Animal Welfare Bien-être des animaux

Genetic engineering of animals: Ethical issues, including welfare concerns

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The genetic engineering of animals has increased significantly in recent years, and the use of this technology brings with it ethical issues, some of which relate to animal welfare — defined by the World Organisation for Animal Health as "the state of the animal…how an animal is coping with the conditions in which it lives" (1). These issues need to be considered by all stakeholders, including veterinarians, to ensure that all parties are aware of the ethical issues at stake and can make a valid contribution to the current debate regarding the creation and use of genetically engineered animals. In addition, it is important to try to reflect societal values within scientific practice and emerging technology, especially publicly funded efforts that aim to provide societal benefits, but that may be deemed ethically contentious. As a result of the extra challenges that genetically engineered animals bring, governing bodies have started to develop relevant policies, often calling for increased vigilance and monitoring of potential animal welfare impacts (2). Veterinarians can play an important role in carrying out such monitoring, especially in the research setting when new genetically engineered animal strains are being developed.

Several terms are used to describe genetically engineered animals: genetically modified, genetically altered, genetically manipulated, transgenic, and biotechnology-derived, amongst others. In the early stages of genetic engineering, the primary technology used was transgenesis, literally meaning the transfer of genetic material from one organism to another. However, with advances in the field, new technology emerged that did not necessarily require transgenesis: recent applications allow for the creation of genetically engineered animals via the deletion of genes, or the manipulation of genes already present. To reflect this progress and to include those animals that are not strictly transgenic, the umbrella term "genetically engineered" has been adopted into the guidelines developed by the Canadian Council on Animal Care (CCAC). For clarity, in the new *CCAC guidelines on: genetically-engineered animals used in science* (currently

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in preparation) the CCAC offers the following definition of a genetically engineered animal: "an animal that has had a change in its nuclear or mitochondrial DNA (addition, deletion, or substitution of some part of the animal's genetic material or insertion of foreign DNA) achieved through a deliberate human technological intervention." Those animals that have undergone induced mutations (for example, by chemicals or radiation — as distinct from spontaneous mutations that naturally occur in populations) and cloned animals are also considered to be genetically engineered due to the direct intervention and planning involved in creation of these animals.

Cloning is the replication of certain cell types from a "parent" cell, or the replication of a certain part of the cell or DNA to propagate a particular desirable genetic trait. There are 3 types of cloning: DNA cloning, therapeutic cloning, and reproductive cloning (3). For the purposes of this paper, the term "cloning" is used to refer to reproductive cloning, as this is the most likely to lead to animal welfare issues. Reproductive cloning is used if the intention is to generate an animal that has the same nuclear DNA as another currently, or previously existing animal. The process used to generate this type of cloned animal is called somatic cell nuclear transfer (SCNT) (4).

During the development of the *CCAC guidelines on: genetically-engineered animals used in science,* some key ethical issues, including animal welfare concerns, were identified: 1) invasiveness of procedures; 2) large numbers of animals required; 3) unanticipated welfare concerns; and 4) how to establish ethical limits to genetic engineering (see Ethical issues of genetic engineering). The different applications of genetically engineered animals are presented first to provide context for the discussion.

Current context of genetically engineered animals

Genetic engineering technology has numerous applications involving companion, wild, and farm animals, and animal models used in scientific research. The majority of genetically engineered animals are still in the research phase, rather than actually in use for their intended applications, or commercially available.

Companion animals. By inserting genes from sea anemone and jellyfish, zebrafish have been genetically engineered to express fluorescent proteins — hence the commonly termed "GloFish." GloFish began to be marketed in the United States in 2003 as ornamental pet fish; however, their sale sparked controversial ethical debates in California — the only US state to prohibit the sale of GloFish as pets (5). In addition to the insertion of

foreign genes, gene knock-out techniques are also being used to create designer companion animals. For example, in the creation of hypoallergenic cats some companies use genetic engineering techniques to remove the gene that codes for the major cat allergen Fel d1: (http://www.felixpets.com/technology.html).

Companion species have also been derived by cloning. The first cloned cat, "CC," was created in 2002 (6). At the time, the ability to clone mammals was a coveted prize, and after just a few years scientists created the first cloned dog, "Snuppy" (7).

With the exception of a couple of isolated cases, the genetically engineered pet industry is yet to move forward. However, it remains feasible that genetically engineered pets could become part of day-to-day life for practicing veterinarians, and there is evidence that clients have started to enquire about genetic engineering services, in particular the cloning of deceased pets (5).

