, so far, only mice contribute ES cells to the germ line, the use of ES cells to manipulate the genome has been rather limited.

2.2.5 Cultured Sperm Cells as Vectors

Another proposed method for introducing a transgene is using cultured sperm cells as vectors. Sperm can be injected into eggs and give rise to fertilization. In initial studies using cultured sperm cell vectors, 30% of the obtained offspring successfully integrated the foreign DNA into the germ-line (Lavitrano *et al.*, 1989). However, such high percent positives have not been reliably reproducible. Similarly, cultured spermatogonial cells, the predecessors of sperm cells, can be introduced to the testis and allow for offspring through standard fertilization (Brinster and Zimmerman, 1994). While this approach proves promising, more experiments using spermatogonial cell cultures are necessary.

Microinjection remains the standard technique for the time being, although the other methods are continually being studied and improved. Embryonic stem cell transfer remains a promising solution and with further studies may, one day, be applicable to a large number of species. Regardless of the preferred method, once the DNA transfer has taken place, all that remains is implantation of the embryo into the surrogate mother. This final step of embryo insertion is all that is left before the creation of a transgenic organism.

2.3 Transgenic Embryo Implantation

2.3.1 Embryo Inspection

Before being inserted into the recipient, the embryos must go through a process of inspection. The embryos are observed under a microscope during the morula or early

blastocyst stage to check for abnormalities. It is also important that the embryos are the correct size and shape for this stage of development. Embryos that pass microscopic inspection are allowed to continue to mature *in vitro* until they reach the blastocyst stage. At this point the transgenic embryos may be frozen for later use, or immediately transferred to the uterus of the recipient female. (Cameron University Embryo Transfer Homepage, 1997) Embryos to be frozen are placed within a protective solution and then stored in a tank of liquid nitrogen (-190°C). They can be thawed and washed at any time when ready to be implanted into the uterus, however many embryos will not survive, or remain viable, due to the physical pressures of the freezing and thawing processes. (Embryo Freezing, 1998)

2.3.2 Embryo Implantation Methods

Embryo insertion is the process by which the transgenic embryos are placed into the uterus of the recipient animal to allow for gestation and birth. If the recipient of the embryos is not the same female from whom the ova were collected, it is necessary to synchronize the estrus cycle of the recipient with that of the donor. The synchronization occurs while the donor is being primed for superovulation, and is accomplished by injecting prostaglandin into the recipient female simultaneous with the final injection of FSH given to the donor animal. This will cause both animals to come into estrus at approximately the same time. Figure 4 (Page 12) above showed the relative amounts of hormones during the estrus cycle of cows. In cows the estrus synchronization must take place between days 5 and 17 in the cycle. There is a window of synchrony between the cycles of the recipient and donor that must be attained in order for the transfer to be

successful. The time span of this window depends on the animal but is generally only a couple days. (Cameron University Embryo Transfer Homepage, 1997)

Once the donor and recipient animals have been estrus synchronized, the recipient is ready to receive the transgenic embryos. The actual embryo transfer can be accomplished using either of two techniques. The surgical technique is generally the most effective, giving rise to pregnancy as much as 75% of the time during the first week after insertion (World Equine Health Network, 1995). However, transferring via the surgical method is also more complicated using the non-surgical transfer, which is similar to the process of artificial insemination.

Surgical embryo transfer requires sedation or tranquilization of the animal. The animal is also given a local anesthetic. The uterus is reached through a laproscopic surgical procedure. An incision is made in the flank, the flesh between the last rib and hip, to expose the uterus. A small puncture is then made through the uterine wall to the lumen (See Figure 2.12). The puncture wound is kept at a minimum, small enough so as not to require suturing. A specially designed sterile pipet, containing the embryos and a small amount of medium, is inserted into the uterus (See Figure 2.13). Once within the uterine lining, the embryos are expelled from the pipet.

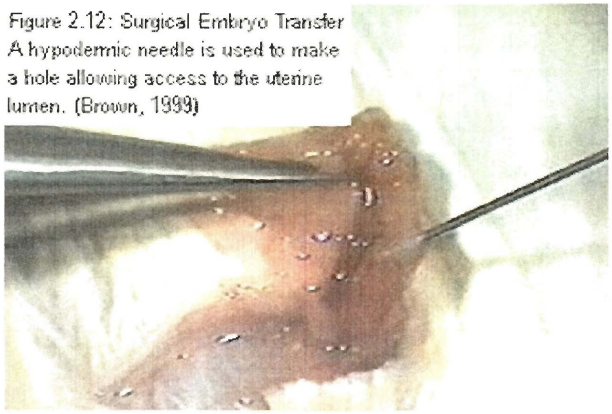

Figure 2.12: Surgical Embryo Transfer
A hypodermic needle is used to make a hole allowing access to the uterine lumen. (Brown, 1999)

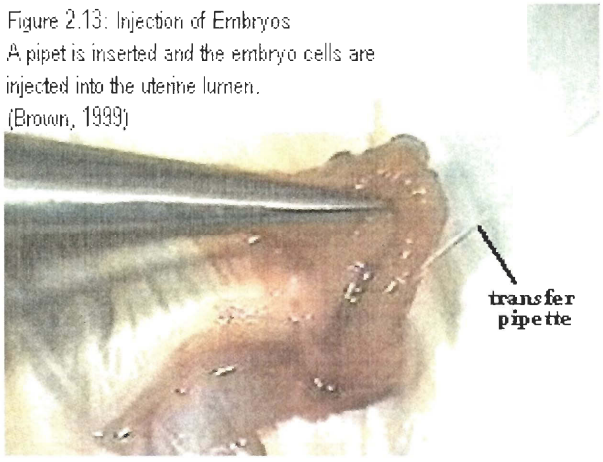

Figure 2.13: Injection of Embryos
A pipet is inserted and the embryo cells are injected into the uterine lumen.
(Brown, 1999)

(World Equine Health Network, 1995)

Non-surgical embryo transfer is performed using the same technique as artificial insemination (AI). The embryos are loaded into a 0.5 cc semen straw and inserted through the vagina into the uterus by using an AI "gun." The straw is loaded so that there is an air bubble and blank medium on either side of the embryo-containing medium. The scientist inserts the gun through the vagina penetrating the cervix so that the tip reaches the inside of the uterus. It is then "fired" releasing the embryos and medium into the uterus. Upon releasing the embryos, it is important to slightly withdraw the gun so as not to puncture the uterine lining with the tip of the gun. (World Equine Health Network, 1995)

Usually, with either process, several embryos are transferred into the recipient female to increase the chances of a successful pregnancy. The number of embryos inserted depends both on the procedure used and the species of the animal. If pregnancy does not occur, the above procedures are repeated until a successful pregnancy is obtained. After a successful transfer and pregnancy, gestation is allowed to continue as normal, and the birth of a transgenic animal is most anxiously awaited.

2.3.3 Testing & Breeding

Once the offspring have been born, it is necessary to analyze them to determine which have successfully accepted the DNA transgene. In mice and other small animals a DNA sample is often obtained by removing a small portion of the tail. When studying other animals, blood is usually drawn for this purpose (Southern Illinois University, 1999). The DNA is then isolated from the cells and, if necessary, amplified by a process

known as the Polymerase Chain Reaction (PCR). The first step in PCR is to heat the DNA so that the two strands of the molecule separate, exposing the bases that encode the DNA sequence. Primers are added that are complimentary to base sequences on either side of the target gene. Taq polymerase then aids in the replication of the DNA molecule starting at the site where the primer has bound to the original DNA strand. This process is repeated until the targeted DNA sequence has been copied in sufficient quantity. (Cooper, 1997)

A **Southern Blot** test is often used to determine if the transgene is present in each animal. The DNA is fragmented using a restriction endonuclease. This carefully chosen enzyme is targeted to cut the DNA at a specific base sequence wherever it occurs in the molecule. The DNA is thus cut into segments of varying size. Using **gel electrophoresis** the fragments are separated based on size and charge. The DNA fragments are loaded at the end of a slab of the gel nearest the negative terminal. After application of electric current, the fragments separate based on the attraction to the positive terminal. Larger molecules are slowed by the gelatinous environment and migrate the least distance. The fragments are transferred in place onto a **filter membrane**, and a **radioactive probe**, specific to the target gene, is used to locate the transgene if it exists in the DNA from the specimen. (Cooper, 1997)

Sometimes, additional tests such as Northern Blots, RT-PCR, or Western Blots are done to determine if the gene, once integrated into the animal's DNA, is being expressed. This will ascertain whether the animal is producing the protein that the gene encodes. Each true transgenic animal is then mated to determine if the transgene can be passed along to its offspring. If the new offspring have inherited the gene correctly it

shows that the transgene was successfully integrated into the stem cells of the transgenic animal.

