CREATION AND USE OF TRANSGENIC ANIMALS IN PHARMACEUTICAL AND BIOMEDICAL RESEARCH: ANIMAL WELFARE AND ETHICAL CONCERNS

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ABSTRACT: The creation of transgenic animals has application in the following areas of pharmaceutical and biomedical research: the production of biopharmaceuticals for human use; the production of organs for xenotransplantation; and the generation of animal models for human genetic diseases. Nuclear transfer technology offers a more precise and efficient way of performing genetic modification and creating transgenic animals than the more traditional method of pronuclear microinjection. This paper will review nuclear transfer as a means of producing transgenic animals; introduce advantages nuclear transfer technology offers in the field of animal transgenesis; and highlight some of the animal welfare issues and ethical concerns raised by the generation and use of transgenic animals in the aforementioned fields of study. Finally, the influence of objectifying language and terminology used to describe transgenic animals will be considered, and the impact of phrases such as "living bioreactor" and "spare part supplier" examined.

I. INTRODUCTION

Recent developments in animal transgenesis and cloning have application in several areas of pharmaceutical and biomedical research including: the production

of biopharmaceuticals for human use; the production of organs for xenotransplantation; and the generation of animal models for human genetic diseases. In each field of research, nuclear transfer offers a more precise and efficient way of performing genetic modification and creating transgenic animals, thereby presenting certain advantages over the more traditional method of pronuclear microinjection. Efficient application of transgenic technologies will reduce the negative impact of such methods upon animals used in research; however, the effect of the transgene itself continues to jeopardize animal welfare. Regardless of the method of genetic modification utilized, serious animal welfare and ethical concerns remain. Examples set forth within the aforementioned fields of research will illustrate the importance of the ethical debate and provide a foundation for understanding concerns arising from the genetic modification of animals for human benefit.

The influence of the terminology and language used to describe transgenic animals will also be examined. For example, terms such as "living bioreactor" and "spare part supplier" connote the non-living and may negatively impact one's perception of the inherent value of these living creatures. Use of objectifying terminology and desensitizing words invoke fear that transgenic animals will be viewed as mere commodities, and will perhaps limit society's ethical questioning of these technologies. As science paces forward, a reexamination of fundamental ethical questions in light of emerging technologies will be vital to discern the impact upon animal welfare and to guide society into the future.

II. METHODS OF GENE TRANSFER: PRONUCLEAR MICROINJECTION VS. NUCLEAR TRANSFER

Several methods are used to produce transgenic animals including pronuclear microinjection, embryonic stem-cell mediated gene transfer, viral vectors, sperm-mediated transgenesis and somatic cell nuclear transfer (NT). Gene transfer by pronuclear microinjection has been the principal method used to produce transgenic farm animals; therefore, this discussion will focus on a comparison of microinjection with the more recent and promising method of NT.

A. Pronuclear Microinjection

Traditionally, transgenic animals have been created by pronuclear microinjection of one-cell embryos. This process uses a fine needle to inject DNA into recently fertilized eggs, which are then cultured and implanted into surrogate mothers. This technique, however, has proved to be rather inefficient. Successful integration of the transgene into the host genome is a hit-or-miss event—approximately 1 to 5 percent of resulting offspring carry the transgene and only a proportion of the transgenic progeny express the added gene in a desired manner and at a high level.[1] Furthermore, integration of the transgene into the host DNA is a random process, which may occur anywhere within the genome. The expression of the transgene is influenced by sequences surrounding its insertion

site; therefore random insertion may produce position effects that contribute to the unpredictable and variable expression of the transgene between transgenic founders.[2] Multiple lines of animals must therefore be tested for proper gene expression when this technique is employed. Random incorporation may also induce mutations that disrupt the function of host DNA coding sequences. Since insertional mutations are oftentimes recessive, their deleterious effects cannot be detected until the animals are bred to transgenic relatives.[3]

Pronuclear injection also leads to the generation of mosaics, which impedes growth of the transgenic herd. If the transgene is incorporated into the host genome before the zygote undergoes its first division, copies of the added gene should appear in all cells of the developing animal, including its eggs or sperm. However, if the transgene is not integrated into the host chromosome until after the zygote has divided, the added DNA will appear in some, but not all, cells of the developing animal. The resulting mosaics will produce two different kinds of germ cells—some contain the added transgene, while others do not.[4] Therefore, even if an individual animal expresses the transgene, it may not transmit the transgene to its offspring.

Various reproductive manipulations (e.g., superovulation, artificial insemination, embryo collection, and embryo transfer) are used to produce transgenic offspring and breeding animals may be repeatedly exposed to these procedures. As noted above, only a small percentage of embryos created by pronuclear injection carry the transgene of interest. In order to reduce the number of non-transgenic pregnancies developing to term, recipient cows, for example, may be subject to transvaginal amniocentesis to verify whether transgenes have integrated into the genome.[5] Non-transgenic fetuses are aborted and the surrogate reused as a recipient. While this approach limits the number of animals used as recipients, it also raises welfare concerns, as individual animals may be repeatedly subject to "procedures likely to cause pain and distress."[6] In contrast, as discussed below, only those cells exhibiting the desired genetic modification are selected to create embryos with NT. The use of NT to create transgenic animals, therefore, could eliminate the problem of repeated elective abortion and reuse of recipient animals.[7]

B. Nuclear Transfer

NT promises to facilitate genetic transformations and increase the efficiency of transgenesis to 100 percent. In pronuclear microinjection, the transgene is injected into the pronucleus of a single-celled fertilized egg; however, in NT, genetic material is transferred to cell lines in culture. After addition of the transgene, cells can be cultured further and analyzed to be certain they contain the added genetic material. The genetic material from the nucleus of the cultured donor cell can then be transferred to an enucleated recipient egg. Embryos are implanted in surrogate mothers and all animals born will be transgenic. NT also eliminates the problem of founder mosaicism. Genetic modification of the donor nucleus together with NT should introduce the genetic change into every cell of the resulting offspring, including its eggs or sperm.