Wild animals. The primary application of genetic engineering to wild species involves cloning. This technology could be applied to either extinct or endangered species; for example, there have been plans to clone the extinct thylacine and the woolly mammoth (5). Holt et al (8) point out that, "As many conservationists are still suspicious of reproductive technologies, it is unlikely that cloning techniques would be easily accepted. Individuals involved in field conservation often harbour suspicions that hi-tech approaches, backed by high profile publicity would divert funding away from their own efforts." However,

cloning may prove to be an important tool to be used alongside other forms of assisted reproduction to help retain genetic diversity in small populations of endangered species.

Farm animals. As reviewed by Laible (9), there is "an assorted range of agricultural livestock applications [for genetic engineering] aimed at improving animal productivity; food quality and disease resistance; and environmental sustainability." Productivity of farm animal species can be increased using genetic engineering. Examples include transgenic pigs and sheep that have been genetically altered to express higher levels of growth hormone (9).

Genetically engineered farm animals can be created to enhance food quality (9). For example, pigs have been genetically engineered to express the $\Delta 12$ fatty acid desaturase gene (from spinach) for higher levels of omega-3, and goats have been genetically engineered to express human lysozyme in their milk. Such advances may add to the nutritional value of animal-based products.

Farm species may be genetically engineered to create disease-resistant animals (9). Specific examples include conferring immunity to offspring via antibody expression in the milk of the mother; disruption of the virus entry mechanism (which is applicable to diseases such as pseudorabies); resistance to prion diseases; parasite control (especially in sheep); and mastitis resistance (particularly in cattle).

Genetic engineering has also been applied with the aim of reducing agricultural pollution. The best-known example is the Enviropig™; a pig that is genetically engineered to produce an enzyme that breaks down dietary phosphorus (phytase), thus limiting the amount of phosphorus released in its manure (9).

Despite resistance to the commercialization of genetically engineered animals for food production, primarily due to lack of support from the public (10), a recent debate over genetically engineered AquAdvantage™ Atlantic salmon may result in these animals being introduced into commercial production (11).

Effort has also been made to generate genetically engineered farm species such as cows, goats, and sheep that express medically important proteins in their milk. According to Dyck et al (12), "transgenic animal bioreactors represent a powerful tool to address the growing need for therapeutic recombinant proteins." In 2006, ATryn® became the first therapeutic protein produced by genetically engineered animals to be approved by the Food and Drug Administration (FDA) of the United States. This product is used as a prophylactic treatment for patients that have hereditary antithrombin deficiency and are undergoing surgical procedures.

Research animals. Biomedical applications of genetically engineered animals are numerous, and include understanding of gene function, modeling of human disease to either understand disease mechanisms or to aid drug development, and xenotransplantation.

Through the addition, removal, or alteration of genes, scientists can pinpoint what a gene does by observing the biological systems that are affected. While some genetic alterations have no obvious effect, others may produce different phenotypes that can be used by researchers to understand the function of the affected genes. Genetic engineering has enabled the creation of human disease models that were previously unavailable. Animal models of human disease are valuable resources for understanding how and why a particular disease develops, and what can be done to halt or reverse the process. As a result, efforts have focused on developing new genetically engineered animal models of conditions such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, and cancer. However, as Wells (13) points out: "these [genetically engineered animal] models do not always accurately reflect the human condition, and care must be taken to understand the limitation of such models."

The use of genetically engineered animals has also become routine within the pharmaceutical industry, for drug discovery, drug development, and risk assessment. As discussed by Rudmann and Durham (14): "Transgenic and knock out mouse models are extremely useful in drug discovery, especially when defining potential therapeutic targets for modifying immune and inflammatory responses…Specific areas for which [genetically engineered animal models] may be useful are in screening for drug induced immunotoxicity, genotoxicity, and carcinogenicity, and in understanding toxicity related drug metabolizing enzyme systems."

Perhaps the most controversial use of genetically engineered animals in science is to develop the basic research on xenotransplantation — that is, the transplant of cells, tissues, or whole organs from animal donors into human recipients. In relation to organ transplants, scientists have developed a genetically engineered pig with the aim of reducing rejection of pig organs by human recipients (15). This particular application of genetic engineering is currently at the basic research stage, but it shows great promise in alleviating the long waiting lists for organ transplants, as the number of people needing transplants currently far outweighs the number of donated organs. However, as a direct result of public consultation, a moratorium is currently in place preventing pig organ transplantation from entering a clinical trial phase until the public is assured that the potential disease transfer from pigs to humans can be satisfactorily managed (16). According to Health Canada, "xenotransplantation is currently not prohibited in Canada. However, the live cells and organs from animal sources are considered to be therapeutic products (drugs or medical devices)…No clinical trial involving xenotransplantation has yet been approved by Health Canada" (see http://www.hc-sc.gc.ca for details).