3. Uses of Transgenic Animals

3.1 Transpharming

Transgenic animals serve a variety of purposes in the modern world. One of these uses is in the field of **transpharming**, where transgenic animal models are engineered to produce a certain protein in their milk or other bodily fluid. Generally, this protein is of human descent and is obtained for the treatment of certain diseases (See Figure 3.1). Others, such as the transgenic dairy cow produced by GenPharm International Inc. in 1990 (See page 7) serve supplemental purposes. Overall, the ability to produce almost any necessary protein is an extremely useful and valuable technological development. A special benefit of engineering an animal to produce the desired product in its milk is the animal does not need to be sacrificed in order to obtain the product.

Drug	Animal	Value/Animal/Yr*
AAT	sheep	$15,000
tPA	goat	75,000
Factor VIII	sheep	37,000
Factor IX	sheep	20,000
Hemoglobin	pig	3,000
Lactoferrin	cow	20,000
CFTR	sheep, mouse	75,000
Human Protein C	pig	1,000,000

*Current market price of the drug and supply produced by one animal.

Drug descriptions:	
AAT	alpha-1-antitrypsin, inherited deficiency leads to emphysema
tPA	tissue plasminogen activator, treatment for blood clots
Factors VIII, IX	blood clotting factors, treatment for hemophilia
Hemoglobin	blood substitute for human transfusion
Lactoferrin	infant formula additive
CFTR	cystic fibrosis transmembrane conductance regulator, treatment of CF
Human Protein C	anticoagulant, treatment for blood clots

Figure 3.1: Transpharming Drugs
A table of drugs made in transgenic animals. (Betsch, 1995)

3.1.1 Anticoagulants

Scientists at Louisiana State University and Tufts University School of Veterinary Medicine produced the first cloned transgenic goat, in 1998. This goat was used in transpharming to produce the human protein antithrombin III (rhAT III). This protein is the transgenic form of the natural human protein AT III normally found in the blood. Currently, rhAT III is used as an **anticoagulant**, which prevents the clotting of blood during certain types of surgery, including cardiopulmonary bypass surgeries. With this new protein, surgeons have the ability to perform surgery without having to worry about a lack of anticoagulants, which are normally obtained from human blood donors. (Genzyme Transgenics Corporation, 1999)

3.1.2 Cancer Fighting Drugs

Goats have also been used as an efficient means of producing cancer-fighting drugs. BR-96, a relatively recent cancer drug, attacks cancer cells specifically within the human body without the side effects associated with chemotherapy and radiation therapy. In the past, this drug could only be produced in mice or large bioreactors, both of which are extremely expensive. In 1996, scientists at Bristol-Meyers Squibb and Genzyme Transgenics collaborated to produce a transgenic goat that secretes human protein in its milk that can be used to produce BR-96. This method of production allows the scientists to produce larger quantities of this important drug at a decreased cost. "When it comes to producing a life-saving protein," said Thomas Smith of Genzyme Transgenics, "in abundant supply and cheaply, this kind of technology holds great promise for the year

2000 and beyond," (McRae, 1996).

3.2 Disease Models

Transgenic disease models are animals in which a transgene has been incorporated that will allow the recipient to display certain human disease symptoms. These models are valuable in researching what leads to the onset of certain diseases. These models also allow for the testing and development of new drug treatments, which will lead to the saving of time and money on research as well as many human lives.

3.2.1 Arthritis

DNX Transgenic Sciences, in collaboration with Dr. George Kollias, developed several mouse models that consistently show the symptoms of arthritis. One such model, known as the "DNX Transgenic Model-TNF-a mouse," expresses human tumor necrosis factor (TNF). TNF is a cytokine that has been shown to lead to the development of human rheumatoid arthritis. These mice exhibit the same chronic symptoms of human arthritis in their forepaws and hind paws. These mice should prove to be a valuable model in the fight against human arthritis and in the production of new drugs to relieve the stiffness associated with the disease. (DNX Transgenics Sciences, 1998)

3.2.2 Bovine Spongiform Encephalopathy (Mad Cow Disease)

Transgenic mice have also been used as a model for Bovine Spongiform Encephalopathy (BSE), commonly known as "mad cow disease." Dr. Stanley Prusiner, Dr. Fred Cohen, and colleagues working at UCSF developed a type of transgenic mouse

that displays the symptoms associated with BSE. By inserting a bovine gene into the mouse strain, the mice have become susceptible to mad cow disease. In fact, the mice develop the disease at a much quicker rate than cattle whereas non-transgenic mice are almost completely resistant to BSE. BSE is believed to be caused by a type of infectious protein termed a **prion**. Prions are also associated with a human disease similar to BSE called Creutzfeldt-Jakob disease (CJD). These new mice may not only be valuable as a disease model for BSE, but also as an experimental model for all diseases believed to be caused by prions. (Cable News Network Inc., 1997)

3.2.3 Sickle Cell Anemia

Sickle-Cell Anemia is a serious and debilitating genetic disorder caused by a mutation in the human gene for beta-globin, a component of hemoglobin, which is a protein that is important in carrying oxygen through the body in the blood stream. It is estimated that some 100,000 people are born each year with sickle-cell disease, most of them being of African descent (Yarris, 1997). Approximately 70,000 Americans suffer from sickle cell anemia (Leary, 1997). In October of 1997, two separate teams from the University of Alabama in Birmingham and University of California at Berkeley developed mouse models for Sickle-Cell disease. Earlier models existed, however they were not successful in effectively modeling the disease symptoms. This is because the earlier models simply expressed the gene that causes Sickle Cell in humans, however the mice continued to produce normal mouse hemoglobin that counteracted the effects of the transgene. The models produced in 1997 had knocked out the normal mouse hemoglobin gene in addition to inserting the Sickle-Cell gene, and the result was a success. The two

teams have decided to cooperate on the venture, which raises hopes for advances in treating and possibly curing Sickle-Cell Disease.

3.2.4 Huntington's Disease

Also in 1997, The Jackson Laboratory announced its release of the first major mouse model that could be used to study Huntington's Disease (HD). The disease is a genetic disorder that causes severe brain degeneration. Onset of HD begins usually during mid-life, in the 30's or 40's. The gene for Huntington's is a **dominant allele**. This means that a child of a Huntington's Disease victim has a 50% chance of inheriting the disease. The inheritability of this gene is quite tragic, as most HD victims have already had children before the symptoms begin to show. In 1997 it was estimated that 30,000 Americans have the gene for HD and another 150,000 are at risk of having the disease (The Jackson Laboratory, 1997). In the end, the disease is inevitably fatal.

The HD mice developed by Jackson Labs exhibit decreased brain size, and the uncontrollable and random muscle twitching typical of HD onset. The disorder is caused by an abnormal amount of **trinucleotide** repeats in a certain region of chromosome 4. The three-nucleotide sequence CAG is found repeated many times in the DNA of HD patients whereas these repeats are absent in non-affected individuals. The abnormal protein resulting from the repeated sequence has been linked to the cause of HD. This important mouse disease model is currently being studied, and it could be the key to a discovery of a cure for this horrible genetic disease. (The Jackson Laboratory, 1997)

3.2.5 Cystic Fibrosis

Another genetic disease being combated by improved treatments as a result of disease models is Cystic Fibrosis (CF), a genetic disorder that results in a build up of mucus in the lungs, eventually causing death in most victims at a young age. CF results from a defect in the gene for a chloride ion channel known as CFTR (Snouwaert *et al.*, 1992). Chloride ion channels are responsible for regulating the flow of chloride ions into and out of a cell. If a channel is not functioning correctly it can have drastic consequences, as seen in victims of the CF disorder. One in 31 unknowing Americans is a carrier of the gene that causes CF. (Cystic Fibrosis Foundation, 2000)

As mentioned earlier (See page 8) a mouse model for CF was announced in 1994, and followed by many subsequent CF mouse models with variations on the method of disrupting the CFTR gene. Several different CF mouse models exist to date, and new treatments are continually being discovered. It is hoped that these models will continue to improve our ability to treat CF patients, as well as eventually lead to a cure for this disease. (Cystic Fibrosis Foundation, 2000)