An additional benefit of NT is the apparent reduction in the number of animals and surgical procedures necessary to generate founders,[8] as only those cells with the desired genetic change are selected as donor cells. Producing transgenic animals by NT uses less than half the experimental animals than does pronuclear injection. Work performed by the Roslin Institute and PPL Therapeutics between 1989 and 1996, for example, required an average of 51.4 ewes per transgenic lamb produced by pronuclear microinjection.[9] Only 20.8 ewes were required per transgenic lamb produced by NT using donor fetal fibroblasts as reported by these groups in a later study.[10] As the researchers stated, "The most important difference is that no recipients are wasted gestating nontransgenic lambs in the nuclear transfer technique."[11] Furthermore, when zygotes are used in pronuclear injection, their sex is not known. There is a 50 percent chance the resulting offspring will be male, and for certain applications (i.e., the production of biopharmaceuticals in the milk), transgenic females are desired. Selection of a female transgenic cell line for NT eliminates the possibility of male offspring.

Importantly, NT permits production of transgenic founder animals in the first generation.[12] Normally, one must wait for a transgenic animal produced by pronuclear injection to mature and reproduce. However, once a transgenic cell line has been identified that expresses a human protein at the desired level, NT permits production of a number of transgenic founders in a single step.[13] Once the founder herd or flock is established, the genetically modified animals could then breed naturally to establish a transgenic line. This factor is particularly relevant for those companies engaged in the production of pharmaceuticals in the milk of transgenic animals. By reducing the time needed to produce a founder herd or flock of lactating females, the time to large-scale protein production, clinical trials, and commercial production will also decrease. As noted by one commercial biotech company, "Where it would normally require 44 months to reach production flock status in sheep, (78 months in cows), nuclear transfer technology can reach production flock status in 18 months for sheep, (33 months for cows)."[14]

While pronuclear microinjection permits only the addition of genetic material to the zygote, cells in culture used for NT can be manipulated to not only add new genetic material, but to delete or substitute specific genes. This will prove to be of significance in the field of xenotransplantation. A very strong immune response is stimulated when pig organs are transplanted into human recipients, leading to hyperactue rejection of the transplanted organ. Pig tissues display a carbohydrate epitope that reacts with human antibodies, stimulating this immune response.[15] A targeted deletion of the gene encoding the enzyme that produces this epitope should diminish hyperacute rejection.[16]

Furthermore, gene targeting[17] helps avoid those problems associated with the random incorporation of DNA observed with pronuclear injection. The position of the gene within the genome affects expression of the transgene; therefore, pre-selection of transgenic integration sites and precise placement of transferred genetic material into the host genome permits more predictable and controlled gene expression. In

addition, the use of a clonal population of transgenic cells as nuclear donors will guarantee the same transgene insertion site for each clone, thereby decreasing animal to animal variation in transgene expression levels.[18] Finally, cultured nuclear donor cells can be frozen and used when desired to generate identical cloned transgenic offspring over a prolonged period of time.[19]

III. ETHICAL ISSUES AND ANIMAL WELFARE CONCERNS

Several general questions are raised in an examination of ethical issues in the field of animal transgensis and cloning: Does animal cloning in pharmaceutical and biomedical research raise new ethical questions or are previously introduced issues being re-examined in the context of a new, developing technology? If our concerns are similar to those elicited by other uses of animals in pharmaceutical research, does cloning intensify or heighten these concerns? If we find there are objections that are unique to cloning for pharmaceutical and biomedical research, do those concerns stem from the nature of cloning itself, or do they arise from the consequences—potential harms and benefits—of this research? Finally, will routine cloning and mass production of genetically identical copies have a negative impact on the value of animal life? In other words, will cloning further advance the commodification of living creatures? Many ways in which our society currently uses animals promote commodification. Therefore, we may again want to ask whether cloning exacerbates this concern more so than other uses of animals.

A. Pharming

Biopharming refers to the production of pharmaceuticals from genetically modified plants or animals. Although the focus of this discussion will be on the production of human proteins in the milk of transgenic animals, there is also the potential for the production of pharmaceuticals in the urine, blood, or eggs.

A number of therapeutically valuable human proteins can be produced in the milk of transgenic sheep, goats, cattle, and even rabbits and pigs. Examples include human factor IX, used to treat hemophilia B[20]; alpha-1-antitrypsin to help counteract lung damage in patients suffering from emphysema and cystic fibrosis[21]; and antithrombin, a plasma protein with anticoagulant and anti-inflammatory properties.[22] Secretion of human proteins in the milk of transgenic animals has resulted in increased volume output and lower cost per unit as compared to traditional cell culture systems.[23] It has been estimated that several hundred transgenic pigs could provide enough factor IX to treat all the world's hemophiliacs;[24] and, theoretically, a herd of 600–700 transgenic cows could produce quantities of human serum albumin that would satisfy worldwide demand.[25] A second advantage over the use of cell culture systems is that the mammary gland is capable of producing complex proteins that require posttranslational modifications for full bioactivity. Finally, proteins produced in the milk of transgenic animals are free of potentially infectious agents that may be associated with human blood products.

The creation of transgenic animals that produce human therapeutic proteins in their milk appears to offer significant human benefit with arguably minor intervention in the animal—particularly once a herd or flock of founder animals has been established. Performance of a cost/benefit analysis is more difficult, however, in fields of animal biotechnology, "because the costs and benefits will be experienced by two different groups with different interests—human beings and animals."[26] There are also disparate subdivisions in our society that perceive and value the risks and benefits to humans and animals differently. Given these diverging value systems, the weighing of risks and benefits can vary and the ultimate outcome will depend upon which group performs the analysis.