Ethical issues of genetic engineering

Ethical issues, including concerns for animal welfare, can arise at all stages in the generation and life span of an individual genetically engineered animal. The following sections detail some of the issues that have arisen during the peer-driven guidelines development process and associated impact analysis consultations carried out by the CCAC. The CCAC works to an accepted ethic of animal use in science, which includes the principles of the Three Rs (Reduction of animal numbers, Refinement of practices and husbandry to minimize pain and distress, and Replacement of animals with non-animal alternatives wherever possible) (17). Together the Three Rs aim to minimize any pain and distress experienced by the animals used, and as such, they are considered the principles of humane experimental technique. However, despite the steps taken to minimize pain and distress, there is evidence of public concerns that go beyond the Three Rs and animal welfare regarding the creation and use of genetically engineered animals (18).

Concerns for animal welfare

Invasiveness of procedures. The generation of a new genetically engineered line of animals often involves the sacrifice of some animals and surgical procedures (for example, vasectomy, surgical embryo transfer) on others. These procedures are not unique to genetically engineered animals, but they are typically required for their production.

During the creation of new genetically engineered animals (particularly mammalian species) oocyte and blastocyst donor females may be induced to superovulate via intraperitoneal or subcutaneous injection of hormones; genetically engineered embryos may be surgically implanted to female recipients; males may be surgically vasectomized under general anesthesia and then used to induce pseudopregnancy in female embryo recipients; and all offspring need to be genotyped, which is typically performed by taking tissue samples, sometimes using tail biopsies or ear notching (19). However, progress is being made to refine the genetic engineering techniques that are applied to mammals (mice in particular) so that less invasive

methods are feasible. For example, typical genetic engineering procedures require surgery on the recipient female so that genetically engineered embryos can be implanted and can grow to full term; however, a technique called non-surgical embryo transfer (NSET) acts in a similar way to artificial insemination, and removes the need for invasive surgery (20). Other refinements include a method referred to as "deathless transgenesis," which involves the introduction of DNA into the sperm cells of live males and removes the need to euthanize females in order to obtain germ line transmission of a genetic alteration; and the use of polymerase chain reaction (PCR) for genotyping, which requires less tissue than Southern Blot Analysis (20).

Large numbers of animals required. Many of the embryos that undergo genetic engineering procedures do not survive, and of those that do survive only a small proportion (between 1% to 30%) carry the genetic alteration of interest (19). This means that large numbers of animals are produced to obtain genetically engineered animals that are of scientific value, and this contradicts efforts to minimize animal use. In addition, the advancement of genetic engineering technologies in recent years has lead to a rapid increase in the number and varieties of genetically engineered animals, particularly mice (21). Although the technology is continually being refined, current genetic engineering techniques remain relatively inefficient, with many surplus animals being exposed to harmful procedures. One key refinement and reduction effort is the preservation of genetically engineered animal lines through the freezing of embryos or sperm (cryopreservation), which is particularly important for those lines with the potential to experience pain and distress (22).

As mentioned, the number of research projects creating and/or using genetically engineered animals worldwide has increased in the past decade (21). In Canada, the CCAC's annual data on the numbers of animals used in science show an increase in Category D procedures (procedures with the potential to cause moderate to severe pain and distress) — at present the creation of a new genetically engineered animal line is a Category D procedure (23). The data also show an increase in the use of mice (24), which are currently the most commonly used species for genetic engineering, making up over 90% of the genetically engineered animals used in research and testing (21). This rise in animal use challenges the Three Rs principle of Reduction (17). It has been reasoned that once created, the use of genetically engineered animals will reduce the total number of animals used in any given experiment by providing novel and more accurate animal models, especially in applications such as toxicity testing (25). However, the greater variety of available applications, and the large numbers of animals required for the creation and maintenance of new genetically engineered strains indicate that there is still progress to be made in implementation of the Three Rs principle of Reduction in relation to the creation and use of genetically engineered animals (21).