3.2.6 Alzheimer's Disease

Transgenic mice have also served as models for Alzheimer's disease. Doctor David Adams at Worcester Polytechnic Institute, and scientists at Transgenic Sciences Inc. created the first animal model for the inherited form of Alzheimer's disease in 1995 (Games *et al.*, 1995). Alzheimer's disease affects four million Americans and costs the country over $100 million dollars per year (Fisher, 1999). The disease destroys mental capacity, causes memory loss, and is eventually fatal. Deposits of amyloid plaques in the brain cause Alzheimer's disease. The model was developed by cloning the gene for the

human amyloid protein. The gene was then put under the control of a promoter sequence that allows for high levels of beta-amyloid expressions in the brain. When the mice became older, they began to show the plaque formations, damaged nerves, and other signs of the disease. This model showed definitively that deposits of high levels of amyloid in the brain is sufficient for the initiation of inherited Alzheimer's disease. (Games *et al.*, 1995)

The Alzheimer's mouse model was recently used by researchers at Elan Pharmaceuticals to develop a vaccine to prevent and reverse the presence of familial Alzheimer's disease in mice. The transgenic mice were vaccinated with a beta-amyloid protein that was linked to the immune system. The vaccinated mice did not develop any plaque deposits in the brain. Mice who were vaccinated after exhibiting symptoms of Alzheimer's disease displayed a lack of the accumulated plaque in their brain. Their immune systems developed antibodies against the amyloid, which cleared out the amyloid plaques (Schenk *et al.*, 1999). It is believed that this vaccine can be adapted for human use and for treating the sporadic version of Alzheimer's as well. This vaccine is now heading for clinical trials and appears to be very promising in treating and potentially curing the disease (Fisher, 1999).

3.3 Transgenic Food Production

3.3.1 The Problem

The human population is growing at an increasingly enormous and startling rate. Unfortunately, the natural resources of the Earth are present in the same abundance today as they were at the start of human civilization. Technological advances have allowed the

human race to more effectively harvest these resources for their consumption. However, the rate of consumption continues to grow, as the amount of our resources is depleting over time. According to "Tomorrow's Food Today", after the year 2050 it is predicted that the growing population will have surpassed the amount of food and natural resources the earth is capable of supplying using our current technology (See Figure 3.2) (1999). The solution they propose is to use the available resources more efficiently and minimize the waste that humans produce (See Figure 3.3). Transgenic animals show great promise in helping us overcome this natural resource deficit.

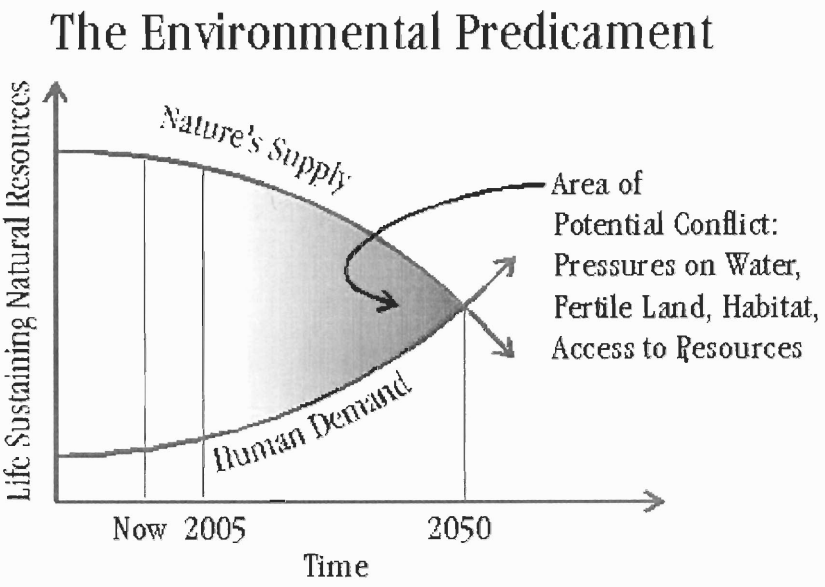

Figure 3.2: Supply Problem
It is predicted that by 2050 the human demand for natural resources will outstrip nature's supply.
(Tomorrow's Food Today, 1999)

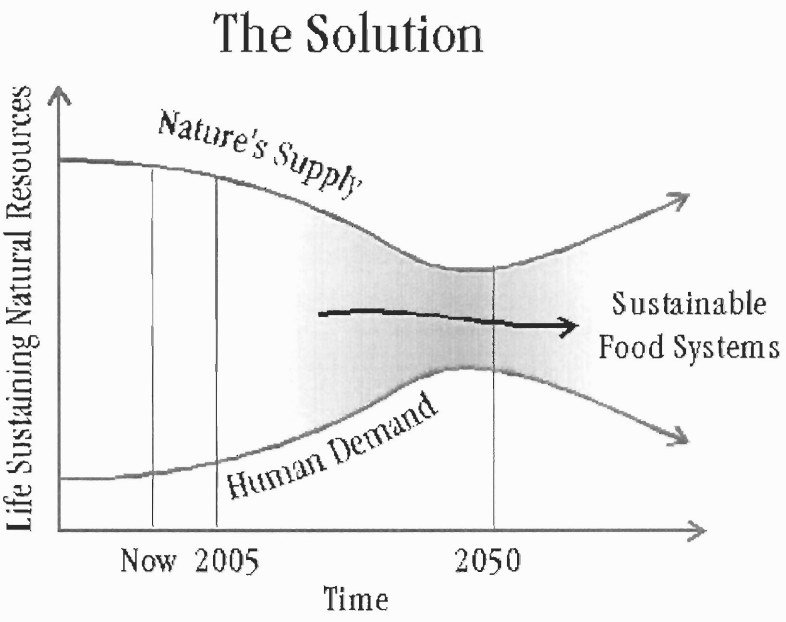

Figure 3.3: The Solution
We must strive to decrease our use of resources as well as increase the supply.
(Tomorrow's Food Today, 1999)

3.3.2 Fish

The deficit of the fish market is particularly severe. To help solve this problem, AquAdvantage has developed a salmon that grows anywhere from 400%-600% faster than a normal fish in the first year. The AquAdvantage salmon uses its own natural growth hormone and does not exhibit any foreign hormones or other artificial substances. The scientists working for this company created a genetic change by inserting a gene promoter sequence from another edible fish into the genome of the salmon. This promoter sequence causes the salmon to secrete its natural growth hormone in the liver. Non-transgenic salmon will only produce growth hormone in the pituitary gland and thus the transgenic salmon will result in rapid growth and more harvestable meat at an earlier point in time. When left to grow for the normal life span of salmon, the transgenic salmon will not exceed the expected size of wild salmon. Further research has shown that the level of production of growth hormone stays within the normally expected range, however, the transgenic salmon reach mature size earlier in their life cycle. This process results in the ability for an increased amount of food since the fish can be grown and harvested at a faster rate than usual. (Entis, 1997)

3.3.3 Livestock

Several transgenes have been produced to investigate the benefit of modifying the genome of farm animals. Genes related to growth are among those being inserted. Recent studies include the insertion of IGF-I genes, which are activated in muscle cells. IGF-I is normally produced by growth-hormone action in a cell and may be responsible for some of its growth-promoting activity (Solomon and Punsel, 1997). Transgenes have

also been inserted to produce specific antibodies, so that offspring would have disease protection from the time of birth. The complex chain structure of antibodies has resulted in limited success to date; thus another approach to disease resistance has been taken. By inserting viral genes, the animal produces the proteins that usually result from a viral infection and an immune response follows. One such experiment in chickens provided considerable protection against the **avian leukosis** virus by blocking the cell surface receptors. (Crittenden and Salter, 1990)

Dairy cows have been shown to produce 10-25% more milk in the presence of recombinant bovine somatotropin (bST). So far the FDA has approved the use of recombinant bST produced in bacteria to increase milk production. Recombinant hormones have also been used in pigs to increase pork leanness and reduce the amount of fatty tissue. This is the result of the insertion of recombinant porcine somatotropin (pST) into pigs (Brewer and Kendell, 1999). The hormone pST "redirects dietary energy away from fat deposition in the direction of lean muscle tissue production," (Brewer and Kendell, 1999).