Most people would likely agree that animals do have interests not to be caused pain and suffering. The question remains whether, and to what extent, these interests may be sacrificed for human interests.[27] Furthermore, in assessing potential risks to animals, all intermediate steps and all animals used in the creation of a founder animal must be considered in addition to the final genetically modified sheep, goat, pig, or cow. Reproductive procedures including administration of drugs to donor animals to induce superovulation, retrieval of donor eggs, and implantation of genetically modified embryos into surrogate mothers, as well as the accompanying stress of handling and post-operative pain must all enter the final analysis. This has been termed "procedural distress," and contrasts to other forms of animal experimentation in that several generations of animals may be subjected to pain, distress, and suffering during production of the final model.[28]

Some contend that NT is simply an extension of selective breeding that has been practiced throughout history. This argument, however, seems to imply that the status quo is an ethically acceptable standard, and it is from this baseline that new developments in animal biotechnology should be judged.[29] Many would argue that there are ethical objections to conventional breeding practices that have serious animal welfare implications. For example, turkeys are bred with such large breasts that they cannot naturally breed, and double muscling in cattle has led to problems during calving. Although man has consistently altered the genetic makeup of animals through selective breeding, and species change naturally through evolution, notable differences do exist between these "natural" events and direct genetic modification. First, transgenesis and cloning permit the transfer of genes between widely different species, while it is exceedingly difficult to cross species boundaries in selective breeding. The production of sheep and goats containing human genes that code for the production of human proteins in their milk, for example, could never be accomplished through selective breeding. Second, often unpredictable and extreme genetic changes may occur rapidly, in a single generation, providing little time to observe potentially deleterious effects upon the animals.[30] Selective breeding, in contrast, is a more gradual process that allows changes in animals to be observed and monitored over several generations. Finally, as with any developing technology, scientists and researchers do not have the benefit of previous experience and scientific knowledge, and unforeseen outcomes may generate substantial animal

suffering. This presents a difficult challenge to animal use committees and ethics committees evaluating proposed research protocols.

In general, the efficiency of reproductive cloning in animals remains low and cloned animals produced by NT have displayed a variety of anatomical and physiological abnormalities, high birth weight, and high pre- and postnatal mortality.[31] Other cloned animals do, however, appear quite normal.[32] Pregnancy complications can cause fetal loss and also result in increased morbidity and mortality in surrogate mothers.[33] Increased size of the fetus causes distress to both mother and fetus during parturition and often necessitates a C-section delivery. Researchers should take steps to document the health, physiology, and behaviors of their cloned animals, and studies should continue throughout the animals' life span.[34] Since the effects of genetic manipulation may not be apparent at all stages of life, animals must be studied at different stages, including the oldest age likely to be reached during usage.[35] Even those cloned animals that appear normal in the early stages of life should be monitored as they age and reproduce[36] as unanticipated side effects may not appear for several generations. Finally, it is important to monitor transgenic animals producing human proteins in their milk to determine if excessive production of an unnatural protein may cause any chronic health problems.[37]

One argument that calls into question the genetic modification of animals focuses not on the technology employed, but on the effect the transgene may have on the physical or physiological state of the animal. This appears to be a valid objection, particularly if the animal's metabolism is changed in a way that is not in the animal's interest or the protein is expressed in an organ or at a level that results in harm to the animal.[38] Bernard Rollin has set forth the "principle of conservation of welfare" as the standard of welfare for agricultural biotechnology, which states,

> Genetic engineering should not be used in ways that increase or perpetuate animal suffering. . . . Any animals that are genetically engineered for human use . . . should be no worse off, in terms of suffering, after the new traits are introduced into the genome than the parent stock was prior to the insertion of the new genetic material.[39]

Applying the principle of conservation of welfare to transgenesis in biomedical research, it is important to note that the methods utilized in transgenesis do not necessarily have an adverse effect on animals' welfare; however, successful integration and expression of the transgene may negatively impact animal welfare.[40] To illustrate this point, certain human proteins produced in the milk of transgenic animals may cause harm to the animal if the transgene is expressed ectopically or the recombinant protein leaks from the mammary gland to the blood.[41] For example, a transgenic cow containing the gene for human erythropoietin has been created but never allowed to produce milk because studies have shown that human erythropoietin can have fatal effects in mice when it circulates in the blood.[42]

This example demonstrates potentially fatal consequences for a transgenic animal. However, as valuable human proteins proceed through clinical trials and are exploited commercially, will less severe effects on animal welfare be tolerated?

In pigs, for example, there has been evidence of abnormal mammary development due to expression of the transgene, which may have caused painful lactation.[43] Will financial incentives overtake concerns for the proper care and welfare of transgenic animals and will animal suffering of this magnitude be viewed acceptable? Will we be inclined to push transgenic animals even harder by increasing the frequency of milking or length of lactation? High rates of milk production in dairy cows have been related to an increased incidence of mastitis. Will this risk to the transgenic animal be recognized in a welfare analysis?

B. Xenotransplantation

Xenotransplantation is the transfer of cells, tissues, or whole organs from one species to another. The shortage of human organs and tissues available for transplantation is the most notable rationale given for xenotransplantation. It is estimated, for example, that there are currently over 17,000 patients awaiting liver transplants in the U.S.[44] In response to the shortage of human organs available for transplantation, researchers have expressed interest in using organs from animals to treat human patients. Pigs are favored as a potential source of transplantable organs because their organs are physiologically similar to those of humans; porcine organs are of an appropriate size; and pigs reproduce quickly and give birth to a large number of offspring.[45] An immediate immunological barrier to xenotransplantation, however, is the hyperacute rejection reaction provoked by porcine organs transferred to human recipients.[46] Pigs, therefore, must be genetically altered before their organs can be transferred to humans. NT is regarded as a means of introducing genetic modifications into an appropriate strain of pigs, in an attempt to combat rejection mechanisms. Researchers hope that cloning will enable them to knock out the pig gene that triggers rejection by the human body, as well as insert human genes more accurately to "humanize" pig organs to counter other human defense mechanisms.[47]

Additional animal welfare concerns related to xenotransplantation include the manner in which pigs are housed, handled, and treated prior to slaughter and organ retrieval. To minimize the risk for transmission of pathogens to human recipients, specific pathogen free (SPF) pigs are used as organ sources. In order to obtain SPF pigs, the pregnant sow is anesthetized shortly before she is to give birth and the entire uterus containing the piglet embryos is removed in a sterile "bubble."[48] (Alternatively, piglets may be born by cesarean section.) Piglets are then reared in isolation for fourteen days and the sow is typically slaughtered. Pigs are intelligent, social, and highly inquisitive animals and it has been demonstrated that piglets subjected to extremely early weaning, as is the case with SPF pigs, develop abnormal behaviors.[49]