Unanticipated welfare concerns. Little data has been collected on the net welfare impacts to genetically engineered animals or

to those animals required for their creation, and genetic engineering techniques have been described as both unpredictable and inefficient (19). The latter is due, in part, to the limitations in controlling the integration site of foreign DNA, which is inherent in some genetic engineering techniques (such as pronuclear microinjection). In such cases, scientists may generate several independent lines of genetically engineered animals that differ only in the integration site (26), thereby further increasing the numbers of animals involved. This conflicts with efforts to adhere to the principles of the Three Rs, specifically Reduction. With other, more refined techniques that allow greater control of DNA integration (for example, gene targeting), unexpected outcomes are attributed to the unpredictable interaction of the introduced DNA with host genes. These interactions also vary with the genetic background of the animal, as has frequently been observed in genetically engineered mice (27). Interfering with the genome by inserting or removing fragments of DNA may result in alteration of the animal's normal genetic homeostasis, which can be manifested in the behavior and well-being of the animals in unpredictable ways. For example, many of the early transgenic livestock studies produced animals with a range of unexpected side effects including lameness, susceptibility to stress, and reduced fertility (9).

A significant limitation of current cloning technology is the prospect that cloned offspring may suffer some degree of abnormality. Studies have revealed that cloned mammals may suffer from developmental abnormalities, including extended gestation; large birth weight; inadequate placental formation; and histological effects in organs and tissues (for example, kidneys, brain, cardiovascular system, and muscle). One annotated review highlights 11 different original research articles that documented the production of cloned animals with abnormalities occurring in the developing embryo, and suffering for the newborn animal and the surrogate mother (28).

Genetically engineered animals, even those with the same gene manipulation, can exhibit a variety of phenotypes; some causing no welfare issues, and some causing negative welfare impacts. It is often difficult to predict the effects a particular genetic modification can have on an individual animal, so genetically engineered animals must be monitored closely to mitigate any unanticipated welfare concerns as they arise. For newly created genetically engineered animals, the level of monitoring needs to be greater than that for regular animals due to the lack of predictability. Once a genetically engineered animal line is established and the welfare concerns are known, it may be possible to reduce the levels of monitoring if the animals are not exhibiting a phenotype that has negative welfare impacts. To aid this monitoring process, some authors have called for the implementation of a genetically engineered animal passport that accompanies an individual animal and alerts animal care staff to the particular welfare needs of that animal (29). This passport document is also important if the intention is to breed from the genetically engineered animal in question, so the appropriate care and husbandry can be in place for the offspring.

With progress in genetic engineering techniques, new methods (30,31) may substantially reduce the unpredictability of

the location of gene insertion. As a result, genetic engineering procedures may become less of a welfare concern over time.

Beyond animal welfare

As pointed out by Lassen et al (32), "Until recently the main limits [to genetic engineering] were technical: what it is *possible* to do. Now scientists are faced with ethical limits as well: what it is *acceptable* to do" (emphasis theirs). Questions regarding whether it is acceptable to make new transgenic animals go beyond consideration of the Three Rs, animal health, and animal welfare, and prompt the discussion of concepts such as intrinsic value, integrity, and naturalness (33).

When discussing the "nature" of an animal, it may be useful to consider the Aristotelian concept of *telos,* which describes the "essence and purpose of a creature" (34). Philosopher Bernard Rollin applied this concept to animal ethics as follows: "Though [*telos*] is partially metaphysical (in defining a way of looking at the world), and partially empirical (in that it can and will be deepened and refined by increasing empirical knowledge), it is at root a moral notion, both because it is morally motivated and because it contains the notion of what about an animal we *ought* to at least try to respect and accommodate" (emphasis Rollin's) (34). Rollin has also argued that as long as we are careful to accommodate the animal's interests when we alter an animal's *telos,* it is morally permissible. He writes, "…given a *telos,* we should respect the interests which flow from it. This principle does not logically entail that we cannot modify the *telos* and thereby generate different or alternative interests" (34).

Views such as those put forward by Rollin have been argued against on the grounds that health and welfare (or animal interests) may not be the only things to consider when establishing ethical limits. Some authors have made the case that genetic engineering requires us to expand our existing notions of animal ethics to include concepts of the intrinsic value of animals (35), or of animal "integrity" or "dignity" (33). Veerhoog argues that, "we misuse the word *telos* when we say that human beings can 'change' the *telos* of an animal or create a new *telos*" — that is to say animals have intrinsic value, which is separate from their value to humans. It is often on these grounds that people will argue that genetic engineering of animals is morally wrong. For example, in a case study of public opinion on issues related to genetic engineering, participants raised concerns about the "nature" of animals and how this is affected (negatively) by genetic engineering (18).