3.4 Other Uses of Transgenic Animals

3.4.1 Biomonitoring

Oak Ridge Centers for Manufacturing Technology have proposed an ingenious new use for transgenic animals in the wild. Their proposal included producing transgenic fish to act as a type of biomonitor to detect toxic chemicals and mutagens in their environment. The scientists would insert a gene that is sensitive to toxic chemical exposure. The fish would be engineered to show visible and immediate evidence of

exposure to mutagenic toxins. The inserted gene would allow scientists to determine which toxins or mutagens are present in the environment. This technique could be applied to almost any animal in any type of ecosystem. The prospect of using transgenic animals as biomonitors is an exciting new technology that could allow us to identify certain pollutants in nature. (Oak Ridge Centers for Manufacturing Technology, 1996)

3.4.2 Xenotransplantation

Transgenic animals can potentially serve a variety of useful purposes in the area of medicine. More specifically, these animals can be used in the field of surgery and organ transplantation. To date, the success rate of organ transplants is rather small. Especially when the organs come from an animal other than another human. Also, close to 4,000 people die every year waiting for an organ to become available (Duke News Service, 1997). Transgenic animals are being looked in to as possible donors in the future. Since we can implant genes from species to species, we would be able to implant genes for human blood into a pig so that the organs will not be rejected when introduced into a human being.

Pigs are being looked to as prime subjects for this new technology. Heart valves from these animals are already being used to replace human valves that wear out or are damaged. According to Jeffrey Platt, an immunologist at Duke University, "Pigs are good potential donor animals because their organs are about the same size as human organs and work like human organs. In addition, much is already known about raising pigs, and there is a ready supply of the animals" (Duke News Service, 1997). With the advancements of transgenics, pigs could potentially be the next step in treating humans.

Implanting genes for human blood into a pig would create a suitable environment for the animal's organs, plus, it would solve the problem of rejection when that animal's organ is implanted into a human host. All of the proteins and blood would be the same and the organ already functions as it would in a human body.

4. Ethical Issues in Transgenic Technology

The ability to manipulate the genome of animals has opened doors that will profoundly change the way humans perceive the world around them, and the way in which people will live in the next century. Animals can be used to produce food more efficiently, to model diseases and help find cures, to track changes in the environment and in many different ways save human lives. However, along this road through the wonders of transgenics, questions can arise that reach beyond the realm of pure science. There is a shift from seeing animals as creatures, to seeing animals as machines. Ethical debates have arisen over the use of genetic technology on animals.

4.1 Animal Rights

The animal rights movement is well known for its protests in the use of animals for food or as sources of fur for clothing. Likewise, many animal rights activists are against the use of transgenic animals based on the same principles. In order to understand why many animal rights activists are against the use of animals in research, one must look at the basic tenets of the movement.

4.1.1 Peter Singer

An Australian philosophy professor named Peter Singer founded the modern animal rights movement. Briefly, Singer's argument is that since animals can feel pain and can suffer, they have certain rights that must be respected. He uses the analogy of a

young child, or a mentally retarded human being, and states that these beings are able to feel pain, and thus have certain rights. For instance, one cannot purposely inflict pain on them without justification. Singer argues that animals, such as apes and monkeys, are not significantly different (in a moral sense) from the child, or the retarded human. We can tell that animals feel pain by the reactions that animals have to certain stimuli, and the fear they show when a painful stimulus is threatened. Because of this, animals have certain rights, like humans, that must be taken into account. (Kopel, 1998)

Singer's argument, as the basis of the animal rights movement, can be extended to argue that animals should not be used in transgenics. More extreme groups argue that animals used for the purpose of agriculture are being exploited. The main concern here is that the conditions where livestock are forced to live are unhealthy and cause the animals undue suffering. There are also concerns about the killing of the animals and the methods commonly used in slaughterhouses. However, it must be pointed out that these slaughterhouse arguments do not apply to transgenic animals used for transpharming where only the milk is harvested.

4.1.2 The Moral Standing of Animals

There two important issues that directly impact the discussion of transgenic animals: 1) the debate over the moral standing of animals, and 2) whether any of the transgenic animals feel pain. Many members of the animal rights movement would give animals a relatively high moral standing by giving them rights almost (if not totally) equivalent to humans. On the other hand, another argument sees animals as part of the ecosystem, the prey to the human predator, objects that are there for humanity to

consume and to fill its needs. There are also varying degrees between these two extremes, however, for simplicity we will tackle them as two basic sides in the debate.

Humans have been using animals for various purposes throughout history, and probably the most basic human need that animals fulfill is food. In one sense humans have only acted naturally, treating lower classes of organisms as prey much like a lion views a gazelle, or a frog a fly. If it is true that animals have the right not to be hurt or killed, then lions and frogs are as guilty of violating their preys' rights as humans are. It seems absurd to accuse a frog of violating the rights of a fly by taking it for food, as the argument for animal rights implies. One response to this line of reasoning is that the frog does not raise the fly, keep it in captivity and restrain its freedom to live. Thus, the fly has a chance to survive, whereas the beef cow does not. This response puts a different spin on the moral understanding of animals. The argument is based on the statement that all animals have a right to live freely, to have a chance of survival. On what basis do animals have this right? Does an animal always suffer just because it is being held captive? If it could be shown that captivity violates an animal's right to live without pain, the argument would be strong and valid. To the contrary, we cannot ascertain that animals are able to consciously understand the concepts of captivity and freedom. Secondly, many animals that are raised in captivity live under better conditions than animals in the wild. They have a constant supply of food, adequate protection and shelter, and in some cases, the best veterinary care in the world.

This interesting issue of animals in captivity can be approached from a different direction. All animals, including humans, have adapted in ways that give them advantages in their natural environment. Animals other than humans have claws, sharp

teeth, bright colors, strength, antlers, and other adaptations that help them survive. Humans have none of these features, but humans have evolved the ecological advantage of reasoning and logic. The raising of livestock is simply a result of using this reasoning ability, as humans survive better when they have a consistent supply of food.

The moral standing of animals has direct consequences on the use of genetic engineering technologies. It is often argued that animals, as a part of nature, have significant moral standing that makes it unethical to alter their genetic makeup artificially. This argument does not hold if one accepts traditional agricultural techniques as morally acceptable. For centuries, humans have been raising and breeding animals to better fit their needs. Through a process called selective breeding, humans have been altering the genetics of animals for thousands of years. For example, racehorses are bred for speed or stamina, milk cows are bred for increased milk production, and beef cattle are bred for their meat. Dogs are selected to maintain their purebred lines, which is yet another artificial manipulation of genes. Genetic technology is simply another way to alter these genes to benefit human kind. Transgenics also has the ability to make significant genetic changes in a much shorter period of time than traditional breeding methods.

4.1.3 Animals in Medical Testing

Due to the argument that animals can feel pain and thus have the right not to be inflicted with pain unnecessarily, the issue of the use of animals in medical testing should be discussed. This debate directly affects the movement for transgenic animals because of the use of certain transgenic strains as disease models. Animals have been used in

many different types of scientific testing, from genetics to medical. Few will argue that it is unethical to use fruit flies as the subjects of genetic crosses used to study inheritance patterns, and likewise many will not argue against the use of mice in similar educational experiments. Mice are also one of the most important creatures used in medical testing for treatments to diseases and inoculations. It seems that in American culture, the use of mice for testing of a medical nature has been justified by the saving of human lives. The creation of disease models in transgenic animals simply allows these studies to be more accurate, lowering the number of test animals, and thus the number of animal lives that will be lost in the fight to end disease. Disease models are important for the scientific community, as they allow us to study diseases more closely in a living animal model and test drugs and medications before they are used on humans.

One final fear regarding animals' rights, and their moral standing, is the animal eugenics movement. Some fear that humans will go too far, altering animals beyond the natural limits of the species. The "natural" species will be selected against, since the new transgenic strain is more useful to humans. This is, in effect, a form of transgenic discrimination and manipulation that has caused the populace to cry out that humans are "playing God." In the next section we will see how the use of genetic technology on animals has massive religious implications.

4.2 Religious Issues

The production of transgenic animals raises numerous concerns from the religious institutions of our society. Although extremely helpful and potentially lifesaving, arguments for the use of transgenic animals incur a severe blow when viewed from a

religious standpoint. Issues such as playing God and disobeying the Divine Law, and the sacred nature of certain animals by some religions need to be taken into consideration when discussing the production of a genetically altered creature.