Pigs also develop abnormal behaviors in confinement if not given the opportunity to root or build nests.[50] This suggests additional welfare issues since pigs intended for use as organ sources might be housed in extremely barren environments that are easily sanitizable.[51] The UK's Home Office Code of Practice for organ-source

pigs recommends that pigs be housed in stable social groups, provided adequate space to move around freely, and provided environmental enrichment, such as straw or other materials for manipulation, to satisfy pigs behavioral needs in terms of rooting and investigative behavior.[52] While it is recognized that the requirement to maintain SPF status may compromise the animal's behavioral needs to some extent, justification is needed if such a compromise becomes essential for a xenotransplantation protocol. The National Research Council has noted, "There are no comparable standards for pigs intended for xenotransplantation in the U.S., and the lack of standardization of housing and care among U.S. facilities for these pigs is a source of concern."[53]

As mentioned previously, pigs are highly inquisitive and intelligent animals. An important question to ask is: Will transgenic organ source pigs be kept isolated and confined in sterile environments, "with no opportunity to fulfill their behavioral and psychological needs?"[54] Furthermore, should the psychological suffering (i.e., frustration, anxiety, loneliness, boredom, fear) of transgenic animals, such as pigs, be acknowledged in a discussion of welfare issues? In addressing this concern, we may draw on the concept of an animal's telos, a term adapted from Aristotle's philosophy and defined by Bernard Rollin as,

> [T]he set of needs and interests which are genetically based, and environmentally expressed, and which collectively constitute or define the 'form of life' or way of living exhibited by that animal, and whose fulfillment or thwarting matter to the animal. The fulfillment of telos matters in a positive way, and leads to well-being or happiness; the thwarting matters in a negative way and leads to suffering.[55]

In this context, to prevent animal suffering and enhance happiness means that attention must be paid to more than the physical—the "behavioural, functional and cognitive drives"[56] of an animal of a given species are additional factors to be recognized in evaluating its welfare. Because pigs are naturally social animals, to isolate such an animal "does not cause it physical pain, but can cause psychological suffering because its telos is being ignored or violated."[57] Similarly, Gary Comstock, director of the Research Ethics Program at North Carolina, has also addressed the concept of "respecting" animals. He contends, "[t]he key to respecting animals, . . . , is respecting their right to satisfy their primary desires."[58]

Another phenomenon to examine in the area of xenotransplantation is gradualism, "in which progressive increments are gradually made in an area of technology, each step being justified on the basis that it represents only a small change from the last."[59] However, when the overall change is evaluated after a period of time has passed, it may appear that an unacceptable change has taken place when compared with the original starting point. Consider, for example, the use of porcine heart valves in human patients, which has become common practice. Simply because this particular use of pig heart valves is generally accepted, does not mean we should condone all other uses of these animals. A rather significant leap is made from the acceptance of pig valves to the idea that whole

animal organs may be transplanted to humans; however it may be presented as simply another small step. The human benefits of xenotransplantation are still largely potential, but even if xenotransplantation becomes medically feasible, it does not answer the question whether it is ethically acceptable. We must refer each step back to more fundamental values and focus not only on what the next step represents in its similarity to the last step. What is required is that we "step back and look at the complete sequence of steps and ask if the final end is in fact acceptable."[60]

Finally, an argument set forth in favor of raising pigs to supply organs is the so-called "ham sandwich" argument. Throughout history, animals have been raised as a source of labor, food, and clothing. It is estimated, for example, that 94.5 million pigs were born, raised, and slaughtered in the U.S. in the year 2002.[61] Since we slaughter pigs for food in order to live, how can we object to raising them and killing them for organs to save human life? One response to this question has been presented as a "naturalness" argument, which has been set forth as follows: All would agree that everyone must eat in order to live. Although it is debatable whether humans must eat animals, it could be said that it is "natural" to do so. This argument does not necessarily extend to the transplantation of animal organs to humans. Organ replacement has become possible only through human skill and scientific and medical advances. "It is not natural to use an animal as a spare part [supplier]. It is human artifice." [62] Although not arriving at the conclusion that it is wrong to use an animal in this way, the authors propose that "in ethical terms it is not the same as eating an animal."[63]

C. Models for Human Genetic Diseases

The third area in which NT will impact the generation of transgenic animals is the creation of animal models for human genetic diseases. This area poses perhaps the most serious animal welfare concerns since gene targeting and cloning may allow production of models for many debilitating human genetic diseases. Transgenic animal models will likely have no option but to suffer, no matter what the end. Is the pain and suffering of the animal justified by the potential benefit to human beings, or does this intervention fall into the category of "harms of a certain degree which ought under no circumstances to be inflicted on an animal?"[64]

Although some contend there is no ethical difference between chemically or surgically inducing a disease condition in a laboratory animal and modifying its genetic structure so as to cause it to develop a particular human disease, it appears as though certain differences do exist. Transgenic animals created to model human genetic diseases will be genetically programmed to suffer the effects of disease from birth. In contrast, those animals with disease conditions created in the laboratory will suffer effects of the disease only from the time it is actually induced.[65] Second, the advantages put forth to support genetic inducement of disease are the reliability and repeatability of the effect.[66] These factors themselves almost guarantee that the animal will suffer adverse effects of the disease condition they are created to

model. If an experiment does not proceed for some reason, i.e., lack of continued funding, genetically altered animals will develop the disease condition and likely suffer symptoms even though the study has not proceeded and no useful data has been generated; whereas, experiments using animals whose disease condition has been chemically or surgically induced can be curtailed and additional animal suffering can be avoided. Finally, it has been suggested that moral questions arising from the development of animal models by transgenesis do not differ in kind from the questions arising from the development of models by chemical or surgical inducement, however they do differ in degree. "Transgenics provides the potential for generating vast numbers of animals modeling genetic disease and other diseases with devastating symptoms."[67]

Many researchers support the use of NT to create genetically uniform animal models of human diseases. They contend that these animals are more accurate disease models and therefore should generate more precise and reliable data. When scientists test a certain chemical agent or medical procedure, they will know that differences in test results are due to the procedure or drug, and not to genetic differences between the research animals.[68] As a result, the number of animals needed for research should decrease. Others however disagree.