An alternative view put forward by Schicktanz (36) argues that it is the human-animal relationship that may be damaged by genetic engineering due to the increasingly imbalanced distribution of power between humans and animals. This imbalance is termed "asymmetry" and it is raised alongside "ambivalence" as a concern regarding modern human-animal relationships. By using genetically engineered animals as a case study, Schicktanz (36) argues that genetic engineering presents "a troubling shift for all human-animal relationships."

Opinions regarding whether limits can, or should, be placed on genetic engineering are often dependent on people's broader worldview. For some, the genetic engineering of animals may not put their moral principles at risk. For example, this could perhaps be because genetic engineering is seen as a logical continuation of selective breeding, a practice that humans have been carrying out for years; or because human life is deemed more important than animal life. So if genetic engineering creates animals that help us to develop new human medicine then, ethically speaking, we may actually have a moral obligation to create and use them; or because of an expectation that genetic engineering of animals can help reduce experimental animal numbers, thus implementing the accepted Three Rs framework.

For others, the genetic engineering of animals may put their moral principles at risk. For example costs may always be seen to outweigh benefits because the ultimate cost is the violation of species integrity and disregard for the inherent value of animals. Some may view *telos* as something that cannot or should not be altered, and therefore altering the *telos* of an animal would be morally wrong. Some may see genetic engineering as exaggerating the imbalance of power between humans and animals, whilst others may fear that the release of genetically engineered animals will upset the natural balance of the ecosystem. In addition, there may be those who feel strongly opposed to certain applications of genetic engineering, but more accepting of others. For example, recent evidence suggests that people may be more accepting of biomedical applications than those relating to food production (37).

Such underlying complexity of views regarding genetic engineering makes the setting of ethical limits difficult to achieve, or indeed, even discuss. However, progress needs to be made on this important issue, especially for those genetically engineered species that are intended for life outside the research laboratory, where there may be less careful oversight of animal welfare. Consequently, limits to genetic engineering need to be established using the full breadth of public and expert opinion. This highlights the importance for veterinarians, as animal health experts, to be involved in the discussion.

Other ethical issues

Genetic engineering also brings with it concerns over intellectual property, and patenting of created animals and/or the techniques used to create them. Preserving intellectual property can breed a culture of confidentiality within the scientific community, which in turn limits data and animal sharing. Such limits to data and animal sharing may create situations in which there is unnecessary duplication of genetically engineered animal lines, thereby challenging the principle of Reduction. Indeed, this was a concern that was identified in a recent workshop on the creation and use of genetically engineered animals in science (20).

It should be noted that no matter what the application of genetically engineered animals, there are restrictions on the methods of their disposal once they have been euthanized. The reason for this is to restrict the entry of genetically engineered animal carcasses into the natural ecosystem until the long-term effects and risks are better understood. Environment Canada (http://www.ec.gc.ca/) and Health Canada (http://www.hc-sc.gc.ca/) offer specific guidelines in this regard.

Implications for veterinarians

As genetically engineered animals begin to enter the commercial realm, it will become increasingly important for veterinarians to inform themselves about any special care and management required by these animals. As animal health professionals, veterinarians can also make important contributions to policy discussions related to the oversight of genetic engineering as it is applied to animals, and to regulatory proceedings for the commercial use of genetically engineered animals.

It is likely that public acceptance of genetically engineered animal products will be an important step in determining when and what types of genetically engineered animals will appear on the commercial market, especially those animals used for food production. Veterinarians may also be called on to inform the public about genetic engineering techniques and any potential impacts to animal welfare and food safety. Consequently, for the discussion regarding genetically engineered animals to progress effectively, veterinarians need to be aware of the current context in which genetically engineered animals are created and used, and to be aware of the manner in which genetic engineering technology and the animals derived from it may be used in the future.

Genetic engineering techniques can be applied to a range of animal species, and although many genetically engineered animals are still in the research phase, there are a variety of intended applications for their use. Although genetic engineering may provide substantial benefits in areas such as biomedical science and food production, the creation and use of genetically engineered animals not only challenge the Three Rs principles, but may also raise ethical issues that go beyond considerations of animal health, animal welfare, and the Three Rs, opening up issues relating to animal integrity and/or dignity. Consequently, even if animal welfare can be satisfactorily safeguarded, intrinsic ethical concerns about the genetic engineering of animals may be cause enough to restrict certain types of genetically engineered animals from reaching their intended commercial application. Given the complexity of views regarding genetic engineering, it is valuable to involve all stakeholders in discussions about the applications of this technology.

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