4.2.1 Sacred Animals

In some religions, various animals are viewed as being sacred and cannot be consumed. This is true in many religions centered in the Middle East and parts of Asia. Islam and Judaism are some of the major believers of sacred animals particularly for certain species. Both consider pigs to be sacred and therefore do not consume porcine products. With this belief, transgenic pigs come under question since we are looking to use these animals to produce pharmaceuticals for human consumption to treat disease. Are followers of Islam and Judaism able to consume these medicines, which have come from their sacred animals? This debate has raged for years and to date there still have been no conclusions drawn. Some religions that hold pigs sacred are still against the production of non-porcine transgenic animals since it would cause people to consume items taken from animals held sacred in other religions. On the other hand, the use of pigs could greatly increase treatments of disease and, for pigs manufacturing human blood, also increase the blood and organ supply levels to a point where humans would not have to worry about lacking those products. With this in mind, the laws of Judaism can be analyzed. They state that in life and death situations, Kosher rules can be set aside, which means that the transfusions or medications from pigs could be used to treat those who are in need. If it will aid in saving the life of a human, why not use a pig or other animal as a way to mass-produce lifesaving drugs?

Following along the same lines of this debate is the right for any patient to refuse a bioengineered treatment. Some religions prevent people from receiving certain treatments, like blood transfusions. Most, however, allow the individual the chance to choose. Any patient that enters a hospital has a given right to refuse any treatment they do not wish to receive. Thus, the introduction of medications and or biological materials from transgenic animals should not be an issue for those who hold certain animals sacred. If they do not wish to have the treatment, they are able to say "no." There is no reason to deny people who want the treatment and need the treatment from getting it because some religions prevent their followers from ingesting certain animal products. In many ways, by preventing the introduction of animal borne pharmaceuticals, the government would be refusing treatments to some because of resulting shortages of certain products. Advancements, such as transgenic technology, that could potentially save millions of lives should be looked into as a method of treatment. A few religions should not control what happens to the entire world. As previously stated, should the people of those religions decide to refuse treatment, it is their choice, but it is unjust for them to argue against others having access to the treatment.

4.2.2 Divine Law

Another religious issue in the debate over the use of transgenic animals is the question of violating Divine Law and playing God. Many believe Divine Law to be the rules that God set down to us as moral and ethical demands. Each religion has their own set of rules, but there are similarities among almost all religions. For instance, the Islamic *Koran* and the Christian *Bible* each state basic laws by which humans should live.

With the production of transgenic animals, many religious leaders claim that we are disobeying the Divine Law by creating new species. They claim God created animals for certain reasons and by changing the animals' DNA, we are essentially taking God's work and changing it. Religious leaders feel we should leave evolution and the changing of genomes to God, since he is our creator and supreme ruler. However, many scientists have taken these claims and dismissed them. Even within some religious sects, questions arise over who interprets Divine Law. Plus, if God is our supreme ruler and did not want things to be changed, some argue he would never have allowed us the ability to generate the technology to produce a transgenic animal. Since the technology exists, it should use it to save lives and produce pharmaceuticals which will cure disease. By doing so, the technology will allow humans to more closely follow their religious ideals of helping each other and preserving human life.

4.3 Slippery Slope Theory

It may be argued that transgenic research should be assessed in the light of its possible applications, as the ability for the misuse of technology exists. The issue arises as to whether the technology could potentially lead to disastrous ethical and moral results. This position has come to be known as the "**slippery slope**" theory (Macer, 1990). This theory assesses transgenic research only in terms of its possible application, and argues that if the implications are ethically unacceptable, the research should be banned or restricted.

4.3.1 Dolly

This slippery slope view certainly seems to have a great deal of appeal among politicians and the general public. This may be illustrated by a recent example, Dolly the sheep. When the story about a sheep being cloned from a single cell became public in 1997, there was a worldwide reaction (Sandoe, 1997). Millions of people became worried that human cloning would follow and politicians had to react. Because of this, moratoriums were passed in Europe banning the cloning of animals.

An interesting aspect of the case is that many people became worried because the relevant cloning experiment was not immediately associated with a specific medical benefit. The Super Mouse of 1982 had similar bothersome reactions from the public, since it had no useful application (Sandoe, 1997). Dolly differs from another Scottish sheep, Tracy, which was developed with the aim of producing a vital pharmaceutical product. Because Tracy was connected to a specific technological purpose, she gave rise to less concern when she appeared in the media.

One thing that seems to be problematic about the research leading to animals such as Dolly is the open-ended set of possible implications. This gives room for speculations about "horror scenarios" of various kinds. Thus, Dolly seems to have given rise to a widespread idea that the cloning techniques will eventually be misused on humans. This slippery slope view may therefore lead to a situation where it becomes much more difficult to justify pure research on transgenic animals than to justify biotechnological research directed specifically at medical applications.

4.3.2 Problems With the Slippery Slope Theory

A problem with the slippery slope approach is that only the possible negative consequences of the research are considered. The intended positive effects and the small probability of the negative effects occurring are not considered. For example, the research that led to the creation of Dolly indeed has some uses for improving transgenesis; just as the research that led to Super Mouse showed that transgenes could be phenotypically expressed. One main technological obstacle to the creation of transgenic animals is gene transfer. With a reliable method for deriving offspring from differentiated mammalian cells, it may be initially possible to target genes on cells in culture prior to transgenesis. Cloning may thus become an essential tool in the creation of transgenic animals. It may be argued that these benefits do not outweigh the proposed negative scenarios; however, the mere fact that unintended scenarios can conceivably occur should not prevent the continuation of research. The probability of adverse effects occurring must be considered, and some weighting or cost-benefit analysis must be conducted.

A second problem with the slippery slope approach is that it relies on the assumption that it is possible to bring the pursuit of knowledge to a halt. This may be possible on a very local scale, however research takes place worldwide. If one country or group of countries bans or restricts a certain kind of research, that research may still take place in other parts of the world. When results from this research are found, they will be available all over the world, including those countries that have banned or restricted the relevant kind of research. Therefore, for research to be stopped, it must be taken to a worldwide conference and agreed upon.

The final problem is that the slippery slope approach only sees the end result of experiments in genetic technology. To many people, the pursuit of knowledge justifies further experimentation. For example, the creation of Dolly could be viewed as showing specialization of cells is reversible; it is possible for a specialized body cell to regain the ability to differentiate into different and useful cell lines (Sandoe, 1997). If confirmed by future research, this will be a fascinating breakthrough in the understanding of the mystery of life.

In order to preserve continuing transgenic research, it is important to explain the role of basic research and to defend the value of scientific knowledge, not only for transgenics, but for other scientific applications as well. Scientific knowledge is valuable in and of itself and carries the promise of an endless number of possible uses. The task is to draw the line between those applications that are ethically problematic or outright unacceptable.

5. Social and Legal Issues in Transgenic Research

5.1 Transgenic Food Production

One application of current transgenic technology involves using transgenic animals as a food source. For example, in livestock and poultry, the possibility exists to engineer animals with superior breeding capabilities. Overall, transgenic food production can produce animals with faster growth rate and leaner meat, increased resistance to diseases, and decreased susceptibility to environmental changes. Although there are many advantages to foods from transgenic animals, the public has not exhibited an acceptance of transgenic technology for food production due to ethical issues and a perception of risk regarding transgenics.

5.1.1 Food Economics

Production of foods by means of transgenics offers a range of tools and products to help farmers increase production and improve income. However, critics say that new technologies may put small farmers' livelihoods at risk. Introduction of growth hormones in the livestock and poultry industries could significantly increase the income of producers. Although overall production could rise, there may be little if any rise in consumption. It is argued that this would lead to lower prices and a substantial loss of income at the farm gate. However, promising new technology cannot simply be ignored because of the problems associated with its introduction. Although there may be difficult changes as the food industry adjusts to the new technology, such changes come with any

technological innovation. Historically, many innovations are initially destructive to jobs and markets. However, in time, they result in more and better new jobs and markets of generally higher quality than those they displace. While transgenic food production could be used to enhance corporate food monopolies, it also offers options to help diversify and stabilize the farm economy. Transgenics can contribute to more self-reliance for farmers and consumers in both industrial and developing nations. It is not the technology itself, but how it is eventually incorporated into the industry that will determine its socioeconomic impact, both in the short and long term.