First, it has been suggested that the total number of animals used in research may actually increase because there are thousands of genetic diseases that may potentially be created in animals. Second, the argument that the number of animals used in experimentation would decrease was the initial justification for the use of genetically engineered model mice in the early 1990s. Evidence shows, however, that there has been a significant increase in the number of mice used in research since the development of transgenic technologies. It appears that all over the world, research centers and animal facilities are filled to capacity with mutant mice and some laboratories are forced to turn down applications for storage simply because of lack of space and funding.[69] Rats and mice are exempt from the Animal Welfare Act and no government agency in the United States requires the reporting of mice numbers used in research. However, one author estimates that the number of mice and rats used in research increased from approximately eleven million to nineteen million in 1993 to eighty million in 2001.[70] Today, genetically transformed laboratory mice can be ordered on-line or via toll-free numbers as though they are mere items listed in a catalogue.[71] Referring back to the concept of gradualism first explored in the discussion of xenotransplantation, "having developed a culture which sees the use of disease model mice as a norm, the progressive extension of this could exceed ethical bounds by imperceptible steps."[72]

Transgenic technologies have contributed greatly to the production of mouse models of human diseases; however, mice sometimes fail to provide a complete model of the human phenotype. The term "phenotype gap" has been used to refer to the gulf between mouse mutant strains available for study and the full range of phenotypes necessary to exploit the mouse as an animal model.[73] Differences in human and mouse life span as well as differences in anatomy and physiology have

contributed to the failure to produce a mouse model that resembles certain human diseases.[74] Certain livestock species are considered better models because they appear to be more similar to humans with respect to size, anatomy, physiology, and life span.[75] For example, mouse models of cystic fibrosis fail to exhibit the same lung pathology seen in humans, and researchers have turned to the sheep as a potential animal model.[76] Other scientists have turned toward creating a porcine or bovine model of the genetic disease ataxia-telangiectasia, as mice do not display the neurodegenerative phenotype seen in humans.[77] Using larger animals as models may raise greater welfare concerns than the use of smaller animals, such as rodents.

Additional limitations of mouse models for various neurogenetic disorders[78] and neurodegenerative diseases[79] have been identified. First, a mouse ortholog to a human gene of interest may not exist.[80] Second, as mentioned above, mouse models do not exhibit the same phenotype observed in humans.[81] Mouse models may exhibit only some symptoms of the disease observed in humans, or they may exhibit no symptoms at all. Furthermore, there are limited cognitive and behavioral tests available for rodents, which may not be applicable to the study of neurodegenerative diseases.[82] As a result, some researchers contend that non-human primates (NHPs) are necessary to study these neurological disorders because mouse models are simply not suitable.[83] In contrast to mouse brains, NHP brains are more complex and display greater similarities to the human brain. Rhesus macaque models are favored because these NHPs display "perceptual, cognitive and behavioral plasticity not observed in mice."[84] The qualities that make NHPs more desirable as models for these diseases are the same qualities that give rise to greater welfare concerns. NHPs are more sentient beings and have higher cognitive capacities and engage in more complex social interactions than small rodent research models.

Although the production of genetically modified cloned NHPs still poses significant challenges, a team at the University of Pittsburgh has made significant steps toward successful therapeutic cloning of nonhuman primate embryos with the hope of producing embryonic stem cells.[85] These researchers are also working towards cloning nonhuman primates as a way to generate genetically uniform animals for experimentation.[86] If researchers are successful in overcoming the obstacles encountered in NHP cloning, concerns arise that the number of primates used in research will increase as we are able to generate models of more and more human genetic diseases. The use of NHPs may rise significantly if they become exploited on a long-term and widespread basis. Not only will these animals suffer debilitating disease symptoms, but the difficulty in satisfying the social and behavioral requirements of NHPs in the laboratory setting will add to their potential for psychological suffering.

The severity of symptoms of many genetic disorders must also enter a welfare analysis. Lesch-Nyhan Syndrome, for example, is a rare genetic disorder caused by a deficiency of the enzyme HPRT.[87] Symptoms of the disease include joint pain, kidney problems, muscle weakness, and uncontrolled spastic muscle

movement.[88] The most striking aspect of the disease is the development of self-mutilating behaviors, such as lip and finger biting, which begin in humans during the second year of life.[89] While there is certainly the potential for significant human benefit arising from the study of this disease, there is a concern over the welfare of animals used as models. Again, researchers may turn to NHP models of this syndrome since mice containing the genetic mutation which leads to Lesch-Nyhan Syndrome do not demonstrate the phenotype typical of this neurogenetic disorder in humans.

Patients diagnosed with Lesch-Nyhan Syndrome do not exhibit symptoms from birth, but develop them later. Death usually occurs in the first or second decade of life due to kidney failure.[90] Therefore, in order for researchers to study the full course of the disease, animals will need to be kept alive for as long as possible. It does not seem possible to study these diseases in acute, terminal, or short-term experiments,[91] and the potential exists for a considerable amount of animal pain, distress, and suffering as these animals will show symptoms displayed by humans with the same syndrome over a long period of time. Although studying diseases such as LN syndrome may benefit humans, it should not be forgotten that the increased use of TG technologies to model human genetic diseases in research animals has its costs.

IV. COMMODIFICATION AND OBJECTIFICATION

Even the absence of welfare problems in transgenic animals, however, does not necessarily imply the absence of a moral problem. In addition to respect for nature, and respect for the natural way, other arguments stem from the mass production of identical genetic copies. Some authors argue that cloning may further dilute the "essence" in copies of the same creature.[92] Similar concerns have been expressed by others, who suggest that NT will encourage animals to be treated increasingly as commodities and will negatively impact the value of animal life. In our market economy, the perceived value of consumer goods decreases as they are produced on a large scale and the number of identical copies increases. As one author has stated in reference to routine cloning for animal production: "To clone routinely would apply a factory model of mass production too far into the realm of living creatures. We need to remind ourselves we are not dealing with identical widgets on a production line, but living creatures, useful to us, but still creatures."[93] The same author notes, however, that small scale special cloning, i.e., use of NT to produce five to ten founder transgenic animals that would then breed naturally, lacks something of the "instrumentality" of other cloning applications.[94] The primary aim of such work is not to clone as such, but to more efficiently perform a genetic modification that could not occur naturally.