5.1.2 Labeling and Safety

Some groups, such as Foundation of Economic Trends headed by Jeremy Rifkin, recommend that new food products produced using recombinant DNA techniques should be labeled (Durso, 1996). This would allow consumers to decide whether they want to use these products. Some people, with regard to personal health issues, have raised additional general health and safety concerns. For example, drinking the milk produced by transgenic dairy cows engineered with synthetic hormones may harm children. In order to help ensure personal safety, rigorous testing is required to gain regulatory approval for new types of foods. Regulations are designed to screen for health and environmental risks that might result from the new trait. There is a precedent of labeling standards that already requires information such as ingredients, grades, and health and safety warnings. Surveys indicate that seventy percent of the people surveyed believe that products of transgenics should be made available, but stipulate that these foods should be labeled so that consumers can choose for themselves (Hoban, 1998). The

biotech industry and many regulators oppose labeling. Along those lines, the United States Food and Drug Administration has determined that genetically engineered food requires no special regulation. They say no technical or scientific reason has emerged which would require it any more than with any other currently used production technique. Current labels are not required to carry information on plant breeding methods, on the use of antibiotics or hormones in livestock production, or on the use of pesticides on crops. A special label for products of transgenics would imply that they are better or worse than others are.

Some critics argue that the process of transgenic food production is risky and dangerous, and thus farmers should continue to employ older and more established means of breeding. However, gene exchanges and mutations occur regularly in nature. According to some researchers, the risks of man-made genetic modifications are no greater than those posed by natural forces or by traditional cross breeding (Thompson, 1997). In fact, genetic engineering may allow for better control of genetic exchanges than traditional methods. Also, because alterations are made quicker, problems can be detected sooner than with slower and more traditional methods.

Transgenesis is a dynamic new force for the improvement of animal productivity. It has the potential to increase agricultural productivity and value, while enhancing environmental protection and sustainability. It can provide healthier, more nutritious food. As with any other technology, the introduction of transgenic food production is viewed with skepticism from its critics and a sense of concern by the public in general. There can be little doubt that it will be progressively employed in agriculture, but the speed with which it is introduced will depend on public acceptance. This will, in turn, be

conditioned by public awareness of the benefits of biotechnology. It will also be affected by the ability of the industry to remedy public concerns and deliver safer and better products.

5.2 Patenting

What a person can patent, how long the patent should last and what the patent implies are all questions that arise as people invent new ideas. In order for something to be patented, it must be novel, useful and non-obvious (Patentability of Inventions, 35 U.S.C. §§ 101-03 (1988)). Plus, intervention by humans must be a critical part of the new idea or invention for it to receive a patent. Phenomena of nature are not patentable. (Funk Bros. Seed Co. v. Kalo Co., 333 U.S. 303, 1980) In general, patents are issued for a period of 17 years and during that time, others cannot profit from the invention and the inventor must share his or her knowledge with others. The granting of a patent does not automatically give commercialization rights to the bearer.

5.2.1 Patented Organisms

With this in mind, there are some underlying problems with patents when brought into the field of transgenic animals. Can a person receive a patent for a genetically altered creature? This question has been under debate for many years and still today has no definite answer. Controversy still pervades the entire field of patenting animals. Where exactly does this controversy lie? It lies in two basic areas; 1) should life itself be patented and 2) if we do patent animals, will scientists then try to patent a human?

Allowing people to obtain patents on living organisms is not a new issue. The

issue has been evident in society for many years, some dating back over a century. Louis Pasteur received a patent for a cultured strain of yeast used in fermenting beer in the 1800's, during which time patents were also offered on various food products. The first patent on an engineered organism did not come until 1980 (Diamond v. Chakrabarty, 447 U.S., 1980), when a patent was awarded for an engineered bacterium that could breakdown crude oil. With this patent came extreme controversy. It was originally rejected on the grounds that the microorganisms are products of nature and therefore not patentable subject matter. This decision, however, was later overturned since the organism fit the definition of a patentable invention.

5.2.2 The Patentability of Life

Although the transgenic patents were awarded, there are still many people who believe life itself should not be patented. From a political standpoint, how can the government regulate whether an organism is patentable? Current guidelines have been set on what can receive a patent, and transgenic organisms fit that definition. Should the government set new standards on what can and cannot be patented? Should they create laws stating that patenting transgenic animals is illegal and cannot be done? Many say yes, but there are also many who say that this would be too much government influence. Many feel that the government already regulates our lives too much and should stay out of this area. If the transgenic animals have useful purposes and could potentially save lives, then they should be created, and the creator should be given the recognition that he or she deserves.

In Body Parts (Gold, 1996), E. Richard Gold presents evidence that patents are

granted when the economic effect is positive and outweighs the negatives. Gold points out that patents, on one hand, create a monopoly for the inventor. However, on the other hand, patents must be awarded in order to "encourage new discovery by providing an economic incentive to invest in research and development." (Gold, 1996) Thus, the patentability of biotechnological inventions, including transgenic animals, must be well established in order for research to continue. No company is going to invest money in research or development projects unless there is an economic incentive in the form of a temporary monopoly. If the government denied patents for transgenic animals, research would screech to a halt.

5.2.3 Human Implications

Along with the question of patenting animals comes the belief that allowing the patenting of animals will eventually lead to a debate over humans. If animals are patentable, then will people be able to take out patents on other humans if they are genetically altered? According to the 14th amendment to the United States Constitution, ownership of a person by another is prohibited. But, as is debatable with animals, will humans who are genetically altered still be humans, or will they be just a product of some scientist? How can the government say that animals are patentable if they are produced through recombinant DNA technology and not allow the same to be true for humans? This issue has begun to take its place in the limelight. Many believe that the patenting of humans is drastically different from that of animals since we are considered to be of a higher intelligence. But even so, some believe there is no way to justify the patenting of one animal and not another. Humans, in many ways, are just like any other animal. We

have a higher mental capacity, but when considering structure on a DNA and molecular basis, we are just like other animals, made up of the same exact material.

5.3 Government Regulation

5.3.1 Standards

Another issue found in the political arena relating to transgenic research and the production of transgenic animals is seen when discussing regulation. Currently there is no set standard for regulating the production of transgenic organisms. There are numerous organizations involved in this research area and too many federal agencies have initiated the regulation process in hopes of a universal set of guidelines. Such agencies include NIH (for bio-medical research), the USDA (for livestock and veterinary studies), NSF (for laboratory research), and AID (for international research centers). All have varying ideas on what should be done with regulation. With all of these agencies involved, one single federal policy is almost impossible to achieve. The closest is a policy set forth by NIH that deals with genetically altered animals. This policy is basically derived from a list of guidelines for Research Involving Recombinant DNA Molecules.

Throughout the world people are in debate over how government should regulate the production of genetically altered animals. Many fear that no regulation at all will lead to experiments that are harmful to both the animals and possibly even humans. If the current state continues, in which there is very little true regulation, many are concerned that the next experiments being proposed will involve humans. In the United States, there are currently very few laws or guidelines preventing this work in humans. On the

other end of the spectrum, however, there are those who believe the government should mind its own business, that it is too involved already, and should partially back out of the regulatory field. Regardless of the position people take, most agree on one thing, government regulation is required, but the controversy lies in how much influence the government should have.

5.3.2 Research Moratoriums

Another area in which there is a debate over regulation deals with the issue of a moratorium on research. Recently, many congressional hearings have dealt with the idea of having a moratorium on research in the field of genetic engineering. The focus has primarily been with regard to transgenic mammals. A moratorium being proposed would only be temporary, lasting until a standard is set on how much influence the government should have and some basic guidelines are set down. Positives to this policy are few and far between. The most important positive would be the prevention of research on humans until laws are established. Although this is a strong argument, there seem to be many more arguments against the incorporation of a moratorium on research. The field of transgenic research lies mainly in a competitive environment. With a moratorium, our nation's competitive edge would be undercut, and progress being made in disease treatment and prevention could suddenly be terminated. Not only could research be terminated in some fields, but the potential for encouragement of new competition and the incorporation of new ideas and technologies could also be lost.

5.4 Funding

Funding for transgenic studies originally came from the government in the form of research grants or pledges. It was rare for the financial aspect to be covered by research firms or by other private investors. Once the potential profits of transgenic research became clear, this gradually began to change and the shift from government financing to private financing is almost complete. Today, the private sector seems to provide much of the funding. The investments are mainly centered about research firms and private pharmaceutical companies looking to produce the best product for the most profit.

Originally, governments supported transgenic projects, but many felt the government was too influential and should back out somewhat. Those who favored government regulation felt that this funding is exactly what needed to be done. They felt the government was doing their job, finding ways to better the lives of people and controlling something potentially dangerous should it fall into the wrong hands. The private businesses, however, began to take some of the control away from the government. They began to fund research projects that became extremely successful and profitable. Because of this, private investors began to emerge in all areas of transgenic research.

The tables turned, and now those who supported the government found themselves fighting for a change. Continuing today, many believe the government has gone too far by allowing the private sector to increase its influence and control over transgenic research. They feel the government needs to reassert interest in the field and

take away some of the power the private financiers have over the studies. Those who support government regulation of the biotech field have expressed concerns that private funding will eventually lead to uncontrolled experiments. Experiments could occur where the companies or research firms produce something dangerous or begin to use this new technology on humans.

Others fear that if government comes in, the competitive edge will be cut off because the government will control what is produced and what is funded. Plus, the government would never be able to afford the amount of financial investments being made by the private sector. Money would run low and progress would come to a standstill. Only those experiments, showing some immediate promise, would be allowed to continue. Moreover, studies being done with transgenic animals might be halted because they are not producing quick, easily obtainable results. By having the government intervene and begin funding the projects, much of the research currently being done would have to be put on hold until the government could allocate the money.

6. Conclusions and Recommendations

6.1 Concerns

There are many concerns continuing to linger in the study of transgenic animals. These concerns range from simple things that can be fixed with no problems, to larger issues that will be debated over and over again for many years to come. The potential for the misuse and abuse of animals being used as test subjects is a very important and crucial concern to this field. Environmental issues also play a huge role in the debate over transgenic animals. Plus, what needs to be addressed along the lines of human consumption of transgenic products is a top priority at this time as well.

6.1.1 Animal Experimentation

Many fear that the use of animals in genetic studies such as these will bring about animal suffering. They fear that transgenic studies will turn into more than just scientific and medical experiments to better society. The potential for the misuse of animals exists as a strong possibility. If scientists are allowed to produce these altered organisms, many feel nothing will be able to prevent them from creating animals that look funny, or have a different color fur. Experiments such as these would have no medical benefit whatsoever and could lead to the uncontrolled suffering of animals.

Animals such as "Supermouse" (See page 6) serve no significant scientific or medical purpose. These types of animals have been the basis for many arguments as to why transgenics should not be allowed. Many feel that other animals will be mutated as these have been, for no reason except to show that it can be done.

There are also many concerns from animal rights activists who believe that many

of the models produced will cause the animal undue suffering. Implanting human genes that cause deadly diseases in humans is cruelty to animals, many argue. This area raises debates over whether or not the animals should have to suffer for scientific and medical advances. Concerned people worry about the animals that are suffering because of a debilitating disease that is incurable at this time. Also, when people dive into this area of debate, many look at the negatives more so than the positives. They see only the number of animals that have died and been killed and not at the number of human lives that have been saved.

6.1.2 Environmental Issues

Another area of much concern involves the environment. The impact that transgenic animals may have on the environment is a point often brought up. First of all, producing transgenic animals changes a species. In essence, scientists produce a new animal when they change the DNA. What impact will these new animals have if they escape or are released into the environment? One of the largest concerns on this topic is the potential for the transgenic animal to compete with the non-transgenic forms. If that occurs, will transgenic animals, due to some new trait that they have, outlive and wipe out the existing animals of the area? Many fear that natural animals will no longer exist one day because the transgenic ones will out survive those that are not artificially mutated.

Environmentalists also wonder about the potential dangers the transgenic animals pose to humans. What happens if someone decides to put a gene into an animal, which causes aggression? Without killing the animal, there would be no way to prevent it from

attacking. This could pose a serious threat to both human lives and also other wildlife. Animals such as these, say many, should be prevented from being formed, but they cannot suggest any ways of stopping this action.

6.1.3 Consumption

Human consumption and product labeling are another area encompassing many debates when related to the production and use of transgenic animals. Since we are taking genes from one animal and placing them in another species, there are possibilities for health concerns to arise. People that are allergic to one type of animal may be affected through use of transgenics. For instance, if a porcine gene is placed into a cow, the person who is allergic to pig meat could have a severe reaction when eating a hamburger produced from the transgenic cow. This causes great concern among various groups including the federal government.

This whole issue leads into a concern over labeling. The FDA has strict guidelines on what products need to have special labels and what products do not need to have the labels. However, with this emerging field of transgenics, the FDA will need to make some serious decisions on what food to label. Will food from transgenic animals need to be specially labeled? So far, the FDA has deemed that transgenic food is not harmful for human consumption. It does not require special labeling at this time, however, as more transgenic food products become available on the market these views may change.

6.1.4 Human Cloning

All of these concerns are extremely valid, but perhaps the most pressing concern for many is the idea of human cloning. With the technology currently in use, the possibility exists to clone a human being. We have seen how animals can be cloned successfully and many people are afraid a human will be the next step in the advancement of our technology. Almost all agree that cloning a human would be immoral and wrong. However, there is nothing strong enough to prevent it from happening if someone really wanted to make it happen. Human cloning is a worldwide concern, which has been debated for many years and will continue to be debated for many more.

6.2 Advantages

6.2.1 Medical Experiments

We have shown that transgenic animals have many potential medical uses, the most important of which include being used for disease models and transpharming. These two technologies have gone through rapid development in the last few years, and some experiments are already showing their usefulness.

There are many cases in which transgenic technology has allowed the creation of models for studying human disease. This technology allows animals, particularly mice, to develop diseases that cannot naturally occur in them. Oncomouse (See page 7) was a pioneering breakthrough in disease model research as it showed a mouse that was susceptible to breast cancer. From this research and others like it, more and more disease

models were produced in mice instead of more advanced creatures for the studies. Perhaps the best example of a disease that could previously have only been studied in larger species is Alzheimer's disease. This disorder has no known counterpart in rodents, and older primates needed to be used. With the advancement of the Alzheimer's disease mouse model (See page 38), a vaccine has been developed that could lead to a cure of the disease in humans. The potential for saving human lives and curing disease through the use of transgenic animals is almost a certainty. As a group, we feel that this overwhelmingly fascinating possibility is one that should not be ignored.

The single most studied transgenic system in livestock species is to modify the protein produced in the mammary gland. Since cows produce a considerable amount of milk, this rich protein source could provide a wealth of pharmaceuticals. Many human diseases are associated with a mutation in a gene that codes for an important protein, for example, a blood-clotting factor. Transgenic animals could be a rich source of these proteins. Since the cells that make the proteins are mammalian, the proteins that are produced are, in many ways, processed in the same manner that they would be in humans. Their presence in milk makes them readily obtainable and relatively easy to prepare. These transgenic producers are readily reproduced and studies have suggested that the transgenes are, for the most part, stable from generation to generation. Production in livestock avoids problems associated with contamination of human diseases. Transpharming could revolutionize the pharmaceutical industry by providing more cost-effective drug supplies in larger quantities.

The human race's ability to cure disease has always been in constant turmoil. First it was penicillin, followed by a range of other antibiotics, which allowed us to

effectively combat just about any bacterial disease. The battle has in many ways moved to the field of viruses, where AIDS has taken center stage amid scores of other life threatening viral diseases. The common cold is a viral infection that is less well known for its lethality, yet we still cannot find a cure. Cures for viruses are in the works, and those works are the gears and wheels of the biotechnological clock ticking away all too slowly. In addition to viruses, genetic diseases continue to evade our best efforts at cures. To make matters worse, our most powerful antibiotics are slowly and steadily losing effectiveness as bacteria continue to adapt an antibiotic resistance. Perhaps the only success story in the fight against disease is the case of small pox, a disease which was totally eradicated through mass immunization and a bit of luck. The only known samples of the virus are hidden away in scientific labs. Transgenic animals could be the keys to eradicating, for good, a growing swarm of other diseases. If we as a society wish to save and prolong human life, then the only justifiable decision is to use transgenic animals in medical research.

6.2.2 Livestock and Agriculture

Any casual observer can tell that the human population is growing remarkably fast. Similarly, it is obvious that the surface of the earth is a constant size, and capable of supplying only a finite number of resources for our consumption. Our ability to feed the growing number of people on this planet is diminishing, and it will soon become necessary to resort to other means of food production. The ability to engineer livestock, including cattle and pigs, to produce more food for consumption on less resources would help to solve this problem. This is exactly the kind of solution that transgenic animals

can present.

We have already seen the advent and use of many types of transgenic plants used in agriculture to resist disease and increase crop yields. This same strategy should be used with animals. Transgenic animals have to possibility of producing meat and milk more efficiently, on less resources, and incorporating biochemical supplements into people's diets. We see no way to avoid the continually growing population, and thus we support the use of transgenic animals in agriculture in order to feed an increasingly hungry world.