A final consideration centers on the choice of terminology used to characterize transgenic animals. The use of phrases such as "living bioreactor" to refer to transgenic animals whose mammary function is used for the production of human proteins, and "spare parts supplier" to describe transgenic pigs whose organs may

be used for xenotransplantation, depicts an extremely instrumental view of these animals. The use of this terminology may contribute to even greater objectification of living animals. As stated by one working group,

> In calling an animal a bioreactor, or spare part supplier, it is described primarily for what it is functionally—as a means to an end—not what it is as an animal. . . . By extrapolating from concepts of the factory, a statement is made that [animals] are more closely related to the non-living world than the living.[95]

Such an instrumental view, which depicts transgenic animals more as production machines than living creatures, conveys a degree of disrespect for these animals. Continued use of these terms may cause them to become more commonplace, affirm questionable attitudes and truncate the ethical debate. Will word choice be responsible for a slow indoctrination and assimilation of such viewpoints? Widespread acceptance of such language may contribute to a sense of normality, obfuscate ethical questioning, and lead to fear of having the insensitivity of the terminology hived on to public acceptance.

V. CONCLUSION

Advances in NT are proceeding at a rapid pace. Accompanying these great strides is the potential for significant animal suffering. Society's views of non-human animals continue to evolve and animal welfare issues concern a growing percentage of our population. Consequently, the impact of emerging technologies on animal welfare will likely influence public acceptance of new scientific breakthroughs. NT offers significant advantages over pronuclear injection for the generation of transgenic animals. Nonetheless, concerns remain regarding both the animals' physical and psychological well-being. Cloning raises issues regarding the increased objectification and commodification of living creatures. Although not unique to the applications of cloning technology discussed above, these matters demand further examination. Importantly, society's view on what is ethically acceptable can change over time. Therefore, it is essential that we revisit old questions, raise new ones, and reassess often as cloning research progresses and animals created by NT are monitored over longer periods of time. The ethical dialogue must remain dynamic as science progresses and the public must be engaged as well as educated. As experience with this technology increases and information is gathered, it should be possible to better anticipate risks and benefits to both humans and non-human animals.

NOTES

1. A. Dove, "Milking the Genome for Profit," *Nature Biotechnology*, 18 (2000) pp. 1045–1049.

2. A. Colman, "Somatic Cell Nuclear Transfer in Mammals: Progress and Applications," *Cloning*, 1 (1999/2000) pp. 185–200.

3. National Research Council. *Animal Biotechnology: Science-based Concerns*, pp. 43, 97 (The Nat'l Academies Press 2002).

4. See I. Wilmut, K. Campbell, and C. Tudge. *The Second Creation: Dolly and the Age of Biological Control* (Farrar, Straus and Giroux 2000) p. 34 (hereinafter *The Second Creation*).

5. M. F. Brink, et al. "Developing Efficient Strategies for the Generation of Transgenic Cattle which Produce Biopharmaceuticals in Milk," *Theriogenology*, 53 (2000) pp. 139–148.

6. National Research Council, *supra* note 3, at p. 94.

7. Ibid.

8. See E. Behboodi, et al. "Transgenic Cloned Goats and the Production of Therapeutic Proteins," in *Principles of Cloning* (Elsevier Science, 2002) pp. 459–474.

9. *The Second Creation*, *supra* note 4, at pp. 237–238.

10. Schnieke, A., et al. "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts," 278 *Science*, pp. 2130–2133 (1997).

11. Ibid.

12. S. L. Stice, et al. "Cloning: New Breakthroughs Leading to Commercial Opportunities," *Theriogenology*, 49 (1998) pp. 129–138.

13. Dove, *supra* note 1, at p. 1046.

14. PPL Therapeutics, "What We Do: Nuclear Transfer," available at http://www.ppl-therapeutics.com/what/what_2_content.html.

15. Dove, *supra* note 1, at p. 1047.

16. Ibid.

17. National Research Council, *supra* note 3, at p. 97. Gene targeting is defined as the controlled integration of transgenes into specific, predetermined locations within the genome.

18. Craig A. Hodges and Steven L. Stice, "Generation of Bovine Transgenics Using Somatic Cell Nuclear Transfer," *Reproductive Biology and Endocrinology*, 1 (2003), available at http://www.rbej.com/content/1/1/81.

19. L. M. Houdebine, *Animal Transgenesis and Cloning*, p. 73 (John Wiley & Sons Ltd. 2003).

20. I. Wilmut, "Cloning for Medicine," *Scientific American*, (Dec. 1998) pp. 58–63.

21. PPL Therapeutics, "Products: Alpha-1-Antitrypsin," available at http://www.ppl-therapeutics.com/products/products_1.html.

22. GTC Biotherapeutics, "ATryn®—Recombinant Human Antithrombin," available at http://www.transgenics.com/products/atryn.html.

23. Dove, *supra* note 1, at p. 1046. ("A transgenic goat, for instance, produces protein at a unit cost of $10–25/g compared with $100–1000/g for cell culture.")

24. "Biotech in the Barnyard: Implications of Genetically Engineered Animals." Proceedings from a workshop sponsored by the Pew Initiative on Food and Biotechnology, available at http://www.pewagbiotech.org/events/0924/proceedings1.pdf.

25. Christopher Bowe, "Biotech Companies Plan to Milk Herds of Cloned Cows for Human Drug Needs," *Financial Times*, Oct. 13, 1999, World News: U.S. and Canada, at p. 6.

26. R. Straughan, "Ethics, Morality and Animal Biotechnology," available at http://www.bbsrc.ac.uk/tools/download/ethics_animal_biotech/ethics_animal_biotech.pdf. See also, "Working Group of the Society, Religion and Technology Project, Church of Scotland," *Engineering Genesis: The Ethics of Genetic Engineering in Non-Human Specie*, ed. Donald and Ann Bruce, (Earthscan 1998) p. 103 [hereinafter *Engineering Genesis*]. It has been proposed that the term "risk-benefit" analysis is preferable to "cost-benefit" analysis, since the latter suggests monetary evaluation and economic worth, and "Ethical assessment can never be quantitative in this sense."