6.3 Recommendations

After considering the above issues, our project group would like to make some recommendations for the use of transgenic animals in future experiments. In general, we feel that allowing the use of transgenic technology within medical and agricultural contexts is not only ethically defensible, but also necessary for the advancement of the human race. However we have concerns that transgenic technology and similar biotechnological information and techniques will be used on humans in a questionable manner. These two beliefs are the cornerstones of our recommendations, directed at both our national government and the global scientific community.

6.3.1 Government

The United States government still retains much power over the products of science and their uses. Laws and regulations may be passed preventing the creation of or experimentation with important and necessary new biotechnological products.

Transgenic animals have come under a lot of scrutiny, both in the media and within the scientific community, and the government will determine how far research will be allowed to continue.

From our research, we recommend that the government support the use of transgenic animals, and their products, in medicine and agriculture. The positive outcome resulting from continued experimentation far outweighs the possible negative consequences. We also recommend that the FDA continue to guide production and protect consumers from potentially unsafe products. We agree with the Administration's stance that transgenic food poses no special threat, and we ask that they extend this policy to encompass the products of transgenic animals in medicine. We also recommend that the government support research into possible uses of transgenic animals in conservation, such as the transgenic fish in section 3.4.1 used to detect toxins and mutagens in the water supply. The potential benefits of using animals in this manner are innumerable.

However, we as a group feel that there are risks that transgenic technology will be used irresponsibly, and applied to human beings. We recognize that scientists are using genetics now to cure human diseases through gene therapy. We support continued research into diseases and conditions that impair the normal physiological function of a human being. However, we feel it is the responsibility of the government to refuse funding for transgenic studies on human beings, including human eugenics and human cloning experiments. The risk that this technology will be used on humans is a real one, and by refusing funding the government is also showing its lack of support for these types of studies.

6.3.2 Scientific Community

The scientific community, on a global scale, is going to be responsible for the majority of the regulation of what types of research occur. National governments simply cannot control this, as scientists who wish to pursue an experiment outlawed in their home country can simply move their lab to a country with less stringent or non-existent regulations.

We propose that the scientific community set the standards for the use of transgenic technology. In studies with animals, we believe the biological community should have regulations that emphasize the same ideas we used in recommending the continuation of research efforts by our government. However, it is the issue of human transgenics and cloning experiments that we feel is best suitably handled by the global scientific community.

We recommend that scientists get together to pledge a worldwide moratorium on transgenic or cloning research on human beings. Once a moratorium is established, we feel it would be extremely difficult for anyone to acquire the materials and financial resources needed to perform such experiments. By uniting the scientists of the world and having them work together to preserve the integrity of the human race, we can best control any possible misuse of biotechnology.

Glossary

anticoagulant (pg. 34) a drug or substance that prevents blood from clotting

avian leukosis (pg. 42) a virus that attacks the white blood cells in birds

central dogma (pg. 2) the idea that genetic information flows from DNA to RNA, and from RNA to protein

complementary (pg. 1) the concept that adenine is always opposite thymine and guanine is always opposite cytosine on two strands of DNA

Cystic Fibrosis Transmembrane-conductance Regulator (pg. 8) a protein that spans the cell membrane and regulates the cell's intake of chloride

deoxyribonucleic acid (pg. 1) the molecule that contains the genetic code and is responsible for the transmission of heredity, commonly known as DNA

dominant allele (pg. 37) a hereditary factor that predominates and determines the traits exhibited by the organism in a case where two conflicting factors are present

double helix (pg. 1) a structure which contains two linear strands spirally intertwined forming a helical shape

embryonic stem cell (ES) (pg. 23) a cell that is cultured from an early embryo that has the ability to develop into any type of cell

embryonic stem cell mediated technique (pg. 6) a technique where a transgenic animal is produced by incorporating the desired sequence of DNA into the germ line cells

estrogen (pg. 12) a type of hormone that induces estrus ("heat") in female animals and it controls changes in the uterus that precede ovulation

expressed (pg. 2) when the trait encoded by a gene is shown in the phenotype of the organism

filter membrane (pg. 31) a nitrocellulose or nylon membrane to which the DNA is blotted during gel electrophoresis to create a replica of the gel

follicle stimulating hormone (pg. 12) a hormone normally secreted in the pituitary gland that causes the ova to develop in females

follicular cumulus cells (pg. 14) cells that surround the ova and protect them

gel electrophoresis (pg. 31) a process by which DNA fragments can be separated based on size and charge using an electric field placed across a gel environment

gene (pg. 1) a unit of heredity or section of DNA encoding a specific trait

genome (pg. 2) a complete set of genes for an organism

in vitro (pg. 18) outside a living organism, in an artificial laboratory environment

in vitro fertilization (pg. 11) combining an egg and sperm outside the organism to create a zygote for later implantation

in vivo (pg. 18) within a living organism

ligases (pg. 17) an enzyme that seals any breaks in a DNA strand or joins two DNA fragments

luteinizing hormone (pg 12) a hormone that promotes the release of estrogen and promotes ovulation

nuclear transfer (pg. 8) the transfer of a cell nucleus from one cell to another

nucleotides (pg. 1) the basic unit of nucleic acids (DNA, RNA) composed of a sugar, a phosphate group, and a nitrogenous base

oncomouse (pg. 6) nickname for a mouse model created in 1988 that was susceptible to human breast cancer

oviducts (pg. 14) tubes via which the ova pass from the ovaries to the uterus

parental donor strain (pg. 10) the strain that is selected to donate the ova through superovulation

passaged (pg. 23) subculturing of cells while outside the organism.

phenotypic (pg. 6) physical characteristic of the organism due to heredity

plasmid vector (pg. 17) a small self-replicating section of DNA that exists outside the nucleus and can be used to transfer segments of DNA from one organism to another

Polymerase Chain Reaction (pg. 5) a technique by which a known sequence of DNA can be amplified to obtain millions of copies, useful in detecting a DNA sequence present in only minute quantities

positive-negative selection (pg. 7) a selection process by which only the desired cells can survive a specific screening/selection procedure

prion (pg. 36) an infectious protein particle

progesterone (pg. 13) a hormone that is responsible for maintaining pregnancy by inhibiting the development and release of additional ova and preserving the uterine lining

prostaglandin (pg. 13) hormone that ends the estrus cycle and is released to restart the estrus cycle

promoter/enhancer constructs (pg. 18) a segment of DNA located at the beginning of a gene that acts as a "switch" to turn on or off the transcription process

pronuclear microinjection (pg. 5) the process by which a DNA transgene is injected into the male pronucleus of the ovum

pronucleus (pg. 17) either of the male or female nuclei in the ovum just after fertilization and prior to nuclear fusion

radioactive probe (pg. 31) a sequence of DNA complementary to the desired sequence that contains radioactive nucleotides which can be traced

recombinant-DNA (pg. 3) a DNA molecule that has had foreign genetic code inserted into it

restriction enzymes (pg. 17) enzymes that cut strands of DNA into segments at specific locations

retroviral vectors (pg. 4) a virus with an RNA genome that carries a gene of interest which is reverse-transcribed into DNA that it inserts into the genetic material of a cell

ribonucleic acid (pg. 2) a single stranded nucleic acid that is responsible for carrying the genetic code from the DNA and translating the code into the correct protein

semi-conservative replication (pg. 2) a replication process where one molecule of DNA splits to produce two new DNA molecules, and each of the new molecules contains one strand from the original

slippery slope (pg. 50) the theory that argues against starting down a moral "slope" for fear of slipping to the immoral and/or dangerous consequences at the "bottom"

Southern Blot (pg. 31) a method using radioactive probes to detect specific DNA sequences after gel electrophoresis

Supermouse (pg. 6) a transgenic mouse produced in 1982 that grew to an enormous size due to a transgene for rat growth hormone

superovulation (pg. 10) a process by which a female is stimulated with hormones to release multiple ova simultaneously for collection

thymidine kinase (pg. 24) An enzyme that allows a cell to utilize an alternate metabolic pathway for incorporating thymidine into DNA and is used as a selectable marker to identify cells that have incorporated transgenes through positive-negative selection

transgametic technology (pg. 8) The method by which a gene is inserted into the unfertilized oocyte or egg which incorporates the gene into the maternal germline

transgene (pg. 4) a foreign gene inserted into a transgenic animal

transpharming (pg. 33) a concept by which transgenic animals produce a desirable protein in their bodily fluids so that it can be collected without killing the animal

trinucleotide (pg. 36) a sequence three nucleotides in length

vector (pg. 6) a molecule or virus used to transfer a DNA sequence

zona pellucida (pg. 25) A jelly-like outerlayer surrounding the ovum

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