27. "Genetic Engineering: Animal Welfare and Ethics," a discussion paper from the *Boyd Group* (Sept. 1999), available at http://www.boyd-group.demon.co.uk/genmod.htm [hereinafter *Boyd Group*].

28. A. Van't Hoog, et al., "Dolly's Deceiving Perfection: Biotechnology, Animal Welfare, and Ethics," *J of Applied Animal Welfare Science*, 3 (2000) pp. 63–69.

29. *Engineering Genesis*, *supra* note 26, at p. 91.

30. Ibid. ("Direct modification of the genome can produce more novel, surprising and wide-ranging phenotypic effects, with greater potential to compromise welfare, in one step."). Pronuclear injection is less precise than NT, as the transferred genetic material is randomly integrated into the recipient animal's genome. Therefore, the unpredictability of adverse effects on animal welfare is arguably a more relevant concern when microinjection is used to create transgenic animals.

31. *Scientific and Medical Aspects of Human Reproductive Cloning* (National Academy Press, 2002) p. 40. See also, F. B. Garry, "Postnatal Characteristics of Calves Produced by Nuclear Transfer Cloning," *Theriogenology*, 45 (1996) pp. 141–152. Anatomical and physiological abnormalities include lung and cardiovascular problems, joint and limb abnormalities, immune system dysfunction, and liver and kidney problems.

32. See, Robert P. Lanza, et al., "Cloned Cattle Can Be Healthy and Normal," *Science*, 294 (2001) pp. 1893–1894.

33. *Scientific and Medical Aspects of Human Reproductive Cloning*, *supra* note 31, at p. 40. Pregnancy complications include abnormal placentation, pregnancy toxemia, and hydroallantois.

34. I. Wilmut, "Are there any Normal Cloned Mammals?" *Nature Medicine*, 8 (2002) pp. 215–216.

35. D. Broom, "The Effects of Biotechnology on Animal Welfare," in *Animal Biotechnology and Ethics*, ed. Alan Holland and Andrew Johnson (Chapman & Hall 1998) p. 73.

36. J. Cibelli, et al. "The Health Profile of Cloned Animals," *Nature Biotechnology*, 20 (2002) pp. 13–14.

37. A. George, "Animal Biotechnology in Medicine," in *Animal Biotechnology and Ethics*, ed. Alan Holland and Andrew Johnson (Chapman & Hall 1998) 32.

38. *Engineering Genesis*, *supra* note 26, at pp. 135–136.

39. B. E. Rollin, *The Frankenstein Syndrome: Ethical and Social Issues in the Genetic Engineering of Animals* (Cambridge University Press 1995) p. 179 [hereinafter *The Frankenstein Syndrome*].

40. See Paul B. Thompson, "Research Ethics for Animal Biotechnology," in *Ethics for Life Scientists*, ed. M. Korthals and R. J. Bogers (Springer 2004) pp. 112–113.

41. L. M. Houdebine, "Transgenic Animal Bioreactors," *Transgenic Research*, 9 (2000) pp. 305–320.

42. *Engineering Genesis*, *supra* note 26, at p. 118. To eliminate or reduce potentially deleterious side effects, a genetic change can be limited to certain tissues of interest. See M. B. Dennis, "Welfare Issues of Genetically Modified Animals," *ILAR J*, 43 (2002) pp. 100–109. Milk-specific gene promoters are used to direct expression of the human gene only in the mammary gland of the transgenic animal.

43. National Research Council, *supra* note 3, at p. 102.

44. "U.S. Transplantation Data. United Network for Organ Sharing Data," available at http://www.unos.org/data/default.asp?displayType=usData.

45. *The Second Creation*, *supra* note 4, at 255.

46. See J. A Platt, "Primer on Xenotransplantation," in *Xenotransplantation 8*, ed. Jeffrey L. Platt (ASM Press 2001).

47. D. M. Bruce, "Ethics Keeping Pace with Technology," in *Beyond Cloning: Religion and the Remaking of Humanity*, ed. Ronald Cole-Turner (Trinity Press Int'l 2001) pp. 34–49. As noted above, *supra* p. 6, pig tissues display a carbohydrate epitope that reacts with human antibodies, stimulating hyperacute rejection of the transplanted organ. Researchers hope to lessen this immune response by deleting the gene encoding the enzyme that produces this epitope. One researcher has noted, however, "the [carbohydrate] structure may provide some essential biological function in pigs and thus destroying the alpha-1,3 GT enzyme could be deleterious to the animals." Dove, *supra* note 1, at 1047, quoting Irina Polajaeva (former head of the Cell Biology Group and project manager for the Porcine Nuclear Transfer Program at PPL Therapeutics Inc.). This reemphasizes the need for long-term monitoring of modified animals.

48. J. D'Silva, "Campaigning against Transgenic Technology," in *Animal Biotechnology and Ethics* 98, ed. Alan Holland and Andrew Johnson (Chapman & Hall 1998).

49. National Research Council, *supra* note 3, at p. 103, citing D. M. Weary, et al., "Responses of Piglets to Early Separation from the Sow," *Applied Animal Behavior Science*, 63 (1999) pp. 289–300. See also, Alexander Tucker, et al., "The Production of Transgenic Pigs for Potential Use in Clinical Xenotransplantation: Microbial Evaluation, in *Xenotransplantation*, 9 (2002) pp. 191–202. These authors described custom built nursery isolator tanks designed to provide a germ free environment while attempting to minimize any impact on piglet behavior or welfare. The isolator tanks were designed to hold five piglets each and provided a heated enclosed bed area, feeding area, a storage area, and piglet segregation area. The tanks were constructed of stainless steel with PVC canopies. Unopened bags of sterile water were positioned in the bed area to provide comfort and a nosing substrate, thereby minimizing behavioral stereotypes such as umbilicus sucking and belly nosing.

50. National Research Council, *supra* note 3, at p. 103.

51. Ibid. See also, Nuffield Council on Bioethics, "Animal-to-Human Transplants: The Ethics of Xenotransplantation," (March 1996), at p. 64, available at http://www.nuffieldbioethics.org/fileLibrary/pdf/xenotransplantation.pdf. (5.20 "Even if isolation is not required, in order to keep animals free from infection, the environment will have to be kept relatively sterile and therefore be easy to clean. So it is likely to consist of monotonous textures and to be free of items which might enrich the life of the animal, but which might also harbour infectious organisms. Human contact, which can be advantageous for animals in captivity, may have to be minimized since human beings harbour some diseases (such as influenza) that can be passed on to pigs.")

52. Her Majesty's Government, "Home Office Code of Practice for the Housing and Care of Pigs Intended for Use as Xenotransplant Source Animals" (2000), available at http://www.homeoffice.gov.uk/docs/xenopig.pdf.

53. National Research Council, *supra* note 3, at p. 103.

54. *The Frankenstein Syndrome*, *supra* note 39, at p. 195.

55. B. Rollin, "On Telos and Genetic Engineering," in *Animal Biotechnology and Ethics* 162 (1998), ed. Alan Holland and Andrew Johnson (Chapman & Hall).

56. See Thompson, *supra* note 40, at p. 114.

57. Straughan, *supra* note 26.

58. *Biotech in the Barnyard*, *supra* note 24.

59. See *Engineering Genesis*, *supra* note 26, at p. 80.

60. Ibid., p. 137.

61. National Pork Producers Council, "Issue paper #4," June 5, 2003, available at http://www.nppc.org/issue_brief/2003/brief_060503.html.

62. *Engineering Genesis*, *supra* note 26, at p. 137.

63. Ibid.

64. M. Banner, "Report of the Committee to Consider the Ethical Implications of Emerging Technologies in the Breeding of Farm Animals" (Banner Report). Ministry of Agriculture, Fisheries and food, HMSO, London (1995).

65. See Boyd Group, *supra* note 27.

66. *Engineering Genesis*, *supra* note 26, at p. 139.

67. *The Frankenstein Syndrome*, *supra* note 39, at p. 204.

68. *The Second Creation*, *supra* note 4, at p. 245.

69. J. Knight and A. Abbott, "Full House, *Nature*, 417 (2002) pp. 785–786.

70. L. Carbone, *What Animals Want: Expertise and Advocacy in Laboratory Animal Welfare Policy* (Oxford University Press 2004) p. 27. See also, Dennis, *supra* note 42, at pp. 102–103. The author noted an increase in mouse populations at the University of Washington of more than 23 percent per year during the eight year period from 1993–2001. In addition to the increase in numbers of animals on studies, the author attributes this growth in numbers of mice used in research to the following: First, a large number of animals are necessary to create each genetically modified line which is then bred to produce animals to study. Second, in order to maintain a genetically modified line, it is necessary to continue breeding animals

that are not studied. These animals have compromised health but do not produce any data directly.

71. See The Jackson Laboratory, Jax® Mice Online Order Request Form, available at https://secureweb.jax.org/jaxmice/order/orderform.pdf.

72. *Engineering Genesis*, *supra* note 26, at p. 70.

73. Steve D. M. Brown and J. Peters, "Combining Mutagenesis and Genomics in the Mouse—Closing the Phenotype Gap," *Trends in Genetics*, 12 (1996) pp. 433–435.

74. Kelly S. Swanson, et al., "Genomics and Clinical Medicine: Rational for Creating and Effectively Evaluating Animal Models," *Experimental Biology and Medicine*, 229 (2004) pp. 866–875.

75. Alison J. Thomson and Jim McWhir, "Biomedical and Agricultural Applications of Animal Transgenesis," *Molecular Biotechnology*, 27 (2004) pp. 231–244.

76. Ann Harris, "Towards an Ovine Model of Cystic Fibrosi"s, *Human Molecular Genetics*, 6 (1997) pp. 2191–2193.

77. Hodges, *supra* note p. 18. See also, Swanson, *supra* note 74, at pp. 870–871.

78. Robert B. Norgren, "Creation of Non-human Primate Neurogenetic Disease Models by Gene Targeting and Nuclear Transfer," *Reproductive Biology and Endocrinology*, 2 (2004) pp. 40–48, available at http://www.rbej.com/content/2/1/40. Examples of neurogenetic disorders include Kallman's syndrome, Lesch-Nyhan's disease, and Ataxia-Telangiectasia.

79. Anthony W. S. Chan, "Transgenic Nonhuman Primates for Neurodegenerative Diseases," *Reproductive Biology and Endocrinology*, 2 (2004) pp. 39–46, available at http://www.innovitaresearch.org/news/04082501.html. Examples of neurodegenerative diseases include Parkinson disease, Alzheimer's disease, and Huntington disease.

80. Norgren, *supra* note 78. See, B. Alberts, et al., "Molecular Biology of the Cell" 4th ed. (Garland Science, New York 2002) p. 22. An ortholog has been defined as, "Genes in two separate species that derive from the same ancestral gene in the last common ancestor of those two species."

81. Norgren, *supra* note 78.

82. Chan, *supra* note 79.

83. See Norgren, *supra* note 78. See also, Chan, supra note 79.

84. Norgren, *supra* note 78.

85. "Efforts to Clone Primates Move Forward: Results Represent Significant Development toward Therapeutic Cloning of Stem Cells," *UPMC News Bureau*, available at http://newsbureau.upmc.com/PDF/SchattenPrimateCloneStudy2004.pdf.

86. Ibid.

87. National Institute of Neurological Disorders and Stroke, NINDS Lesch-Nyhan Syndrome Information Page, available at http://www.ninds.nih.gov/disorders/lesch_nyhan/lesch_nyhan_pr.htm.

88. National Organization for Rare Disorders, Lesch-Nyhan Syndrome, available at http://www.rarediseases.org/search/rdbdetail_abstract.html?disname=Lesch%20Nyhan%20Syndrome.

89. National Institute of Neurological Disorders and Stroke, *supra* note 87.

90. Ibid.

91. *The Frankenstein Syndrome*, *supra* note 39, at p. 204.

92. Van't Hoog, *supra* note 28, at p. 65.

93. D. Bruce, "Polly, Dolly, Megan, and Morag: A View From Edinburgh on Cloning and Genetic Engineering," *J of the Society for Philosophy and Technology*, 3 (Winter 1997), available at http://www.scholar.lib.vt.edu/ejournals/SPT/v3n2/BRUCE.html.

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